Environmental and Microbial Biotechnology

Inamuddin
Mohd Imran Ahamed
Ram Prasad *Editors*

Application of Microbes in Environmental and Microbial Biotechnology

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Environmental and Microbial Biotechnology

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Editors
Inamuddin
Faculty of Engineering and Technology,
Department of Applied Chemistry,
Zakir Husain College of Engineering
and Technology
Aligarh Muslim University
Aligarh, India

Mohd Imran Ahamed
Faculty of Science, Department of Chemistry
Aligarh Muslim University
Aligarh, India

Ram Prasad
Department of Botany
Mahatma Gandhi Central University
Motihari, Bihar, India

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About the Editors

Inamuddin is an assistant professor at the Department of Applied Chemistry, Aligarh Muslim University, Aligarh, India. He has extensive research experience in multidisciplinary fields of analytical chemistry, materials chemistry, electrochemistry, renewable energy, and environmental science. He has published about 177 research articles in various international scientific journals, 18 book chapters, and 115 edited books with multiple well-known publishers. His current research interests include ion exchange materials, a sensor for heavy metal ions, biofuel cells, supercapacitors, and bending actuators.

Mohd Imran Ahamed received his Ph.D. degree on the topic "Synthesis and characterization of inorganic-organic composite heavy metals selective cation-exchangers and their analytical applications" from Aligarh Muslim University, Aligarh, India, in 2019. He has published several research and review articles in journals of international recognition. He has also edited various books which are published by Springer, CRC Press Taylor & Francis Asia Pacific, and Materials Science Forum LLC, USA. He has completed his B.Sc. (Hons) in chemistry from Aligarh Muslim University, Aligarh, India, and M.Sc. (organic chemistry) from Dr. Bhimrao Ambedkar University, Agra, India. His research works include ion-exchange chromatography, wastewater treatment and analysis, bending actuator, and electrospinning.

Ram Prasad is currently working as an Associate Professor at the Department of Botany, Mahatma Gandhi Central University, Motihari, Bihar, India. Dr. Prasad previously served as an Assistant Professor at Amity University, Uttar Pradesh, India; Visiting Assistant Professor at Whiting School of Engineering, Department of Mechanical Engineering at Johns Hopkins University, USA; and Research Associate Professor at the School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou, China. Dr. Prasad has more than one hundred fifty publications to his credit, including research papers, review articles, and book chapters; has edited or authored several books; and has five patents issued or pending. His research interests include applied microbiology, plant–microbe interactions, sustainable agriculture, and nanobiotechnology.

Application of Endophyte Microbes for Production of Secondary Metabolites

1

Seyyed Sasan Mousavi and Akbar Karami

Abstract

Herbs live in association with microbes with diverse levels of relationship. This association motivates perceptions on herb microbiome, and novel theories in herb evolution would be expanded in view of endophytes. Exploration of structurally new natural products considerably eases the detection of biologically active components, to successful progress of novel medicines. Endophytes colonize the interior tissues of various herb genera which have been demonstrated to make a lot of structurally varied secondary metabolites, which are valuable resources for pharmaceutical industries. Endophytes are any kind of microorganisms that live in an herb but may be categorized in diverse methods including functional types (endosyms, endosympaths, endopaths); taxonomic grouping, such as bacteria, fungi, and viruses and their subtaxa; the herb organ that they are living in (stem, radix or seed endophytes); or their mode of transmission (horizontally or vertically). They comprise components of plant microecosystems that dwell asymptomatically and symbiotically within plant tissue systems. Certain endophytes and their specific hosts have established a unique correlation that can expressively control plant metabolites and affect the physicochemical properties of medicinal plant-based crude drugs. Endophytes exhibit an eco-friendly alternative to promote herb development and also for serving as viable supplies of new bioactive natural products. Endophyte metabolites related to different structural types including alkaloids, terpenes, phenolics, flavonoids, glycosides, etc. have different therapeutic effects. These metabolites represent various medical functions including fungicidal, bactericidal, antiviral, antitumor, antidiabetic, insecticidal, immunosuppressive,

S. S. Mousavi · A. Karami (✉)
Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran
e-mail: akbarkarami@shirazu.ac.ir

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antioxidant, etc. Several of these natural products are used as immune-suppressant and to suppress the infectious and parasitic disorders, cancer, and hypertension.

Keywords

Anticancer · Antimicrobials · Endophytic microbes · Medicinal herbs · Secondary metabolites

1.1 Introduction

Herbs interact with different microbial groups and assist in preserving the biodiverseness and balance of the environment. There could be various kinds of microbial groups with regard to the locality, including epiphytic and endophytic fungi (Lindow and Brandl 2003; Yin et al. 2016; Bhardwaj et al. 2018). Fungal endophytes exist inside herb tissues without suffering hosts (Ludwig-Müller 2015). It is evaluated that there are about one million endophytes in plants (Wilson et al. 1997) that generate different compounds under limited growth space, particular natural ecosystems, and specific lifestyles. The word endophyte belongs to a bacterium or a fungus that colonizes in the herb's different parts, while it does not show pathogenic impacts on its host(s). Wilson (1995) stated that "endophytes are fungus or microorganisms, that in whole or a portion of their ontogenesis, attack the structures of alive herbs and induce invisible and symptomless infections in herb organs with no signs of illness." A conception of environment is one common portion of herb-microb ecology framework that may completely be clarified by realizing the environmental factors (Frank et al. 2017). Endophytes live in all herbs; hence, they are explained to be considerable in nature (Doty 2008; Khan and Doty 2011). However, studies stated the presence of endophytes in herb cells and cavities in various species (Backman and Sikora 2008). In general, endophytic microorganisms arise from the rhizosphere or phyllosphere and enter to the herbs via natural openings or wounds. In these entrances, various enzymes including cellulase, pectinase, and proteinase, that break down cell membrane and penetrate via radixes, are involved (Sturz and Nowak 2000; Wang and Dai 2011; Lemanceau et al. 2017). These microorganisms are achieved from all herb organs that appear to produce no external symptom for the existence of any lifestyles within them. They have sparked a large attention in the herb microbiome (endophytes) and how these microorganisms can affect the growth and the potential of an herb to withstand various stressed situations (Reid and Greene 2013). Endophytes act in these ways: (1) enhance nutrients acquired by herbs (White et al. 2012; Paungfoo-Lonhienne et al. 2010; Prieto et al. 2017; Beltran-Garcia et al. 2014), (2) protect herbs from diseases and herbivores (Soares et al. 2016; Gond et al. 2015; Verma et al. 2018b), (3) enhance tolerance to stress in herbs (Redman et al. 2002; Irizarry and White 2018), (4) regulate herb growth (Irizarry and White 2018; Verma et al. 2017, 2018a), (5) decrease weed development (White et al. 2018), and (6) cumulate active medicinal metabolites (Kusari et al. 2012a). Endophytic fungi also yield several biologically active compounds (Schulz et al. 2002). Endophytes can synthesize bioactive compounds for the competence with co-occurring endophytes, host, and diseases to colonize the host and also for nutrition (Clay 1988). They function as important origins for structurally special, bioactive natural compounds including alkaloids, flavonoids, phenolics, steroids, and terpenes, with vast ability for the exploration of new remediation (Tan and Zou 2001). They function as extremely influential producers of fungicidal, bactericidal, virucidal, and cytocidal compounds (Wiyakrutta et al. 2004; Terhonen et al. 2019).

1.2 Origin and Evolution of Endophytes

Various classes of microorganisms including fungi and bacteria are described as endophytes of herbs (Bandara et al. 2006). Asymptomatic fungal endophytes are universal, plentiful, and taxonomically diversified residents in all herbs (Saikkonen et al. 1999, 2016; Rodriguez et al. 2009). Fossils represent that endophytes were inhabited in herbs for about millions of years (Krings et al. 2007). This association begins when a region is colonized by herbs, which have a crucial part in evolution (Kozyrovska 2013). This evolutionary process caused variations in cells and molecules of hosts (Aravind et al. 2010; Costa and de Melo 2012; Karmakar et al. 2019). Endophytic fungi could be useful in antagonistic to mutual scopes (Giauque et al. 2019), making a framework for symbionts, essential for conception and application of endophytes. Different patterns for understanding the impacts of endophytic fungi on herb hosts are records of evolutionary (Giauque et al. 2019), habitation modifications (Rodriguez et al. 2009), and environmental or physiologic characteristics (Giauque et al. 2019). In the past decade, the availability of eukaryotic genomes and information about whole prokaryotic genomes has made it convenient to study HGT (horizontal gene transfer) between distantly related species, for organismal evolution and ecological adaptation (Latz et al. 2018). In the evolution of species, HGT is considered to be a key, evolutionary mechanism for conferring novel characteristics and helping adaptation to various ecosystems. However, examinations on gene transfer between herbs and endophytes were limited. The HGT phenomenon, regarded as a function favored by evolution, aids the acquisition of new characteristics by the associated species. Investigations considering the significance of HGT have been recognized between *Alternaria* and *Fusarium* fungi, nematodes and insects, humans and bacterial pathogens, and herbs and fungi (Tiwari and Bae 2020). Many investigations on the HGT phenomenon in prokaryotic evolution revealed a possible mechanism for acquiring new characteristics (Hawkins et al. 2019). Moreover, the transmission and integration of the transferred genes would prepare some useful features, viz., adaptation to ecosystem disturbances and acquisition of novel attributes/functions. Recently, the availability of complete genomes facilitated the analysis of HGT and its roles in the adaptation and evolution of bacterial, fungal, and eukaryotic genomes (Barreiro et al. 2019). Genetic disposition role in the evolution of endophytes was also

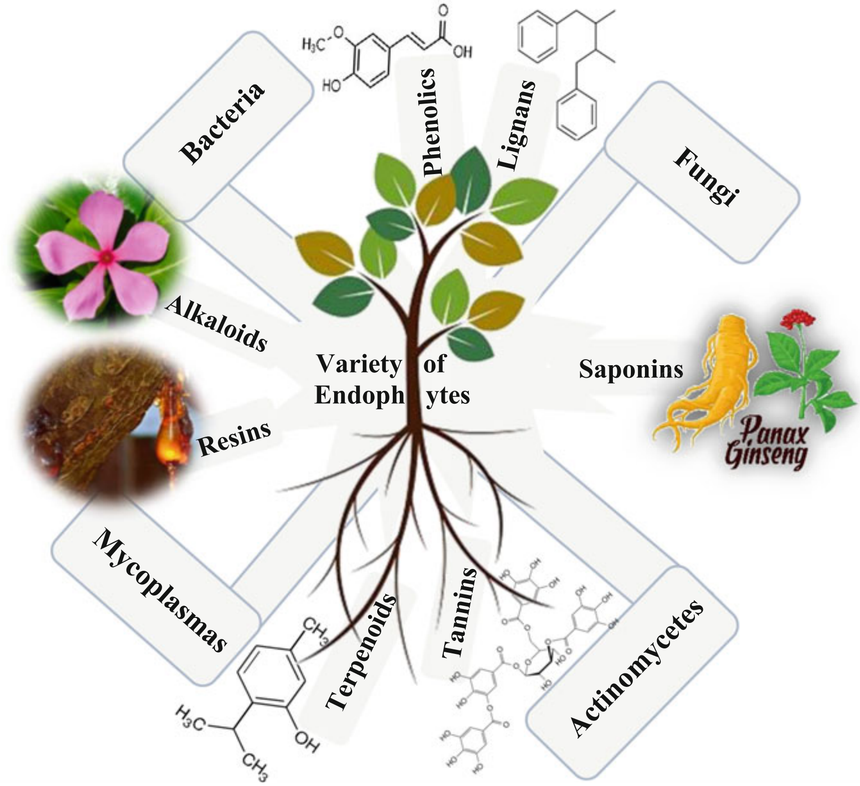


Fig. 1.1 Endophytes and the natural compound classes integrated with them

demonstrated by Freeman and Rodriguez (1993). In the pattern of endophyte inclusion to the root area, the radix external layer comes into a portion of the soil-radix microbial community, adequate for drift of endophytes into the xylem (Darbyshire and Greaves 1973; Old and Nicolson 1978; Sturz and Nowak 2000). Thus, a continuum of radix-associated microorganisms exists that are capable to colonize the rhizosphere, the radix cortex, and other herb parts (Sturz and Nowak 2000). The symbiosis between endophyte species and host plants is integrated with different factors (Harman and Uphoff 2019; Rho et al. 2018). Many endophytic microorganisms are species specific, and genetical discrepancy between host and endophyte may restrict the colonization (Zhou et al. 2018). Kinds of endophytes and the bioactive component classes associated with them are shown in Fig. 1.1.

1.3 Endophyte Diversity

The microorganisms might change host development and interactions with the ecosystem and also influence the diversity and composition of the microbiome community (Seabloom et al. 2019). There is a huge biodiversity of endophytes, in about 300,000 terrestrial host-plant species (Selim et al. 2017). Each host species has at least one endophyte microorganism. Endophytic microfungi are varied polyphyletic classes of organisms and could develop well in various organs of herbs, viz., shoot, leaf, and/or radix (Faeth and Fagan 2002; Yasser et al. 2020). The composition and diversity of a host's microbiome could change host physiology, development, and behavior (Seabloom et al. 2019). For instance, fungal endophytes could supply a vast fitness advantages to their herb hosts, including increased tolerance to stress and herbivores and also resource-use efficiency (Rodriguez et al. 2009; Busby et al. 2016; Buckley et al. 2019).

The biodiversity of endophytes is higher in comparison with the variation of herbs, vertebrates, and pests (Rana et al. 2020). The various classes of microorganisms were investigated for their integration with a variety of epiphytic, endophytic, and rhizospheric host herbs (Yadav et al. 2018, 2020; Seabloom et al. 2019; Zhou and Xu 2018).

A huge variation of endophytic microorganisms as archaea regard to the Euryarchaeota and fungi regard to the *Ascomycota*, *Basidiomycota*, and *Mucoromycota* are reported. Endophytic bacteria are also varied and big class of microorganisms stated from *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia* (Rana et al. 2020) (Fig. 1.2). The biggest phylum between bacteria was Proteobacteria, while *Acidobacteria*, *Bacteroidetes*, and *Deinococcus-Thermus* had minimum separated endophytes (Fig. 1.3). Given observed variations in endophytes between wet and dry locations, it has been shown that climate differences and ecological stresses would have various impacts on the endophytes and the host herb situation (Giauque and Hawkes 2016).

In contrast, in rainy tropical regions, with no significant climate difference among years, variations in endophytic fungi are induced by variations in host herb age or life phase (Higgins et al. 2014). So it is obvious that temporal and spatial differences should track host and climatic parameters over time (Maček et al. 2019).

1.4 Close Relationship Between Endophytes and Medicinal Herbs

Endophytes inhabit in the healthy organs of alive herbs and are essential compounds of herb microclimates. Medicinal plants and the diversity of the co-microbiota associated with these herbs remain poorly understood (Martinez-Klimova et al. 2017). The relation among endophytes and herbs is a mutually beneficial interaction (Cui et al. 2017). Recently, it is understood that endophytes have a major part in influencing the composition and content of the unprocessed medicines via a specific

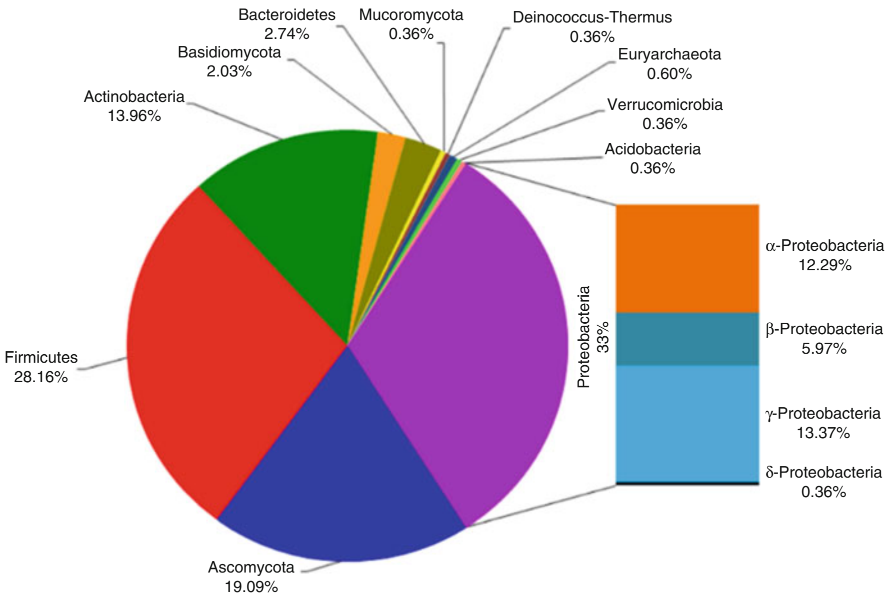


Fig. 1.2 Abundance of endophytic microbes belonging to diverse phylum (Rana et al. 2020)

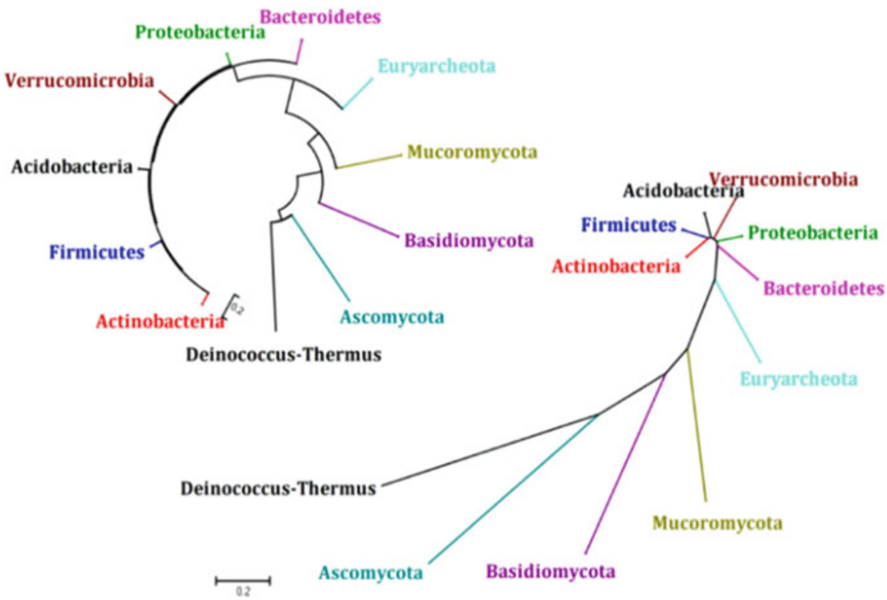


Fig. 1.3 Phylogenetic tree showing the relationship among different groups of endophytic microbiomes isolated from different host plant (Rana et al. 2020)

endophyte-herb relationship (Nalini and Prakash 2017). Endophytic fungi colonize their hosts without causing harm to them, contributing to enough situations for survival under various stresses, including high temperatures and nutrient insufficiency (Khidir et al. 2010). Endophytes construct secondary products, including phytohormones. Some fungal endophytes have stated that make different groups of auxins, including indole acetic acid (IAA) (Jagannath et al. 2019). The relationship between endophytes and their respective hosts might enhance in complexity if the hosts are medicinal herbs (Ogbe et al. 2020). The antioxidative function of endophytic fungi could be because of the secretion of phenolics and flavonoids into the growth medium. It was showed previously that endophytes associated with medicinal herbs have the ability to provide host-like bioactive chemical components. Endophytes might co-evolve with herb hosts and undergo species-specific interactions (Afridi et al. 2019; Dzoyem et al. 2017). Some examples of various endophytes and their host herbs are exhibited in Table 1.1.

Endophytes exhibit the ecologically favored association between herbs and microorganisms, supplying numerous benefits for the herb and the ecosystem. Endophytic associations have potential applications in agriculture, industries, and pharmaceuticals. The host herb protects the organisms, and the organisms synthesize compounds that enhance nutrients' absorption, influencing the herb development and growth enhancement (Giauque and Hawkes 2016; Shikano 2017; Kowalski et al. 2015). Some studies have reported on the production of virucidal, bactericidal, and fungicidal components by fungal endophytes (Gunatilaka 2006; Tejesvi et al. 2011). Exploiting these interactions would ease the perfect production of novel medicines by changing the development situations of therapeutic herbs by using specific class of endophytes (Firáková et al. 2007). In 2008, Moricca and Ragazzi stated that the type of relationship between endophytes and herbs is managed by the genes of both organisms and changed by the ecosystem. The relationship among endophytes and herbs happens at a metabolic degree that some types of relationships are practicable: (1) the endophytic fungi causes herb metabolism, (2) the herb causes endophytic fungi metabolism, (3) the herb and endophytic fungi distribute metabolic pathways between each other, (4) the host herb might synthesize endophytic fungi metabolites, and (5) the endophytic fungi could produce herb-derived medicinal compounds (Ludwig-Müller 2015).

Benefits by fungi seem to depend on the host species, host genotype, and environmental situations (Saikkonen et al. 1999). The endophytes also affect herbs by nitrogen fixation, phosphorus solubilization, improving water-nutrient accessibility and usage, causing resistance to various stresses, biological control of herbivores, and producing phytochemicals (Walia et al. 2017; Xia et al. 2015; Santos et al. 2018).

Table 1.1 Various endophytes and their host plants

Endophytic fungi	Host plant	References
Verruconis strain SYPF 8337T	*Panax notoginseng*	Zhang et al. (2018)
Bacillus cereus and *B. subtilis; Penicillium chrysogenum* and *P. crustosum.*	*Teucrium polium*	Hassan (2017)
Muscodor tigerii	*Cinnamomum camphora*	Saxena et al. (2015)
Alternaria sp.	*Corylus avellana*	Michalczyk et al. (2015)
Cladosporium oxysporum	*Moringa oleifera*	Raj et al. (2015)
Nigrograna mackinnonii	*Guazuma ulmifolia*	Shaw et al. (2015)
Colletotrichum gloeosporioides	*Piper nigrum*	Chithra et al. (2014)
Penicillium resedanum LK6	*Capsicum annuum*	Khan et al. (2013)
Perenniporia tephropora	*Taxus chinensis var. mairei*	Wu et al. (2013a)
Colletotrichum gloeosporioides	*Tectona grandis*	Senthilkumar et al. (2013)
Fusarium redolens	*Taxus wallichiana*	Garyali et al. (2013)
Cephalotheca faveolata	*Eugenia jambolana*	Giridharan et al. (2012)
Cladosporium oxysporum	*Moringa oleifera*	Zhao et al. (2012)
Bacillus subtilis, Myxormia sp.	*Angelica sinensis*	Yang et al. (2012)
Chaetomium globosum L18	*Curcuma wenyujin*	Wang et al. (2012)
Arbuseular mycorrhiza	*Salvia miltiorrhiza*	Meng and He (2011)
Penicillium baarnense, Penicillium frequentans	*Curcuma zedoaria*	Qun et al. (2011)
Leucocoprinus gongylophorus	*Cordia alliodora*	Bittleston et al. (2011)
Thielavia subthermophila	*Hypericum perforatum*	Kusari et al. (2009)
Phomopsis sp.	*Camptotheca acuminate*	Lin et al. (2009)
Chaetomium sp.	*Salvia officinalis*	Debbab et al. (2009)
Alternaria sp.	*Ginkgo biloba*	Qin et al. (2009b)
Alternaria sp.	*Rosa damascena*	Kaul et al. (2008)
Alternaria sp.	*Polygonum senegalense*	Aly et al. (2008a)
Hypoxylon truncatum	*Artemisia annua*	Gu et al. (2007)
Sebacina vermifera	*Nicotiana attenuata*	Barazani et al. (2007)
Phomopsis cassiae	*Cassia spectabilis*	Silva et al. (2006)

(continued)

Table 1.1 (continued)

Endophytic fungi	Host plant	References
Chaetomium globosum	*Ephedrafa sciculata*	Bashyal et al. (2005)
Muscodor albus	*Cinnamomum zeylanicum*	Strobel et al. (2001)

1.5 Endophytes and Secondary Metabolites

Medicinal herbs are hopeful sources for the expansion of natural drugs, promoting an enhancement in the use of these herbs worldwide. In recent years, numerous novel metabolites from fungi have extracted and stated to make lead components for novel drug discovery (Palanichamy et al. 2018). Bioactive medicinal compounds are major origin of antidiabetic, antineoplastic, antioxidant, immunosuppressive, fungicidal, bactericidal, insecticidal, anti-nematode, and virucidal drugs (Tan and Zou 2001; Strobel and Daisy 2003; Strobel et al. 2004; Gunatilaka 2006; Zhang et al. 2006; Verma et al. 2009; Aly et al. 2010, 2011; Brader et al. 2014). Endophytic fungi from medicinal herbs can be a good source of functional compounds (Huang et al. 2008; Tejesvi et al. 2007). There is a positive relation between endophytes and medicinal herbs, in metabolite production due to genetic recombination with the host during evolution (Khan et al. 2017). They behave as important origins for structurally special, active non-chemical compounds including alkaloids, phenolics, steroids, flavonoids, and terpenes, with vast ability for new remediations (Liu et al. 2016). Mutualism between endophytes and host herbs might have advantages for both partners (Kogel et al. 2006; Hoysted et al. 2019). Detailed endophyte-plant interaction strategies are shown in Fig. 1.4. Endophytes provide various bioactive compounds and natural products with distinctive structure, such as alkaloids, isocoumarins, phenylpropanoids, lignans, glycosides, flavonoids, phenols, steroids, and aliphatic metabolites (Tan and Zou 2001; Kaul et al. 2013; Rathod et al. 2013; Palanichamy et al. 2018).

1.6 Terpenoids

Terpenes are wide group of medicinal metabolites utilized in the aroma and flavor industries and have considerably utilized in biotransformation process by microbes with focus on the recognition of novel flavor ingredients (Bicas et al. 2009). Terpenoids serve in herb-fungus relationships as both are constitutive and clearly caused chemical defenses (Viiri et al. 2001; Yan et al. 2018). Fungi could biotransform terpenoids and release them in herb organs (Demyttenaere and De Kimpe 2001) or in the surrounding atmosphere by volatilization (Pandey et al. 1993; Giamperi et al. 2002; Gómez-Lama Cabanás et al. 2014). A diterpenoid, namely,

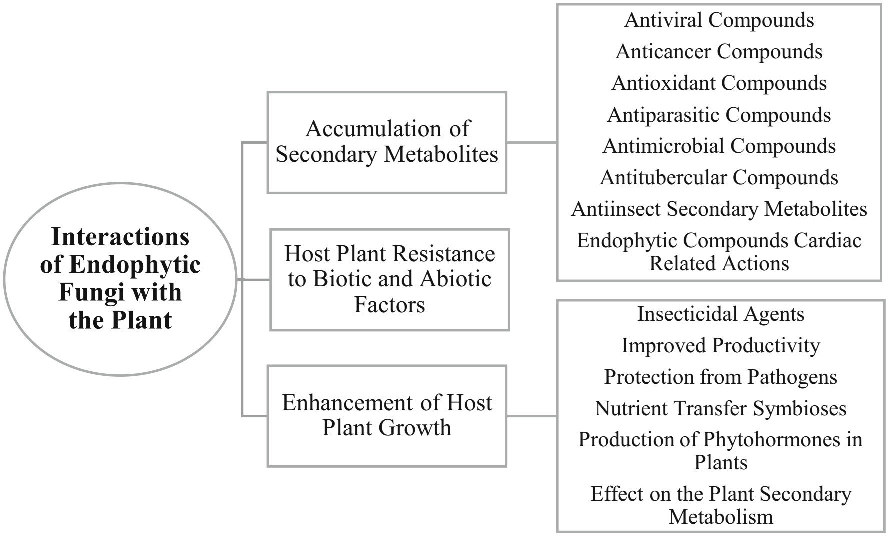


Fig. 1.4 Common endophyte-plant interaction strategies

paclitaxel (Taxol), a natural metabolite, is extracted from *Taxus brevifolia* in the 1960s. This compound showed notable antitumor potential, specifically ovarian, uterine, and breast cancer with high degree. In an investigation, sesquiterpene derivatives were separated from *Phomopsis cassiae*, an endophytic fungus isolated from *Cassia spectabilis* (Silva et al. 2006). Biotransform activity of fungal endophytes separated from *Huperzia serrata*, detected in the conversion of huperzine A to produce active sesquiterpenoid hybrids, namely, huptremules A–D (Ying et al. 2014). Monoterpene preaustinoids made by *Penicillium* sp., endophytic to *Melia azedarach*, presented bactericidal function against *E. coli*, *S. aureus*, *P. aeruginosa*, and *Bacillus* sp. (Dos Santos and Rodrigues-Fo 2003). The separation of *Penicillium* species, specifically *Penicillium brevicompactum*, from *Taxus brevifolia* have been stated in 2000 (Stierle and Stierle 2000). It is reported that they manufacture a terpene, namely, mycophenolic acid. This component is fungicidal and utilized in the curing of dengue fever. Evaluation of microfungi separated from internal parts of radix and shoot of *Coleus forskohlii* for forskolin extraction, a labdane diterpenoid, exhibited that *Rhizoctonia bataticola* was capable to produce forskolin and, interestingly, send out into the medium (Mir et al. 2015). The usages of forskolin extend from alleviation of glaucoma, anti-HIV or anticancer activities, curing of high blood pressure and cardiac problems to weight loss and lipolysis (Pateraki et al. 2017). Thirteen triterpenoids were gained from the fermented *Kadsura angustifolia* after successive separation and purifications using different column chromatography procedures (Qin et al., 2019a). The fungus *Aspergillus fumigatus*, an endophyte of *Ligusticum wallichii*, made novel sesquiterpene compounds, fumagillin A and B (Li et al. 2020). Another new terpenoid, a 14-nordrimane-type sesquiterpene, phomanolide was achieved from the culture

Table 1.2 Fungi-medicinal plant interactions which produce terpenoids

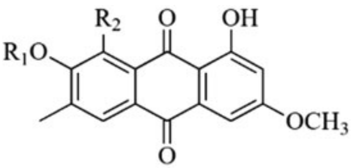
	Fungal endophyte	Plant host	References
Terpenoids	*Coriolopsis* sp.	*Ceriop stagal*	Chen et al. (2017)
	Pseudolagarobasidium acaciicola	*Bruguiera gymnorrhiza*	Wibowo et al. (2016)
	Fusarium oxysporum SY0056	*Ginkgo biloba L.*	Cui et al. (2012)
	Xylaria sp.	*Piper aduncum*	Silva et al. (2010)
	Eutypella sp.	*Etlingera littoralis*	Isaka et al. (2009)
	Phomopsis sp.	*Plumeria acutifolia*	Xu et al. (2008)
	Phyllosticta spinarum	*Platycladus orientalis*	Wijeratne et al. (2008)
	Pestalotiopsis terminaliae	*Terminalia arjuna*	Gangadevi and Muthumary (2008)
	Phomopsis cassiae	*Cassia spectabilis*	Silva et al. (2006)
	Periconia sp.	*Taxus cuspidate*	Kim et al. (2004)
	Periconia atropurpurea	*Xylopia aromatica*	Teles et al. (2006)
	Pestalotiopsis microspora	*Taxus wallichiana*	Stierle et al. (1993)
	Taxus brevifolia	*Taxomyces andreanae*	Stierle et al. (1993)

broth of *Phoma* sp. separated from the radix of *Aconitum vilmorinianum* (Liu et al. 2019). New diterpenes, koninginols A–C formed by the endophytic fungus *Trichoderma koningiopsis* A729, were separated from the shoots of *Morinda officinalis* (Chen et al. 2019). Integracide E and isointegracide E (tetracyclic triterpenoids) have been gained from the *Hypoxylon* sp. 6269 that was separated from *Artemisia annua* (Liang et al. 2018). Terpenes from the plant genus *Copaifera*, demonstrated in vitro antiparasitic potential (Izumi et al. 2012). Azadirachtins A and B have isolated from cultures of *Penicillium parvum*, endophytic in *Azadirachta indica* tree possessing insecticidal potential (Chutulo and Chalannavar 2018).

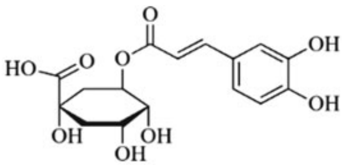
Duan et al. (2016) separated some monoterpenoids with regard to structural investigations and bioactive potentials from the endophytic fungus *Penicillium* sp. colonized on *Gastrodia elata*. Another compound, trichodermin, was obtained from *Trichoderma harzianum*, a microfungus from *Ilex cornuta* (Chen et al. 2007). Trichodermin has stated to preserve against *Alternaria solani* and *Rhizoctonia solani*, the solanaceous plant pathogens (Chen et al. 2007). A sesquiterpene, phomenone, is obtained from *Xylaria* sp., an endophyte colonized with *Piper aduncum* (Silva et al. 2010). Cycloepoxylactone and cycloepoxytriol B were isolated from *Phomopsis* sp., separated from the leaf of *Laurus azorica*. Cycloepoxylactone restricts the growth of *Microbotryum violaceum* and *Bacillus megaterium*, while cycloepoxytriol B suppressed *Chlorella fusca* development (Hussain et al. 2009). The separation and evaluation of an endophytic fungus, *Eupenicillium parvum*, from *Azadirachta indica* A. Juss. manufactures azadirachtin A and B under shake-flask fermentation situations (Kusari et al. 2012b). Some examples of fungi-medicinal plant interactions which result in terpenoid production are listed in Table 1.2.

1.7 Phenolics

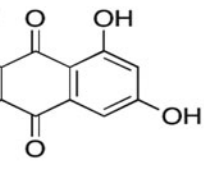
In herbs, phenolics could be formed by various different routes: shikimic acid from carbohydrates or by acetate (Richards et al. 2006; Tinikul et al. 2018). In several occasions, endophytic fungi promote lengthy radix and increase emission of phenolics into the root around area (Malinowski and Belesky 2000; Lunardelli Negreiros de Carvalho et al. 2016). As an example, in the leaf of *Coccoloba cereifera*, an obvious relation between leaf polyphenols and endophyte richness was recognized (Sanchez-Azofeifa et al. 2012). Elicitin, a cysteine-rich extracellular protein secreted by many *Phytophthora* species, was extracted from *Phytophthora palmivora*, a pathogen of *Hevea brasiliensis*, and induced scopoletin, peroxidase isozymes, and total phenolics in cell suspension of *H. brasiliensis*. Moreover, it induces total phenolics and increased resistance against *P. palmivora* on rubber plantlets. Their phenolic compounds, that are usual to the endophytic fungi metabolism and to their host, are showed in Fig. 1.5 (Dutsadee and Nunta 2008; Lunardelli Negreiros de Carvalho et al. 2016). It has also exhibited that the herb growth-promoting endophyte *Burkholderia phytofirmans* PsJN enhanced the levels of phenolic compounds in grapevine seedlings that showed an increased cold tolerance (Barka et al. 2006). Also, it was stated in *Lolium perenne* that colonization with



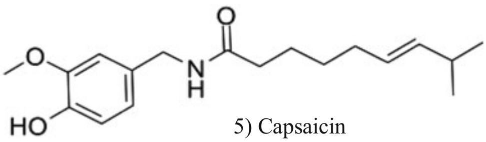
1) R_1=R_2=H, Macrosporin
2) R_1=H, R_2=OH, 3-*O*-Methylalaternin



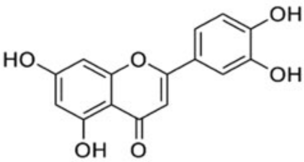
3) 5-*O*-caffeoylquinic acid



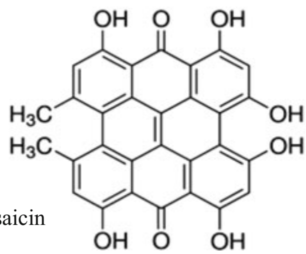
4) Emodin



5) Capsaicin



6) Luteolin



7) Capsaicin

Fig. 1.5 Chemical structures from phenolic secondary metabolites produced by endophytic fungi

Neotyphodium lolii notably affected the phenolics quantity and antioxidant potential, although the impact was understood to be strain-dependent (Qawasmeh et al. 2012). Sommart et al. (2012) separated 14 phenolic compounds from *Garcinia hombroniana* leaves. 1-(2,5-Dihydroxyphenyl)-2-buten-1-one showed fungicidal potential against *Microsporum gypseum* SH-MU-4, anti-malarial activity, and high activity in reducing free radicals. Microsphaerophtalide A and sclerin also exhibited fungicidal potential against *M. gypseum* SH-MU-4. Microsphaerophtalide E also exhibited an activity against *Cryptococcus neoformans*. Casella et al. (2013) investigated endophytic fungi *Lewia infectoria* SNB-GTC2402 and isolated the alterperylenol. This component was active toward *Staphylococcus aureus* ATCC 29213. Endophytic fungi provide important antioxidant components. Theantana et al. (2012) separated 39 fungi from five Thai therapeutic herbs that they made phenolic acids. A phenolic antifungal component, separated from liquid culture of *Colletotrichum gloeosporioides*, is an endophytic fungus of *Artemisia mongolica* and was helpful against *Helminthosporium sativum* (Zou et al. 2000). The endophyte, *Ampelomyces* sp., separated from *Urospermum picroides* provided some phenolics after fermentation (Aly et al. 2008b). Erbert et al. (2012) distinguished phenolics in the endophytic fungus extracts achieved from the red algae *Bostrychia radicans*. Kornsakulkarn et al. (2011) separated javanicin and other 15 phenolics from *Fusarium* sp. BCC14842 that was separated from bamboo leaf. These phenolic compounds include 4-hydroxydihydronorjavanicin, dihydronaphthalenone, diastereomer dihydronaphthalenone, 5-hydroxydihydrofusarubin A, 5-hydroxydihydrofusarubin B, 5-methoxydihydrofusarubin B, 5-hydroxy3-methoxydihydrofusarubin A, 3,5-dimethoxydihydrofusarubin B, 5-hydroxydihydrofusarubin D, 5-hydroxy-3- methoxydihydrofusarubin D, 3,5-dimethoxyihydrofusarubin D, 5-hydroxydihydrofusarubin C, bostrycoidin, anydrofusarubin, and 3-*O*-methylfusarubin. Researches showed that chlorogenic acid is the main phenolic components from some remedial herbs that showed antioxidant activities (Huang et al. 2007a, b; Ray et al. 2020).

In a research, 42 endophytic fungi from leaf and shoot of *Nerium oleander* isolated, and phenolics quantities and radical scavenging functions evaluated. They presented that many endophytic fungi separated from *N. oleander* displayed radical scavenging potential to some degree. The phenolics, such as phenolic acids, flavonoids and several aromatic and metabolites are responsible for radical scavenging potential (Huang et al. 2007a, b). In another study, the microbicidal potential of fungal endophytes colonizing *Emblica officinalis* has been evaluated. Endophytic fungi, *Phomopsis* sp., *Epacris* sp., *Xylaria* sp., and *Diporthe* sp., were separated from various organs of the herb. The antioxidant activity and total phenol were investigated utilizing ethanolic extract of endophytic fungi. Endophytes, *Phomopsis* sp. and *Xylaria* sp., exhibited maximum antioxidant potential and also had the higher contents of phenolics.

1.8 Flavonoids

Flavonoids are a class of secondary metabolites that have notable pharmaceutical potentials, including antioxidant, anticancer, analgesic, bactericidal, and heart protection (Graf et al. 2005; Mehmood et al. 2019). Flavonoids have high radical scavenging potentials; therefore, it has preventive and therapeutic impacts against several usual illnesses (Atmani et al. 2009). Fungal flavonoid-type components have been stated to have involved in herb defense against fungi. Flavonoids are common medicinal compounds which have roles in various pathways including cell signaling, herb development, and reproduction (Taylor and Grotewold 2005; Cui et al. 2018; Harwoko et al. 2019). *Ceriporia lacerata* DMC1106, an endophytic fungus, might make the antitumor flavonoid, 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (Wang et al. 2013). The endophytic fungi strain *Aspergillus nidulans* and *Aspergillus oryzae* were achieved from *Ginkgo biloba* L. and could manufacture flavonoids (Qiu et al. 2010). Total flavonoids in medium culture of endophytic *Aspergillus flavus* L7 was 158.33 mg quercetin/mL (Patil et al. 2015). Liu et al. (2007) achieved an endophytic *Xylaria* sp. from *G. biloba*, and this strain has the potential to make flavonoids. In a study, cultivation of *Epicoccum nigrum*, an endophytic fungus separated from the willow leaf (*Salix* sp.), yielded the flavonol kaempferol and two kaempferol diglycosides. Previously, also the existence of flavonoid glycosides in *E. nigrum* had examined when the fungus was fermented on solid corn medium (Harwoko et al. 2019). As reported on flavonoids from endophytic fungi, for example, *Pestalotiopsis uvicola*, *Aspergillus flavus*, and *Annulohypoxylon squamulosum* have been exhibited to accumulate flavonoids in the form of aglycones or glycosides (Qian et al. 2017; Patil et al. 2015). Also it had been reported that flavonoid monoglycosides or an unusual chlorinated flavonoid, named chlorflavonin, are reported in *Nigrospora oryzae* and *Mucor irregularis* colonized with the medicinal herbs *Loranthus micranthus* and *Moringa stenopetala*, respectively (Harwoko et al. 2019). Flavonoids have stated to have important impact on radix colonization by *Gigaspora* and *Glomus* species (Scervino et al. 2007). Also, the role of flavonoids in radix colonization by endophytic fungi, such as *Aspergillus nodulias* and *Aspergillus oryzae*, has been investigated previously (Qiu et al. 2010). In an investigation, three flavonoids, viz., calycosin, dihydroxyflavone, and pratensein, were recognized in the culture of endophytes Pz11, which isolated from *Asphodelus tenuifolius* root (Mehmood et al. 2019). Bioconversion could influence herb metabolite compositions, as was exhibited for the endophytic *Paraconiothyrium variabile*, that biotransforms glycosylated flavonoids to aglycons, that in turn changes the host *Cephalotaxus harringtonia* metabolomic profile. It was stated that comparison of *Fusarium*-infected wheat cultivars exhibited varied accumulation of benzoxazinoids, phenolics, carotenoids, and flavonoids, whose levels showed varying selective pressures on the fungal pathogen *Fusarium*. Furthermore, the flavonoids, homoorientin, and orientin were reported as key inhibitors of the trichothecene mycotoxin deoxynivalenol produced under *Fusarium* infection (Cui et al. 2018). Researchers stated that Alt a 1, a host-selective phytotoxin, is an allergenic protein present in *Alternaria alternata* that causes asthma. They stated that Alt a 1 exist in the spores. The Alt a 1 ligand was recognized as a methylated flavonoid which prevents herb radix development and detoxifies reactive oxygen species (Garrido-Arandia et al. 2016). In another study, researchers stated that the endophytic fungi *Aspergillus niger* GZ-4 from sugarcane leaves produce flavonoids in which can be measured by UV spectrophotometry with rutin as reference substance (Zhou et al. 2016).

1.9 Alkaloids

Endophytic fungi could produce various compounds, including alkaloids. Alkaloids are essential metabolites, for chemical characteristics, and varied bioactivities, including fungicidal, antitumor, and virucidal (Bastias et al. 2017). Endophytes are good origins of new and active alkaloid products. Several worthy alkaloids with pharmacologically functional potentials have achieved from fungal endophytes and can be emphasized as a crucial source for drugs (Wang et al. 2011a; Zhou et al. 2020). An endophytic fungus *Alternaria* sp. separated from *Catharanthus roseus* phloem had the potential to produce vinblastine, first reported by Guo and Kunming (1998). Lingqi et al. (2000) proficiently found an endophyte, *Fusarium oxysporum* from *C. roseus* phloem, which produced vincristine. The endophytic fungus in the leaf of *C. roseus* also makes the same alkaloid, vincristine (Xianzhi et al. 2004). In another research stating that the various *C. roseus* parts harbor a plethora of endophytic fungi for the production of vinca alkaloids showed that just endophytic fungi harboring in the leaf of *C. roseus* were capable of vinblastine and vincristine production. These endophytes have been recognized as *Fusarium oxysporum*, *Talaromyces radicus*, and *Eutypella* sp. (Kharwar et al. 2008; Kumar et al. 2013; Palem et al. 2015; Kuriakose et al. 2016). Evaluating the endophytic fungus, *F. solani* from *C. roseus* for vinca alkaloids analysis also investigated. The fungus was recognized to make vincristine and vinblastine (Kumar et al. 2013). Endophytic fungi isolated from *Vinca minor* produced vincamine that is utilized in the pharmacological industry as a vasodilator (Yin and Sun 2011). Alkaloids are also principal for protection of the herb against herbivores (Bush et al. 1982; Siegel et al. 1990). As a best-described example, Clavicipitaceous fungi produce lolitrems, the neurotoxic indole-diterpenoid alkaloids, which intoxicate cattle grazing on the endophyte-infected lawn (Fletcher and Harvey 1981; Gallagher et al. 1984). In a study, Sun et al. (2012), some endophytic fungi obtained from *Datura stramonium* L., that produces crucial tropane alkaloids, viz., scopolamine and hyoscyamine (Naik et al. 2018). Alkaloids produced by endophytic fungi isolated from various medicinal herbs with their biological potential are listed in Table 1.3. Several endophytes fill herbs with components that decrease herbivory by various herbivores. Fungal endophytes in the genus *Epichloë* (*Clavicipitaceae*) intercellularly colonize herbs (i.e., leaves, culms, and seeds) and produce various alkaloids that prevent feeding by herbivores (Panaccione et al. 2014). In a similar way, Fabaceae family crops, endophytic fungi in genus *Undifilum* (*Pleosporaceae*), produce the toxic alkaloid swainsonine, a great anti-herbivore component (Panaccione et al. 2014). In the

Table 1.3 Compounds obtained from endophytic fungi isolated from various medicinal plants (Gómez and Luiz 2018)

Medicinal plant	Endophytic fungi	Product of interest	Pharmacological effects	References
Nerium indicum	*Geomyces* sp.	Vincamine (indole alkaloid)	Cardiovascular and cerebrovascular protective and acetylcholinesterase inhibitor	Na et al. (2016)
Catharanthus roseus	*Fusarium oxysporum, Talaromyces radicus, and Eutypellas pp.*	Vinblastine and vincristine (alkaloids)	Antitumor	Palem et al. (2015), Kumar et al. (2013)
Coleus forskohlii	*Rhizoctonia bataticola*	Forskolin (alkaloid)	Glaucoma, antitumor, anti-HIV, cardiovascular protective	Mir et al. (2015)
Fritillaria cirrhosa	*Fusarium redolens*	Peimisine; imperaline-3-β-D-glucoside (alkaloids)	Antitussive and expectorant	Pan et al. (2015)
Capsicum annuum	*Alternaria alternate*	Capsaicin (alkaloid)	Cardiovascular protective and antitumor	Devari et al. (2014)
Piper nigrum L.	*Colletotrichum gloeosporioides*	Piperine (alkaloid)	Antibacterial, antifungal, hepato-protective, antipyretic, anti-inflammatory, anti-convulsant, inseticidal, andantioxidant	Chithra et al. (2014)
Macleaya cordata	*Fusarium proliferatum* BLH51	Sanguinarine (alkaloid)	Antibacterial, antihelmintic, antitumor, anti-inflammatory	Wang et al. (2014)
Cinchona ledgeriana	*Phomopsis, Diaporthe, Schizophyllum, Penicillium, Fomitopsis* and *Arthrinium*	Cinchona alkaloids	Antiparasitic (malaria)	Maehara et al. (2011, 2013)

context of biological studies, cytotoxic examinations toward the human leukemia and colon cancer cell lines were recorded by an alkaloid chaetominine achieved from *Chaetomium* sp. IFB-E015, an endophytic fungus from *Adenophora axiliflora* (Puri et al. 2006). Another well-known alkaloid is caffeine, which is the methylxanthine alkaloid from *Coffea* sp. plant. This alkaloid has a psychoactive drug observed in endophytes' extracts gained from the herbs *Osbeckia chinensis*, *O. stellata*, and *Potentilla fulgens* (Bhagobaty and Joshi 2011). Taxol is a diterpene alkaloid generated by the endophyte *Metarhizium anisopliae* recognized in the bark of taxus tree (Zhang et al. 2009; Sonaimuthu and Johnpaul 2010; Jalgaonwala et al. 2011). Another endophytic fungal strain is *Penicillium* sp., which is inhabiting in the shoot of *Quercus variabilis* and induces production of Penicidones A-C (Zhang et al. 2012). Another investigation exhibited isolation of different compounds, including (−)-4,6′-anhydrooxysporidinone, (−)-6-deoxyoxysporidinone, and (−)-joxysporidinone from the culture of the endophytic fungus *Cladosporium herbarum* of *Ephedra fasciculata*. All of them showed either no or only weak potentials toward lung, pancreatic, CNS glioma, and breast cancer cell lines (Zhang et al. 2012; Zhan et al. 2007).

In the genus *Crotalaria*, biosynthesis of pyrrolizidine alkaloids, that is important in herb's protection toward herbivores, depends on the nodulation by the genus *Bradyrhizobium* (Irmer et al. 2015). Another investigation showed that *Huperzia serrata*, a medicinal herb existed in tropical regions, could generate Huperzine-A components which are induced by endophytic fungi *Acremonium* sp. and *Shiraia* sp. (Wang et al. 2011b; Zhou et al. 2009). Alkaloids made by endophytes could preserve the host, stimulate useful compositions production, and can be utilized in the pharmaceutical industry and in addition for treating illnesses (Zhang et al. 2012).

1.10 Glycosides

Glycosides is a plentiful secondary metabolites which existing in various herbs (Dembitsky 2004; Evans 2009). In herbs, glycosides are gained mostly from post-modification of the secondary metabolites activated by herb enzymes, glycosyltransferases (Blanchard and Thorson 2006; Firdous et al. 2020). Glycosides are accumulated and transferred in herb's different organs and might have a key role in signaling, in growth controlling, and also in a phytotoxic activity. They are also essential in the herb's defense pathways against pathogens and herbivores (Shang et al. 2018; Notarte et al. 2019). In the series of functional active components from herbs and their respective endophytes, the principal metabolites that could be isolated from *Digitalis lanata* and *Digitalis purpurea* contain digoxin and cardiac glycosides (Ahmed et al. 2012). Glycosides from herbs of the genus *Digitalis* have stated to improve heart function. All 35 endophytic fungi were obtained from shoots and foliage of *Digitalis* genus that mainly were *Alternaria*, *Penicillium*, and *Aspergillus* species and investigated for medicinal metabolite production. Unprocessed extracts of fungal cultures revealed the glycoside digoxin from extracts of mentioned endophytes (Kaul et al. 2013). Another examination also reported about the detection of cardiotonic glycosides from the leaf of *Digitalis purpurea* by HPLC (Kwon et al. 2011a). The quantity of the cardiotonic glycosides from *D. purpurea* was also investigated by Pérez-Alonso et al. (2009). The aim of a study was to achieve *D. purpurea* to regulate the quantity of cardiac glycosides (digoxin, digitoxin, and lanatoside C) as medicinal compounds of industrial importance for the drug productive enterprises. High-performance liquid chromatography examinations showed digoxin and digitoxin existence in all immersion frequencies. Takahashi et al. (2015) isolated two lignin glycoside (compound [α]D^{24}–27.8 and [α]D^{24}–26.7) and two phenolic glycosides ([α]D^{24}–75.0 and [α]D^{24}–31.9) , as well as 15 known compounds (+)-lyoniresinols 3a-*O*-β-D-glucopyranoside and 3a-O-(2″-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside, (–)-isolariciresinol 3a-*O*-β-D-glucopyranoside, 3,4-dimethoxyphenol O-(6′-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside 5″-*O*-4-‴-hydroxybenzoate, 4-hydroxyphenethyl alcohol 7-*O*-(6′-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside 5″-*O*-4‴-hydroxybenzoate, 5″-*O*-3‴,4-‴-dimethoxybenzoate, 5″-*O*-3‴,4‴,5‴-trimethoxybenzoate, 5″-*O*-ferulate and 5″-*O*-3‴,4‴-dimethoxycinnamate, 6-*O*-4″-hydroxybenzoylleonuride, 6-*O*-vanilloylleo-nuride, derwentioside B, catalposide, amphicoside, and 6-*O*-veratrylcatalposide from the branches of *Tabebuia chrysotricha*. Separated lignin glycosides showed moderate antioxidant activities (Takahashi et al. 2015).

1.11 Saponins

Saponins, a class of triterpene glycoside components existing in some herb genera, have aglycone attachments, which constructed utilizing triterpenoid or steroidal frameworks (Carelli et al. 2011). These metabolites are a group of compounds and have been joined to herb tolerance against pathogens (Ito et al. 2002; Xiaocheng et al. 2018). Xu et al. separated *Paecilomyces* sp. from the ginseng and investigated its antifungal and antitumor activities. The examinations represented that *Paecilomyce* sp. and ginseng extracts held the similar component falcarinol, a natural insecticide and antitumor agent (Xu et al. 2008). Two endophytic fungi, *Fusarium* sp. PN8 and *Aspergillus* sp. PN17, have been obtained from *Panax notoginseng*. Saponins made by *Fusarium* PN8 were ginsenoside Rb1, ginsenoside Rd, and 20(S)-ginsenoside-Rg3, while *Aspergillus* PN17 had the potential to produce ginsenoside Re, ginsenoside Rd, and 20(S)-ginsenoside-Rg3. The separated endophytes might be utilized as prospective origins for microbic construction of herbal medicinal compounds and for microbicidal uses (Jin et al. 2017). Park et al. (2012) separated 38 fungal strains from *Panax ginseng* different cultivars that were arranged into *Phoma radicina*, *Fusarium oxysporum*, *Setophoma terrestris*, and *Ascomycota* sp. The main endophytic fungus was *P. radicina* in those ginseng plants (Park et al. 2012). Other researchers also indicated that *Fusarium* sp. could manufacture triterpenoid saponins (Cira et al. 2008; Jiao et al. 2015) that are the important medicinal compounds of *Dipsacus asperoides* and utilized to cure loss of bony tissue, decrease lipids, and keep from oxidation (Wang et al. 2016). During the evaluation of tropical herbs for endophytes' microbes, a *Xylareaceous* fungus was observed harbored on the interior section of *Sapindus saponaria* fruit. The *S. saponaria* fruit provides huge contents of triterpenoid and sesquiterpenoid saponins (Murgu et al. 2008). Scientists separated and recognized 46 endophytic fungi from the taproot, radix, and leaf of *Dipsacus asperoides* (Gong et al. 2019). *Conyza blinii* H. Lév is an herb that has various remedial potentials, but because of its loss of materials, its examination is not progressed (Tang et al. 2020). Various triterpenoid saponins including oleanane-type saponins are isolated from *Conyza blinii* H. Lév (Qiao et al. 2010). Furthermore, *C. blinii* H. Lév has high medical function with antitumor impacts (Ma et al. 2017). In the case of helpful active metabolites from herbs and their respective endophytes, steroidal saponin, diosgenin, from *Dioscorea bulbifera* is the main important compound that can be extracted (Ahmed et al. 2012).

1.12 Polyketides

As another secondary metabolite, polyketides are compounds with polyketomethylene groups, $(CH_2–CO)_n$; such materials were stated to include "multiple keten groups" (Collie 1907; Collie and Wilsmore 1896; Noumeur et al. 2017). Besides, contained components originated from polyketomethylene frameworks, for instance, by adding or reducing of water or by decarboxylation (Bentley and Bennett 1999; Hemphill et al. 2016). Polyketides show a huge class of structurally various secondary metabolites, representing a large array of pharmacologically crucial potentials. *Penicillium janthinellum*, separated as a microfungi endophyte from *Melia azedarach* fruits, produced the familiar polyketides and anthraquinones including citrinin, emodin, omega-hydroxyemodin, and janthinone. The endophytic fungus *Penicillium citrinum* was separated from the *Ceratonia siliqua* shoots. Extracts of *P. citrinum* on various medias produced some components, namely, citriquinochroman, tanzawaic acids G and H, 6-methylcurvulinic acid, ancistrocladeine, and 1,2,3,11b-tetrahydroquinolactacide, that had been explained as a synthetic compound before. Moreover, six polyketides were isolated. Fermentation of the *Ocimum tenuiflorum*-derived fungus *P. citrinum* consequenced in the separation of different polyketides (Lai et al. 2013). Three well-known components, including 4-hydroxymellein, 4,8-dihydroxy-6-methoxy-3methyl-3,4-dihydro-1*H*-isochromen-1-one, and 1-(2,6-dihydroxyphenyl) ethanone, were obtained from the endophytic fungi. A polyketide, 4-hydroxymellein, and a benzopyran 4,8-dihydroxy-6-methoxy-3-methyl-3,4-dihydro-1*H*-isochromen-1-one represented good preventing potential toward leukemic cells, *Bacillus subtilis* and *Aspergillus niger* (Santiago et al. 2014). Some polyketides were separated from the cultures of the endophytic fungal strain *Diaporthe* sp. XZ-07 of *Camptotheca acuminate* (Yuan et al. 2009). Endophytic fungus *Penicillium janthinellum* inhabiting in the fruits of *Melia azedarach* producing polyketide citrinin showed 100% bactericidal potential toward *Leishmania* sp. Antibacterial compound YX-28 was separated from *Ginkgo biloba* L. having potential toward some foodborne and food spoilage microbes, viz., *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Yersinia* sp., *Vibrio* sp., *Candida albicans*, *Penicillium expansum*, and *Aspergillus niger*, especially to *Aeromonas hydrophila*, and was proposed to be utilized as natural preservative in food (Momose et al. 2000). An endophyte, *Alternaria* sp., separated from an herb *Polygonum senegalense*, made some lactone polyketides (Aly et al. 2008a). Regarding one strain/many compounds (OSMAC) method, five novel polyketides, namely, phomopsiketones A-C, (10S)-10-*O*-β-D-4′-methoxymannopyranosyldiaporthin, and clearanol H, were separated from an endophytic fungus, *Phomopsis* sp. sh917, inhabiting in shoots of *Isodon eriocalyx* var. *laxiflora* (Tang et al. 2017). Pinheiro et al. (2017) separated the polyketide monocerin from *Exserohilum rostratum*, an endophytic fungus existing in *Bauhinia guianensis* (Marinho et al. 2005). Endophytic fungus *Penicillium* sp. JP-1 separated from *Aegiceras corniculatum* represented by four polyketides, leptusphaerone, penicillenone, 9-demethyl FR-901235, and leptosphaerone C, exhibited cytocidal effect toward A-549 cells, while penicillenone exhibited cytocidal effect toward P388 cells and arugosin I (Jalgaonwala et al. 2011).

1.13 Coumarins

There are more than 1300 coumarins recognized in herbs, bacteria, and fungi (Umashankar et al. 2015; Sahoo et al. 2018). Coumarins have their special fingerprints as antiviral, antimicrobial (Nitiema et al. 2012), antioxidant, anti-inflammatory (Witaicenis et al. 2010; Kwon et al. 2011b), antiadipogenic (Shin et al. 2010), cytotoxic (Qin et al. 2019b), apoptosis (Bisi et al. 2017), antiprolilferative (Yun et al. 2011), antitubercular, and cytotoxic (Chiang et al. 2010). Mellein and its derivatives, as dihydroisocoumarins, have been identified along with isofraxidin, from *Annulohypoxylon bovei* inhabiting the bark of *Cinnamomum* sp. (Cheng et al. 2011). Two compounds, 5,7-dimethoxy-4-phenylcoumarin and 5,7-dimethoxy-4-*p*-methoxylphenylcoumarin, isolated from *Streptomyces aureofaciens* CMUAc130 were originally made by many species of herbs. These metabolites presented good antifungal and anticancer activity (Taechowisan et al. 2007). The metabolites, which have biological effects, achieved from the culture of *Xylaria* sp. YX-28, an endophytic fungus, separated from *Ginkgo biloba* were recognized as “7-amino-4-methylcoumarin” (Liu et al. 2008). In addition, periconicin B, 6,8-dimethoxy-3-(2′-oxo-propyl)-coumarin, and 2,4-dihydroxy-6-[(1′*E*,3′*E*)-penta-1′,3′-dienyl]-benzaldehyde were separated from *Periconia atropurpurea* extract, an endophyte isolated from the leaf of *Xylopia aromatic* (Teles et al. 2006). The leaf endophytic fungi, *Alternaria* species of *Crotalaria pallida*, have yielded all the suspected three different coumarins including coumarin, *p*-coumaric acid, and 2-hydroxy cinnamic acid in microwave-assisted extraction method. The purified coumarin from fungal extract showed strong antimitotic activity through various mechanisms in onion actively growing root cells. The endophytic fungal coumarin had also showed inhibition of green gram germination by act as a toxic (Umashankar et al. 2015). Seven dihydroisocoumarins, including five brominated (palmaerones A–E) and two chlorinated (palmaerones F–G) compounds, were made by the endophyte *Lachnum palmae*, separated from the wet biomass of *Przewalskia tangutica* (Zhao et al. 2018). Two active ingredients identified as (I) 5, 7dimethoxy-4-*p*-methoxyphenyl coumarin and (II) 5, 7-dimethoxy-4-*p*-phenyl coumarin from endophytic *Streptomyces aureofaciens* separated from radix tissue of *Zingiber officinale* exhibited fungicidal potential toward *Colletotrichum musae* and *Fusarium oxysporum* (Taechowisan et al. 2005). The isolation of 3,4,7-trimethylcoumarin from the shoot of *Trigonella foenum-graecum* was also described (Khurana et al. 1982). An infrequent coumarin derivative, pestalustaine B, was separated from *Pestalotiopsis adusta*, endophyte of the herb *Sinopodophyllum hexandrum* (Xiao et al. 2018). Seven dihydroisocoumarins, including five brominated (palmaerones A, palmaerones B, palmaerones C, palmaerones D, and palmaerones E), were made by *Lachnum palmae*, separated from *Przewalskia tangutica* (Fig. 1.6) (Zhao et al. 2018). All separated metabolites were investigated for inhibitory potential toward *Cryptococcus neoformans*, *Penicillium* sp., *C. albicans*, *B. subtilis*, and *S. aureus* strains (Rustamova et al. 2020).

In an investigation, 23 species of endophytic fungi were separated from symptomless fennel (*Foeniculum vulgare*), lettuce (*Lactuca sativa*), chicory (*Cichorium intybus*), and celery (*Apium graveolen*s) herbs. Among the separated fungi, *Acremonium*, *Alternaria*, *Fusarium*, and *Plectosporium* were recognized in all the collected plants, while *Cylindrocarpon*, *Epicoccum*, *Gliocladium*, *Mortierella*, *Phoma*, *Stemphylium*, and *Verrucaria* were each separated from only one plant (D'Amico et al. 2008). The minimum separation value of endophytic fungi from celery might be due to several of the herb's compounds, including columbianetins and furanocoumarins, that have fungicidal characteristics (D'Amico et al. 2008).

1.14 Steroids

Steroids in herbs compose a varied class of non-chemical metabolites. They are originated from *S*-squalene-2,3-epoxide via acetate-mevalonate pathway (Gunaherath and Gunatilaka 2006). Some steroids including hydroxyl ergosta derivates, oxo ergosta- derivate; acetoxy ergosta derivates and phenylacetoxy ergosta derivates have described as ingredients of *Colletotrichum* sp. culture, achieved from *Artemisia annua*. They have exhibited fungicidal potential toward *Phytophthora capsici* Leonian. Colletotric acid is a metabolite of an endophyte, *Colletotrichum gloeosporioides*, separated from *Artemisia annua*, a plant that is well accepted for artemisinin synthesis (an anti-malarial drug).

The *Colletotrichum* sp. existing in *A. annua* generate compounds which have bioactivity toward human and plant pathogens (Lu et al. 2000). Some steroids including calvasterols A and B and ganodermaside D were separated from the growth of an endophytic fungus *Phomopsis* sp. as achieved from *Aconitum carmichaelii* (Wu et al. 2013a, b). Six components involving the classes of steroids (ergosterol, ergosterol peroxide, neociclocitrinols, cerevisterol, 25-hydroxy-ergosta-4,6,8(14),22-tetraen-3-one, ergosta-4,6,8(14),22-tetraen-3-one), were separated from *Penicillium herquei* achieved from *Melia azedarach* (Marinho et al. 2009). The *Fusarium* sp. is an excellent origin of ergosterol derivatives and other compounds of different groups. Ergosterol derivatives, namely, Fusaristerol B, Fusaristerol C, and Fusaristerol D, have achieved from the endophytic fungus *Fusarium* sp., isolated from the inner parts of *Mentha longifolia* L. radix (Fig. 1.7) (Khayat et al. 2019).

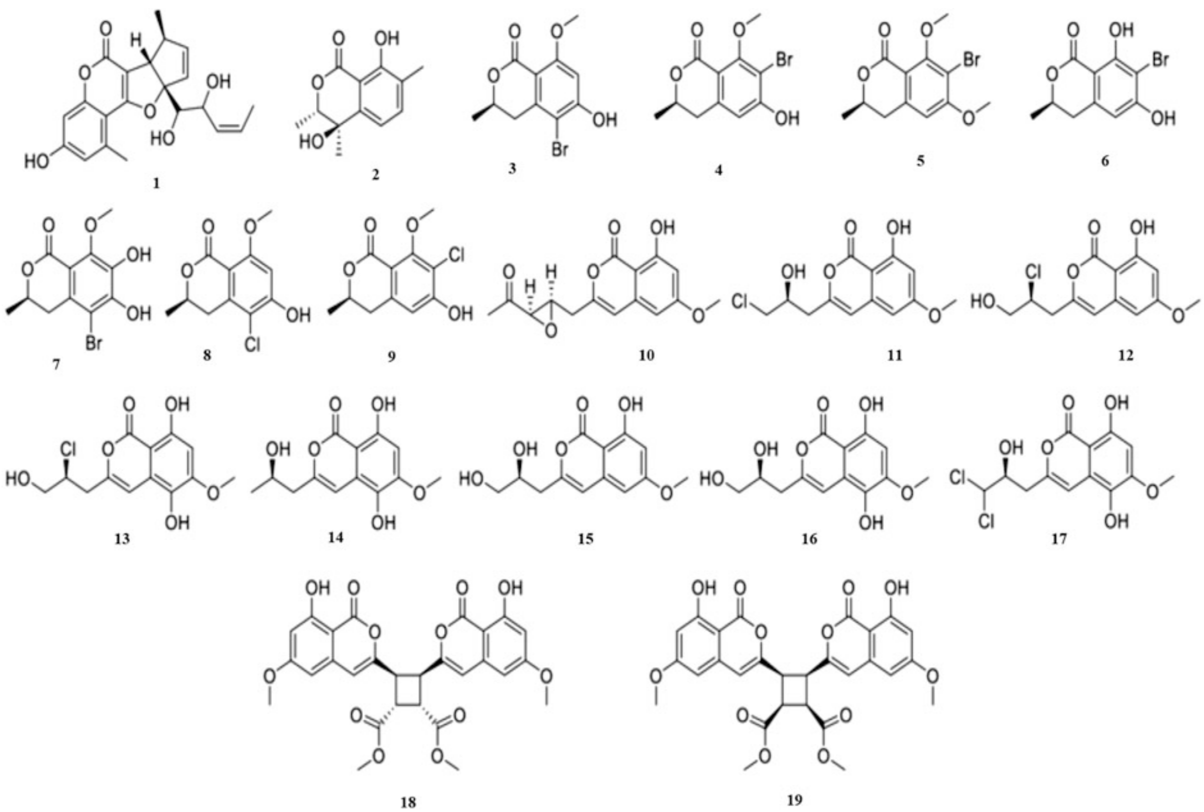


Fig. 1.6 The structures of coumarins 1, isocoumarins 2–19 produced by endophytic fungi. 1 (pestalustaine B); 2 (pestalotiopisorin B); 3 (palmaerones A); 4 (palmaerones B); 5 (palmaerones C); 6 (palmaerones D); 7 (palmaerones E); 8 (palmaerones F); 9 (palmaerones G); 10 (peniisocoumarins A); 11 (peniisocoumarins B); 12 (peniisocoumarins c); 13 (peniisocoumarins D); 14 (peniisocoumarins E); 15 (peniisocoumarins F); 16 (peniisocoumarins G); 17 (peniisocoumarins H); 18 (peniisocoumarins I) and 19 (peniisocoumarins J)

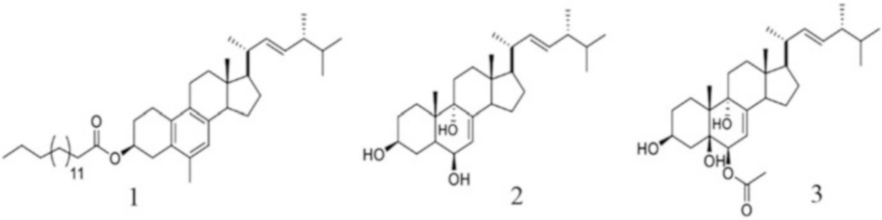


Fig. 1.7 The structures of new steroids (1–3) achieved from the endophytic fungus *Fusarium* sp., isolated from the inner parts of *Mentha longifolia* L. radix

A polyhydroxylated sterol, designated globosterol, together with tetrahydroxylated ergosterol, has separated from the cultures of *Chaetomium globosum* ZY-22, an endophytic fungus, isolated from *Ginkgo biloba* (Qin et al. 2009a).

1.15 Conclusion and Perspectives

Higher plants have the ability to make compounds which have always been a very good origin of pharmaceuticals, insecticides, flavorings, fragrances, and food colorants. Medicinal herbs act like a richest origin of specialized compounds. These secondary metabolites could be explained as components that do not have role in the usual growth and reproduction directly, but they are important in the relation of the herb with its ecosystem. There are regional and environmental obstacles that may reduce the commercial production of natural components. Endophytes have stated to be a good origin of new non-chemical components with various biological potentials and a very good structural diversity. Non-chemical bioactive components made by endophytes have exhibited high ability in human health and safety debates. In all kingdoms, microbes might have been involved in the medicinal metabolite production in "higher hosts." Microbial endophytes can be involved to upgrade herb development and increase productivity directly in economic herbs. Endophytes might assist plants with less fertilizers, pesticides, or weedkillers. They are also very crucial biological origins that require to be investigated in the future to gain ecological balance. They also have a role as great origins of bioactive compounds for various commercial parts and human safety. The demand is to evaluate genomics and the integrated metabolism of the herb-endophyte interaction for the purpose of collecting advantages from this notable association. Due to the great commercial importance of natural products produced by endophytic microbes, scientists attract very much attention for detection of bioactive compounds in the form of antimicrobial, anticancer, antifungal, and antibacterial activity. Endophytes as medicine origin can assist to keep biodiversity and drug resistance, as they are as another origin of medicines. So, in the future, the conventional techniques of medicine discovery might be replaced by endophytes.

References

Afridi MS, Mahmood T, Salam A, Mukhtar T, Mehmood S, Ali J et al (2019) Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: involvement of ACC deaminase and antioxidant enzymes. Plant Physiol Biochem 139:569–577

Ahmed M, Hussain M, Dhar MK, Kaul S (2012) Isolation of microbial endophytes from some ethnomedicinal plants of Jammu and Kashmir. J Nat Prod Plant Resour 2(2):215–220

Aly AH, Edrada-Ebel R, Indriani ID, Wray V, Müller WE, Totzke F et al (2008a) Cytotoxic metabolites from the fungal endophyte Alternaria sp. and their subsequent detection in its host plant Polygonum senegalense. J Nat Prod 71(6):972–980

Aly AH, Edrada-Ebel R, Wray V, Müller WE, Kozytska S, Hentschel U, Ebel R (2008b) Bioactive metabolites from the endophytic fungus Ampelomyces sp. isolated from the medicinal plant Urospermum picroides. Phytochemistry 69(8):1716–1725

Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Diversity 41(1):1–16

Aly AH, Debbab A, Proksch P (2011) Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol 90(6):1829–1845

Aravind R, Eapen SJ, Kumar A, Dinu A, Ramana KV (2010) Screening of endophytic bacteria and evaluation of selected isolates for suppression of burrowing nematode (Radopholus similis Thorne) using three varieties of black pepper (Piper nigrum L.). Crop Protect 29(4):318–324

Atmani D, Chaher N, Atmani D, Berboucha M, Debbache N, Boudaoud H (2009) Flavonoids in human health: from structure to biological activity. Curr Nutr Food Sci 5(4):225–237

Backman PA, Sikora RA (2008) Endophytes: an emerging tool for biological control. Biol Control 46(1):1–3

Bandara WMMS, Seneviratne G, Kulasooriya SA (2006) Interactions among endophytic bacteria and fungi: effects and potentials. J Biosci 31(5):645–650

Barazani O, von Dahl CC, Baldwin IT (2007) Sebacina vermifera promotes the growth and fitness of Nicotiana attenuata by inhibiting ethylene signaling. Plant Physiol 144(2):1223–1232

Barka EA, Nowak J, Clément C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, Burkholderia phytofirmans strain PsJN. Appl Environ Microbiol 72(11):7246–7252

Barreiro C, Gutiérrez S, Olivera ER (2019) Fungal horizontal gene transfer: a history beyond the phylogenetic kingdoms. In: Horizontal gene transfer. Springer, Cham, pp 315–336

Bashyal BP, Wijeratne EK, Faeth SH, Gunatilaka AL (2005) Globosumones A−C, cytotoxic orsellinic acid esters from the Sonoran desert endophytic fungus Chaetomium globosum. J Nat Prod 68(5):724–728

Bastias DA, Martínez-Ghersa MA, Ballaré CL, Gundel PE (2017) Epichloë fungal endophytes and plant defenses: not just alkaloids. Trends Plant Sci 22(11):939–948

Beltran-Garcia MJ, White JF Jr, Prado FM, Prieto KR, Yamaguchi LF, Torres MS et al (2014) Nitrogen acquisition in Agave tequilana from degradation of endophytic bacteria. Sci Rep 4:6938

Bentley R, Bennett JW (1999) Constructing polyketides: from collie to combinatorial biosynthesis. Annu Rev Microbiol 53(1):411–446

Bhagobaty RK, Joshi SR (2011) Metabolite profiling of endophytic fungal isolates of five ethno-pharmacologically important plants of Meghalaya, India. J Metab Syst Biol 2(2):20–31

Bhardwaj S, Verma R, Gupta J (2018) Challenges and future prospects of herbal medicine. Int Res Med Health Sci 1(1):12–15

Bicas JL, Dionisio AP, Pastore GM (2009) Bio-oxidation of terpenes: an approach for the flavor industry. Chem Rev 109(9):4518–4531

Bisi A, Cappadone C, Rampa A, Farruggia G, Sargenti A, Belluti F et al (2017) Coumarin derivatives as potential antitumor agents: growth inhibition, apoptosis induction and multidrug resistance reverting activity. Eur J Med Chem 127:577–585

Bittleston LS, Brockmann F, Wcislo W, Van Bael SA (2011) Endophytic fungi reduce leaf-cutting ant damage to seedlings. Biol Lett 7(1):30–32
Blanchard S, Thorson JS (2006) Enzymatic tools for engineering natural product glycosylation. Curr Opin Chem Biol 10(3):263–271
Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37
Buckley H, Young CA, Charlton ND, Hendricks WQ, Haley B, Nagabhyru P, Rudgers JA (2019) Leaf endophytes mediate fertilizer effects on plant yield and traits in northern oat grass (Trisetum spicatum). Plant Soil 434(1):425–440
Busby PE, Ridout M, Newcombe G (2016) Fungal endophytes: modifiers of plant disease. Plant Mol Biol 90(6):645–655
Bush LP, Cornelius PL, Buckner RC, Varney DR, Chapman RA, Burrus PB, Saunders MJ (1982) Association of N-acetyl Loline and N-formyl Loline with Epichloe typhina in Tall Fescue 1. Crop Sci 22(5):941–943
Carelli M, Biazzi E, Panara F, Tava A, Scaramelli L, Porceddu A et al (2011) Medicago truncatula CYP716A12 is a multifunctional oxidase involved in the biosynthesis of hemolytic saponins. Plant Cell 23(8):3070–3081
Casella TM, Eparvier V, Mandavid H, Bendelac A, Odonne G, Dayan L et al (2013) Antimicrobial and cytotoxic secondary metabolites from tropical leaf endophytes: isolation of antibacterial agent pyrrocidine C from Lewia infectoria SNB-GTC2402. Phytochemistry 96:370–377
Chen LZ, Chen JM, Zheng XS, Zhang JF, Yu XP (2007) Identification and antifungal activity of the metabolite of endophytic fungi isolated from Llex cornuta. Chin J Pesticide Sci 9(2):143–148
Chen LL, Kong FD, Wang P, Yuan JZ, Guo ZK, Wang H et al (2017) Two new tremulane sesquiterpenes from a mangrove endophytic fungus, Coriolopsis sp. J5. Chin Chem Lett 28 (2):222–225
Chen S, Li H, Chen Y, Li S, Xu J, Guo H et al (2019) Three new diterpenes and two new sesquiterpenoids from the endophytic fungus Trichoderma koningiopsis A729. Bioorg Chem 86:368–374
Cheng MJ, Wu MD, Hsieh SY, Hsieh MT, Chen IS, Yuan GF (2011) Constituents of the endophytic fungus Annulohypoxylon boveri var. microspora BCRC 34012. Helvetica Chim Acta 94(6):1108–1114
Chiang CC, Cheng MJ, Peng CF, Huang HY, Chen IS (2010) A novel dimeric coumarin analog and antimycobacterial constituents from Fatoua pilosa. Chem Biodivers 7(7):1728–1736
Chithra S, Jasim B, Sachidanandan P, Jyothis M, Radhakrishnan EK (2014) Piperine production by endophytic fungus Colletotrichum gloeosporioides isolated from Piper nigrum. Phytomedicine 21(4):534–540
Chutulo EC, Chalannavar RK (2018) Endophytic mycoflora and their bioactive compounds from Azadirachta indica: a comprehensive review. J Fungi 4(2):42
Cira LA, González GA, Torres JC, Pelayo C, Gutiérrez M, Ramírez J (2008) Heterologous expression of Fusarium oxysporum tomatinase in Saccharomyces cerevisiae increases its resistance to saponins and improves ethanol production during the fermentation of Agave tequilana Weber var. azul and Agave salmiana must. Antonie Van Leeuwenhoek 93(3):259
Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. Ecology 69(1):10–16
Collie JN (1907) Derivatives of the multiple keten group. J Chem Soc Trans 91:1806–1813
Collie JN, Wilsmore NTM (1896) The production of naphthalene and of isoquinoline derivatives from dehydracetic acid. J Chem Soc Trans 69:293–304
Costa FDC, de Melo IS (2012). Endophytic and rhizospheric bacteria from Opuntia ficus-indica mill and their ability to promote plant growth in cowpea, Vigna unguiculata (L.) Walp. Embrapa Meio Ambiente-Artigo em periódico indexado
Cui Y, Yi D, Bai X, Sun B, Zhao Y, Zhang Y (2012) Ginkgolide B produced endophytic fungus (Fusarium oxysporum) isolated from Ginkgo biloba. Fitoterapia 83(5):913–920

Cui JL, Guo SX, Xiao PG (2017) Interaction between endophytes and host plant and the role of endophytes in genuineness analysis of medicinal plant. Yao xue xue bao= Acta pharmaceutica Sinica 52(2):214–221
Cui JL, Zhang YY, Vijayakumar V, Zhang G, Wang ML, Wang JH (2018) Secondary metabolite accumulation associates with ecological succession of endophytic fungi in Cynomorium songaricum Rupr. J Agric Food Chem 66(22):5499–5509
D'Amico M, Frisullo S, Cirulli M (2008) Endophytic fungi occurring in fennel, lettuce, chicory, and celery—commercial crops in southern Italy. Mycol Res 112(1):100–107
Darbyshire JF, Greaves MP (1973) Bacteria and protozoa in the rhizosphere. Pestic Sci 4 (3):349–360
Debbab A, Aly AH, Edrada-Ebel R, Wray V, Müller WE, Totzke F, Mosaddak M (2009) Bioactive metabolites from the endophytic fungus Stemphylium globuliferum isolated from Mentha pulegium. J Nat Prod 72(4):626–631
Dembitsky VM (2004) Chemistry and biodiversity of the biologically active natural glycosides. Chem Biodivers 1(5):673–781
Demyttenaere J, De Kimpe N (2001) Biotransformation of terpenes by fungi: study of the pathways involved. J Mol Catal B Enzym 11(4–6):265–270
Devari S, Jaglan S, Kumar M, Deshidi R, Guru S, Bhushan S et al (2014) Capsaicin production by *Alternaria alternata*, an endophytic fungus from *Capsicum annum*; LC–ESI–MS/MS analysis. Phytochemistry 98:183–189
Dos Santos RMG, Rodrigues-Fo E (2003) Further meroterpenes produced by Penicillium sp., an endophyte obtained from Melia azedarach. Zeitschrift für Naturforschung C 58(9–10):663–669
Doty SL (2008) Enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179(2):318–333
Duan R, Zhou H, Yang Y, Li H, Dong J, Li X, Ding Z (2016) Antimicrobial meroterpenoids from the endophytic fungus Penicillium sp. T2-8 associated with Gastrodia elata. Phytochem Lett 18:197–201
Dutsadee C, Nunta C (2008) Induction of peroxidase, scopoletin, phenolic compounds and resistance in Hevea brasiliensis by elicitin and a novel protein elicitor purified from Phytophthora palmivora. Physiol Mol Plant Pathol 72(4–6):179–187
Dzoyem JP, Melong R, Tsamo AT, Maffo T, Kapche DG, Ngadjui BT et al (2017) Cytotoxicity, antioxidant and antibacterial activity of four compounds produced by an endophytic fungus Epicoccum nigrum associated with Entada abyssinica. Revista Brasileira de Farmacognosia 27 (2):251–253
Erbert C, Lopes AA, Yokoya NS, Furtado NA, Conti R, Pupo MT, Debonsi HM (2012) Antibacterial compound from the endophytic fungus Phomopsis longicolla isolated from the tropical red seaweed Bostrychia radicans. Bot Mar 55(4):435–440
Evans WC (2009) Trease and Evans' pharmacognosy E-book. Elsevier Health Sciences, Amsterdam
Faeth SH, Fagan WF (2002) Fungal endophytes: common host plant symbionts but uncommon mutualists. Integr Comp Biol 42:360–368
Firáková S, Šturdíková M, Múčková M (2007) Bioactive secondary metabolites produced by microorganisms associated with plants. Biologia 62(3):251–257
Firdous J, Latif NA, Mona R, Mansor R, Muhamad N (2020) Andrographis paniculata and its endophytes: a review on their pharmacological activities. Res J Pharm Technol 13 (4):2029–2032
Fletcher LR, Harvey IC (1981) An association of a Lolium endophyte with ryegrass staggers. N Z Vet J 29(10):185–186
Frank AC, Saldierna Guzmán JP, Shay JE (2017) Transmission of bacterial endophytes. Microorganisms 5(4):70
Freeman S, Rodriguez RJ (1993) Genetic conversion of a fungal plant pathogen to a nonpathogenic, endophytic mutualist. Science 260(5104):75–78

Gallagher RT, Hawkes AD, Steyn PS, Vleggaar R (1984) Tremorgenic neurotoxins from perennial ryegrass causing ryegrass staggers disorder of livestock: structure elucidation of lolitrem B. J Chem Soc Chem Commun 9:614–616

Gangadevi V, Muthumary J (2008) Taxol, an anticancer drug produced by an endophytic fungus Bartalinia robillardoides Tassi, isolated from a medicinal plant, Aegle marmelos Correa ex Roxb. World J Microbiol Biotechnol 24(5):717

Garrido-Arandia M, Silva-Navas J, Ramírez-Castillejo C, Cubells-Baeza N, Gómez-Casado C, Barber D et al (2016) Characterisation of a flavonoid ligand of the fungal protein Alt a 1. Sci Rep 6:33468

Garyali S, Kumar A, Reddy MS (2013) Taxol production by an endophytic fungus, Fusarium redolens, isolated from Himalayan yew. J Microbiol Biotechnol 23(10):1372–1380

Giamperi L, Fraternale D, Ricci D (2002) The in vitro action of essential oils on different organisms. J Essent Oil Res 14(4):312–318

Giauque H, Hawkes CV (2016) Historical and current climate drive spatial and temporal patterns in fungal endophyte diversity. Fungal Ecol 20:108–114

Giauque H, Connor EW, Hawkes CV (2019) Endophyte traits relevant to stress tolerance, resource use and habitat of origin predict effects on host plants. New Phytol 221(4):2239–2249

Giridharan P, Verekar SA, Khanna A, Mishra PD, Deshmukh SK (2012) Anticancer activity of sclerotiorin, isolated from an endophytic fungus Cephalotheca faveolata Yaguchi, Nishim. & Udagawa. Indian J Exp Biol 50(7):464–468

Gómez OC, Luiz JHH (2018) Endophytic fungi isolated from medicinal plants: future prospects of bioactive natural products from Tabebuia/Handroanthus endophytes. Appl Microbiol Biotechnol 102(21):9105–9119

Gómez-Lama Cabanás C, Schilirò E, Valverde-Corredor A, Mercado-Blanco J (2014) The biocontrol endophytic bacterium Pseudomonas fluorescens PICF7 induces systemic defense responses in aerial tissues upon colonization of olive roots. Front Microbiol 5:427

Gond SK, Bergen MS, Torres MS, White JF, Kharwar RN (2015) Effect of bacterial endophyte on expression of defense genes in Indian popcorn against Fusarium moniliforme. Symbiosis 66 (3):133–140

Gong A, Zhou T, Xiao C, Jiang W, Zhou Y, Zhang J et al (2019) Association between dipsacus saponin VI level and diversity of endophytic fungi in roots of Dipsacus asperoides. World J Microbiol Biotechnol 35(3):1–14

Graf BA, Milbury PE, Blumberg JB (2005) Flavonols, flavones, flavanones, and human health: epidemiological evidence. J Med Food 8(3):281–290

Gu W, Ge HM, Song YC, Ding H, Zhu HL, Zhao XA, Tan RX (2007) Cytotoxic benzo [j] fluoranthene metabolites from Hypoxylon truncatum IFB-18, an endophyte of Artemisia annua. J Nat Prod 70(1):114–117

Gunaherath GK, Gunatilaka AL (2006) Plant steroids: occurrence, biological significance and their analysis. Encyclopedia of analytical chemistry: applications, theory and instrumentation. Wiley, New York, pp 1–26

Gunatilaka AL (2006) Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. J Nat Prod 69(3):509–526

Guo B, Kunming LH (1998) A middle vinblastine fungi isolated. J Yunnan Univ 20:214–215

Harman GE, Uphoff N (2019) Symbiotic root-endophytic soil microbes improve crop productivity and provide environmental benefits. Scientifica 2019:9106395

Harwoko H, Hartmann R, Daletos G, Ancheeva E, Frank M, Liu Z, Proksch P (2019) Biotransformation of host plant flavonoids by the fungal endophyte Epicoccum nigrum. ChemistrySelect 4 (45):13054–13057

Hassan SED (2017) Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of Teucrium polium L. J Adv Res 8(6):687–695

Hawkins NJ, Bass C, Dixon A, Neve P (2019) The evolutionary origins of pesticide resistance. Biol Rev 94(1):135–155

Hemphill CFP, Daletos G, Liu Z, Lin W, Proksch P (2016) Polyketides from the mangrove-derived fungal endophyte Pestalotiopsis clavispora. Tetrahedron Lett 57(19):2078–2083
Higgins KL, Arnold AE, Coley PD, Kursar TA (2014) Communities of fungal endophytes in tropical forest grasses: highly diverse host-and habitat generalists characterized by strong spatial structure. Fungal Ecol 8:1–11
Hoysted GA, Jacob AS, Kowal J, Giesemann P, Bidartondo MI, Duckett JG et al (2019) Mucoromycotina fine root endophyte fungi form nutritional mutualisms with vascular plants. Plant Physiol 181(2):565–577
Huang WY, Cai YZ, Hyde KD, Corke H, Sun M (2007a) Endophytic fungi from Nerium oleander L (Apocynaceae): main constituents and antioxidant activity. World J Microbiol Biotechnol 23 (9):1253–1263
Huang WY, Cai YZ, Xing J, Corke H, Sun M (2007b) A potential antioxidant resource: endophytic fungi from medicinal plants. Econ Bot 61(1):14
Huang Z, Cai X, Shao C, She Z, Xia X, Chen Y, Lin Y (2008) Chemistry and weak antimicrobial activities of phomopsins produced by mangrove endophytic fungus Phomopsis sp. ZSU-H76. Phytochemistry 69(7):1604–1608
Hussain H, Akhtar N, Draeger S, Schulz B, Pescitelli G, Salvadori P et al (2009) New bioactive 2,3-epoxycyclohexenes and isocoumarins from the endophytic fungus Phomopsis sp. from Laurus azorica. Eur J Org Chem 2009(5):749–756
Irizarry I, White JF (2018) Bacillus amyloliquefaciens alters gene expression, ROS production and lignin synthesis in cotton seedling roots. J Appl Microbiol 124(6):1589–1603
Irmer S, Podzun N, Langel D, Heidemann F, Kaltenegger E, Schemmerling B et al (2015) New aspect of plant–rhizobia interaction: alkaloid biosynthesis in Crotalaria depends on nodulation. Proc Natl Acad Sci 112(13):4164–4169
Isaka M, Palasarn S, Lapanun S, Chanthaket R, Boonyuen N, Lumyong S (2009) γ-Lactones and ent-eudesmane sesquiterpenes from the endophytic fungus Eutypella sp. BCC 13199. J Nat Prod 72(9):1720–1722
Ito S, Takahara H, Kawaguchi T, Tanaka S, Kameya-Iwaki M (2002) Post-transcriptional silencing of the tomatinase gene in Fusarium oxysporum f. sp lycopersici. J Phytopathol 150 (8–9):474–480
Izumi E, Ueda-Nakamura T, Veiga VF Jr, Pinto AC, Nakamura CV (2012) Terpenes from Copaifera demonstrated in vitro antiparasitic and synergic activity. J Med Chem 55 (7):2994–3001
Jagannath S, Konappa NM, Alurappa R, Chowdappa S (2019) Production, characterization of indole acetic acid and its bioactive potential from endophytic fungi of Cymbidium aloifolium L. J Biol Active Prod 9(5):387–409
Jalgaonwala RE, Mohite BV, Mahajan RT (2011) A review: natural products from plant associated endophytic fungi. J Microbiol Biotechnol Res 1(2):21–32
Jiao X, Lu X, Chen AJ, Luo Y, Hao JJ, Gao W (2015) Effects of Fusarium solani and F. oxysporum infection on the metabolism of Ginsenosides in American ginseng roots. Molecules 20 (6):10535–10552
Jin Z, Gao L, Zhang L, Liu T, Yu F, Zhang Z et al (2017) Antimicrobial activity of saponins produced by two novel endophytic fungi from Panax notoginseng. Nat Prod Res 31 (22):2700–2703
Karmakar R, Bindiya S, Hariprasad P (2019) Convergent evolution in bacteria from multiple origins under antibiotic and heavy metal stress, and endophytic conditions of host plant. Sci Total Environ 650:858–867
Kaul S, Wani M, Dhar KL, Dhar MK (2008) Production and GC-MS trace analysis of methyl eugenol from endophytic isolate ofAlternaria from rose. Ann Microbiol 58(3):443
Kaul S, Ahmed M, Zargar K, Sharma P, Dhar MK (2013) Prospecting endophytic fungal assemblage of Digitalis lanata Ehrh. (foxglove) as a novel source of digoxin: a cardiac glycoside. 3 Biotech 3(4):335–340
Khan Z, Doty S (2011) Endophyte-assisted phytoremediation. Plant Biol 12:97–105

Khan AL, Kang SM, Dhakal KH, Hussain J, Adnan M, Kim JG, Lee IJ (2013) Flavonoids and amino acid regulation in *Capsicum annuum* L. by endophytic fungi under different heat stress regimes. Sci Horticult 155:1–7

Khan AL, Gilani SA, Waqas M, Al-Hosni K, Al-Khiziri S, Kim YH et al (2017) Endophytes from medicinal plants and their potential for producing indole acetic acid, improving seed germination and mitigating oxidative stress. J Zhejiang Univ Sci B 18(2):125–137

Kharwar RN, Verma VC, Strobel G, Ezra D (2008) The endophytic fungal complex of Catharanthus roseus (L.) G. Don. Curr Sci 95:228–233

Khayat MT, Ibrahim SR, Mohamed GA, Abdallah HM (2019) Anti-inflammatory metabolites from endophytic fungus Fusarium sp. Phytochem Lett 29:104–109

Khidir HH, Eudy DM, Porras-Alfaro A, Herrera J, Natvig DO, Sinsabaugh RL (2010) A general suite of fungal endophytes dominate the roots of two dominant grasses in a semiarid grassland. J Arid Environ 74(1):35–42

Khurana SK, Krishnamoorthy V, Parmar VS, Sanduja R, Chawla HL (1982) 3,4,7-Trimethylcoumarin from Trigonella foenum-graecum stems. Phytochemistry 21(8):2145–2146

Kim S, Shin DS, Lee T, Oh KB (2004) Periconicins, two new fusicoccane diterpenes produced by an endophytic fungus Periconia sp. with antibacterial activity. J Nat Prod 67(3):448–450

Kogel KH, Franken P, Hückelhoven R (2006) Endophyte or parasite—what decides? Curr Opin Plant Biol 9(4):358–363

Kornsakulkarn J, Dolsophon K, Boonyuen N, Boonruangprapa T, Rachtawee P, Prabpai S, Thongpanchang C (2011) Dihydronaphthalenones from endophytic fungus Fusarium sp. BCC14842. Tetrahedron 67(39):7540–7547

Kowalski KP, Bacon C, Bickford W, Braun H, Clay K, Leduc-Lapierre M, White J (2015) Advancing the science of microbial symbiosis to support invasive species management: a case study on Phragmites in the Great Lakes. Front Microbiol 6:95

Kozyrovska NO (2013) Crosstalk between endophytes and a plant host within information-processing networks. Biopolym Cell 29(3):234–243

Krings M, Taylor TN, Hass H, Kerp H, Dotzler N, Hermsen EJ (2007) Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses. New Phytol 174(3):648–657

Kumar A, Patil D, Rajamohanan PR, Ahmad A (2013) Isolation, purification and characterization of vinblastine and vincristine from endophytic fungus Fusarium oxysporum isolated from *Catharanthus roseus*. PLoS One 8(9):e71805

Kuriakose GC, Palem PP, Jayabaskaran C (2016) Fungal vincristine from Eutypella spp-CrP14 isolated from Catharanthus roseus induces apoptosis in human squamous carcinoma cell line-A431. BMC Complement Altern Med 16(1):1–8

Kusari S, Zühlke S, Kosuth J, Cellarova E, Spiteller M (2009) Light-independent metabolomics of endophytic Thielavia subthermophila provides insight into microbial hypericin biosynthesis. J Nat Prod 72(10):1825–1835

Kusari S, Hertweck C, Spiteller M (2012a) Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 19(7):792–798

Kusari S, Verma VC, Lamshoeft M, Spiteller M (2012b) An endophytic fungus from Azadirachta indica A. Juss. that produces azadirachtin. World J Microbiol Biotechnol 28(3):1287–1294

Kwon HJ, Sim HJ, Lee YM, Park YD, Hong SP (2011a) HPLC method validation for digitalis and its analogue by pulsed amperometric detection. J Pharm Biomed Anal 54(1):217–221

Kwon OS, Choi JS, Islam MN, Kim YS, Kim HP (2011b) Inhibition of 5-lipoxygenase and skin inflammation by the aerial parts of Artemisia capillaris and its constituents. Arch Pharm Res 34 (9):1561

Lai D, Brötz-Oesterhelt H, Müller WE, Wray V, Proksch P (2013) Bioactive polyketides and alkaloids from Penicillium citrinum, a fungal endophyte isolated from Ocimum tenuiflorum. Fitoterapia 91:100–106

Latz MA, Jensen B, Collinge DB, Jørgensen HJ (2018) Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. Plant Ecol Divers 11(5–6):555–567

Lemanceau P, Barret M, Mazurier S, Mondy S, Pivato B, Fort T, Vacher C (2017) Plant communication with associated microbiota in the spermosphere, rhizosphere and phyllosphere. In: Advances in botanical research, vol 82. Academic Press, New York, pp 101–133
Li S, Chen JF, Qin LL, Li XH, Cao ZX, Gu YC et al (2020) Two new sesquiterpenes produced by the endophytic fungus Aspergillus fumigatus from Ligusticum wallichii. J Asian Nat Prod Res 22(2):138–143
Liang HQ, Zhang DW, Guo SX, Yu J (2018) Two new tetracyclic triterpenoids from the endophytic fungus Hypoxylon sp. 6269. J Asian Nat Prod Res 20(10):951–956
Lin T, Lin X, Lu C, Hu Z, Huang W, Huang Y, Shen Y (2009) Secondary metabolites of Phomopsis sp. XZ-26, an endophytic fungus from Camptotheca acuminate. Eur J Org Chem 2009 (18):2975–2982
Lindow SE, Brandl MT (2003) Microbiology of the phyllosphere. Appl Environ Microbiol 69 (4):1875–1883
Lingqi Z, Bo G, Haiyan L, Songrong Z, Hua S, Su G, Rongcheng W (2000) Preliminary study on the isolation of endophytic fungus of Catharanthus roseus and its fermentation to produce products of therapeutic value. Zhong Cao Yao= Chinese Traditional and Herbal Drugs *31* (11):805–807
Liu X, Dong M, Chen X, Jiang M, Lv X, Yan G (2007) Antioxidant activity and phenolics of an endophytic Xylaria sp. from Ginkgo biloba. Food Chem 105(2):548–554
Liu X, Dong M, Chen X, Jiang M, Lv X, Zhou J (2008) Antimicrobial activity of an endophytic Xylaria sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin. Appl Microbiol Biotechnol 78(2):241–247
Liu X, Dou G, Ma Y (2016) Potential of endophytes from medicinal plants for biocontrol and plant growth promotion. J Gen Plant Pathol 82(3):165–173
Liu SS, Jiang JX, Huang R, Wang YT, Jiang BG, Zheng KX, Wu SH (2019) A new antiviral 14-nordrimane sesquiterpenoid from an endophytic fungus Phoma sp. Phytochem Lett 29:75–78
Lu H, Zou WX, Meng JC, Hu J, Tan RX (2000) New bioactive metabolites produced by Colletotrichum sp., an endophytic fungus in Artemisia annua. Plant Sci 151(1):67–73
Ludwig-Müller J (2015) Plants and endophytes: equal partners in secondary metabolite production? Biotechnol Lett 37(7):1325–1334
Lunardelli Negreiros de Carvalho P, de Oliveira Silva E, Aparecida Chagas-Paula D, Honorata Hortolan Luiz J, Ikegaki M (2016) Importance and implications of the production of phenolic secondary metabolites by endophytic fungi: a mini-review. Mini Rev Med Chem 16(4):259–271
Ma L, Liu H, Qin P, Hu C, Man S, Li Y et al (2017) Saponin fraction isolated from Conyza blinii H. Lév. demonstrates strong anti-cancer activity that is due to its NF-κB inhibition. Biochem Biophys Res Commun 483(1):779–785
Maček I, Clark DR, Šibanc N, Moser G, Vodnik D, Müller C, Dumbrell AJ (2019) Impacts of long-term elevated atmospheric CO2 concentrations on communities of arbuscular mycorrhizal fungi. Mol Ecol 28(14):3445–3458
Maehara S, Simanjuntak P, Kitamura C, Ohashi K, Shibuya H (2011) Cinchona alkaloids are also produced by an endophytic filamentous fungus living in Cinchona plant. Chem Pharm Bull 59 (8):1073–1074
Maehara S, Simanjuntak P, Maetani Y, Kitamura C, Ohashi K, Shibuya H (2013) Ability of endophytic filamentous fungi associated with Cinchona ledgeriana to produce Cinchona alkaloids. J Nat Med 67(2):421–423
Malinowski DP, Belesky DP (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci 40 (4):923–940
Marinho AM, Rodrigues-Filho E, Moitinho MDLR, Santos LS (2005) Biologically active polyketides produced by Penicillium janthinellum isolated as an endophytic fungus from fruits of Melia azedarach. J Braz Chem Soc 16(2):280–283
Marinho AMDR, Marinho PSB, Rodrigues Filho E (2009) Esteroides produzidos por Penicillium herquei, um fungo endofítico isolado dos frutos de Melia azedarach (Meliaceae). Química Nova 32(7):1710–1712

Martinez-Klimova E, Rodríguez-Peña K, Sánchez S (2017) Endophytes as sources of antibiotics. Biochem Pharm 134:1–17
Mehmood A, Hussain A, Irshad M, Hamayun M, Iqbal A, Rahman H et al (2019) Cinnamic acid as an inhibitor of growth, flavonoids exudation and endophytic fungus colonization in maize root. Plant Physiol Biochem 135:61–68
Meng JJ, He XL (2011) Effects of AM fungi on growth and nutritional contents of Salvia miltiorrhiza Bge. under drought stress. Journal of Agricultural University of Hebei, 1:51–55
Michalczyk A, Cieniecka-Rosłonkiewicz A, Cholewińska M (2015) Plant endophytic fungi as a source of paclitaxel. Herba Polonica 60(4):22–33
Mir RA, Kaushik SP, Chowdery RA, Anuradha M (2015) Elicitation of forskolin in cultures of Rhizactonia bataticola—a phytochemical synthesizing endophytic fungi. Int J Pharm Pharm Sci 7(10):185–189
Momose I, Sekizawa R, Hosokawa N, Iinuma H, MAISUI S, Nakamura H et al (2000) Melleolides K, L and M, new melleolides from Armillariella mellea. J Antibiot 53(2):137–143
Murgu M, Santos LFA, Souza GDD, Daolio C, Schneider B, Ferreira AG, Rodrigues-Filho E (2008) Hydroxylation of a hederagenin derived saponin by a Xylareaceous fungus found in fruits of Sapindus saponaria. J Braz Chem Soc 19(5):831–835
Na R, Jiajia L, Dongliang Y, Yingzi P, Juan H, Xiong L et al (2016) Indentification of vincamine indole alkaloids producing endophytic fungi isolated from Nerium indicum, Apocynaceae. Microbiol Res 192:114–121
Naik T, Vanitha SC, Rajvanshi PK, Chandrika M, Kamalraj S, Jayabaskaran C (2018) Novel microbial sources of tropane alkaloids: first report of production by endophytic fungi isolated from Datura metel L. Curr Microbiol 75(2):206–212
Nalini MS, Prakash HS (2017) Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Lett Appl Microbiol 64(4):261–270
Nitiema LW, Savadogo A, Simpore J, Dianou D, Traore AS (2012) In vitro antimicrobial activity of some phenolic compounds (coumarin and quercetin) against gastroenteritis bacterial strains. Int J Microbiol Res 3(3):183–187
Notarte KIR, Devanadera MKP, Mayor ABR, Cada MCA, Pecundo MH, Macabeo APG (2019) Toxicity, antibacterial, and antioxidant activities of fungal endophytes Colletotrichum and Nigrospora spp. Isolated from Uvaria grandiflora. Philipp J Sci 148(3):503–510
Noumeur SR, Helaly SE, Jansen R, Gereke M, Stradal TE, Harzallah D, Stadler M (2017) Preussilides A–F, bicyclic polyketides from the endophytic fungus Preussia similis with antiproliferative activity. J Nat Prod 80(5):1531–1540
Ogbe AA, Finnie JF, Van Staden J (2020) The role of endophytes in secondary metabolites accumulation in medicinal plants under abiotic stress. South Afr J Bot 134:126–134
Old KM, Nicolson TH (1978) The root cortex as part of a microbial continuum. In: Loutit MV, Miles JAR (eds) Microbial ecology. Springer, Berlin, pp 291–294
Palanichamy P, Krishnamoorthy G, Kannan S, Marudhamuthu M (2018) Bioactive potential of secondary metabolites derived from medicinal plant endophytes. Egypt J Basic Appl Sci 5 (4):303–312
Palem PP, Kuriakose GC, Jayabaskaran C (2015) An endophytic fungus, Talaromyces radicus, isolated from Catharanthus roseus, produces vincristine and vinblastine, which induce apoptotic cell death. PLoS One 10(12):e0144476
Pan F, Su X, Hu B, Yang N, Chen Q, Wu W (2015) Fusarium redolens 6WBY3, an endophytic fungus isolated from *Fritillaria unibracteata* var. *wabuensis*, produces peimisine and imperialine-3β-d-glucoside. Fitote rapia 103:213–221
Panaccione DG, Beaulieu WT, Cook D (2014) Bioactive alkaloids in vertically transmitted fungal endophytes. Funct Ecol 28(2):299–314
Pandey RR, Arora DK, Dubey RC (1993) Antagonistic interactions between fungal pathogens and phylloplane fungi of guava. Mycopathologia 124(1):31–39
Park SU, Lim HS, Park KC, Park YH, Bae H (2012) Fungal endophytes from three cultivars of Panax ginseng Meyer cultivated in Korea. J Ginseng Res 36(1):107

Pateraki I, Andersen-Ranberg J, Jensen NB, Wubshet SG, Heskes AM, Forman V, Staerk D (2017) Total biosynthesis of the cyclic AMP booster forskolin from Coleus forskohlii. Elife 6:e23001

Patil MP, Patil RH, Maheshwari VL (2015) Biological activities and identification of bioactive metabolite from endophytic Aspergillus flavus L7 isolated from Aegle marmelos. Curr Microbiol 71(1):39–48

Paungfoo-Lonhienne C, Rentsch D, Robatzek S, Webb RI, Sagulenko E, Näsholm T, Lonhienne TG (2010) Turning the table: plants consume microbes as a source of nutrients. PLoS One 5(7): e11915

Pérez-Alonso N, Wilken D, Gerth A, Jähn A, Nitzsche HM, Kerns G et al (2009) Cardiotonic glycosides from biomass of Digitalis purpurea L. cultured in temporary immersion systems. Plant Cell Tissue Organ Cult 99(2):151–156

Pinheiro EA, Pina JR, Feitosa AO, Carvalho JM, Borges FC, Marinho PS, Marinho AM (2017) Bioprospecting of antimicrobial activity of extracts of endophytic fungi from Bauhinia guianensis. Revista Argentina de microbiologia 49(1):3–6

Prieto KR, Echaide-Aquino F, Huerta-Robles A, Valério HP, Macedo-Raygoza G, Prado FM, White JF Jr (2017) Endophytic bacteria and rare earth elements; promising candidates for nutrient use efficiency in plants. In: Plant macronutrient use efficiency. Academic Press, New York, pp 285–306

Puri SC, Nazir A, Chawla R, Arora R, Riyaz-ul-Hasan S, Amna T et al (2006) The endophytic fungus Trametes hirsuta as a novel alternative source of podophyllotoxin and related aryl tetralin lignans. J Biotechnol 122(4):494–510

Qawasmeh A, Obied HK, Raman A, Wheatley W (2012) Influence of fungal endophyte infection on phenolic content and antioxidant activity in grasses: interaction between Lolium perenne and different strains of Neotyphodium lolii. J Agric Food Chem 60(13):3381–3388

Qian YX, Kang JC, Luo YK, He J, Wang L, Zhang XP (2017) Secondary metabolites of an endophytic fungus Pestalotiopsis uvicola. Chem Nat Comp 53(4):756–758

Qiao X, Zhang X, Ye M, Su YF, Dong J, Han J et al (2010) Rapid characterization of triterpene saponins from Conyza blinii by liquid chromatography coupled with mass spectrometry. Rapid Commun Mass Spectrom 24(22):3340–3350

Qin JC, Gao JM, Zhang YM, Yang SX, Bai MS, Ma YT, Laatsch H (2009a) Polyhydroxylated steroids from an endophytic fungus, Chaetomium globosum ZY-22 isolated from Ginkgo biloba. Steroids 74(9):786–790

Qin JC, Zhang YM, Hu L, Ma YT, Gao JM (2009b) Cytotoxic metabolites produced by Alternaria no. 28, an endophytic fungus isolated from Ginkgo biloba. Nat Prod Commun 4(11) 1934578X0900401106

Qin D, Shen W, Wang J, Han M, Chai F, Duan X et al (2019a) Enhanced production of unusual triterpenoids from Kadsura angustifolia fermented by a symbiont endophytic fungus, Penicillium sp. SWUKD4. 1850. Phytochemistry 158:56–66

Qin QP, Wang ZF, Huang XL, Tan MX, Zou BQ, Liang H (2019b) Strong in vitro and vivo cytotoxicity of novel organoplatinum (II) complexes with quinoline-coumarin derivatives. Eur J Med Chem 184:111751

Qiu M, Xie RS, Shi Y, Zhang H, Chen HM (2010) Isolation and identification of two flavonoid-producing endophytic fungi from Ginkgo biloba L. Ann Microbiol 60(1):143–150

Qun X, Ling-qi Z, Juan Y, Yu-peng LI (2011) β-Elemene from curcuma zedoaria endophytic fungus. Nat Prod Res Dev 23(3):473–475

Raj KG, Manikandan R, Arulvasu C, Pandi M (2015) Anti-proliferative effect of fungal taxol extracted from Cladosporium oxysporum against human pathogenic bacteria and human colon cancer cell line HCT 15. Spectrochim Acta Part A Mol Biomol Spectrosc 138:667–674

Rana KL, Kour D, Kaur T, Devi R, Yadav AN, Yadav N, Saxena AK (2020) Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. Antonie Van Leeuwenhoek 113(8):1075–1107

Rathod D, Dar M, Gade A, Shrivastava RB, Rai M, Varma A (2013) Microbial endophytes: progress and challenges, Biotechnology for medicinal plants. Springer, Berlin, pp 101–121

Ray S, Swapnil P, Singh P, Singh S, Sarma BK, Singh HB (2020) Endophytic Alcaligenes faecalis mediated redesigning of host defense itinerary against Sclerotium rolfsii through induction of phenolics and antioxidant enzymes. Biol Control 150:104355
Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermotolerance generated by plant/fungal symbiosis. Science 298(5598):1581–1581
Reid A, Greene SE (2013) How microbes can help feed the world. Issues. Am Acad Microbiol Colloquium Rep 105:33
Rho H, Hsieh M, Kandel SL, Cantillo J, Doty SL, Kim SH (2018) Do endophytes promote growth of host plants under stress? A meta-analysis on plant stress mitigation by endophytes. Microb Ecol 75(2):407–418
Richards TA, Dacks JB, Campbell SA, Blanchard JL, Foster PG, McLeod R, Roberts CW (2006) Evolutionary origins of the eukaryotic shikimate pathway: gene fusions, horizontal gene transfer, and endosymbiotic replacements. Eukaryotic Cell 5(9):1517–1531
Rodriguez RJ, White JF Jr, Arnold AE, Redman ARA (2009) Fungal endophytes: diversity and functional roles. New Phytol 182(2):314–330
Rustamova N, Bozorov K, Efferth T, Yili A (2020) Novel secondary metabolites from endophytic fungi: synthesis and biological properties. Phytochem Rev 19:425–448
Sahoo I, Devasurmutt Y, Thippeswamy U, Satwadi PR, Ramachandra YL, Ananda KHB, Melappa G (2018) In silico anti-HIV and anticoagulant activity of [60] fullerene conjugated coumarin and p-coumaric acid isolated from endophytic fungi, alternaria species-1. Int J Microbiol Appl 5 (4):81
Saikkonen K, Helander M, Faeth SH, Schulthess F, Wilson D (1999) Endophyte-grass-herbivore interactions: the case of Neotyphodium endophytes in Arizona fescue populations. Oecologia 121(3):411–420
Saikkonen K, Young CA, Helander M, Schardl CL (2016) Endophytic Epichloë species and their grass hosts: from evolution to applications. Plant Mol Biol 90(6):665–675
Sanchez-Azofeifa A, Oki Y, Fernandes GW, Ball RA, Gamon J (2012) Relationships between endophyte diversity and leaf optical properties. Trees 26(2):291–299
Santiago C, Sun L, Munro MHG, Santhanam J (2014) Polyketide and benzopyran compounds of an endophytic fungus isolated from C innamomum mollissimum: biological activity and structure. Asian Pac J Trop Biomed 4(8):627–632
Santos MLD, Berlitz DL, Wiest SLF, Schünemann R, Knaak N, Fiuza LM (2018) Benefits associated with the interaction of endophytic bacteria and plants. Braz Arch Biol Technol:61. https://doi.org/10.1590/1678-4324-2018160431
Saxena S, Meshram V, Kapoor N (2015) Muscodor tigerii sp. nov.-volatile antibiotic producing endophytic fungus from the Northeastern Himalayas. Ann Microbiol 65(1):47–57
Scervino JM, Ponce MA, Erra-Bassells R, Bompadre J, Vierheilig H, Ocampo JA, Godeas A (2007) The effect of flavones and flavonols on colonization of tomato plants by arbuscular mycorrhizal fungi of the genera *Gigaspora* and *Glomus*. Can J Microbiol 53:702–709
Schulz B, Boyle C, Draeger S, Römmert AK, Krohn K (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol Res 106(9):996–1004
Seabloom EW, Condon B, Kinkel L, Komatsu KJ, Lumibao CY, May G et al (2019) Effects of nutrient supply, herbivory, and host community on fungal endophyte diversity. Ecology 100(9): e02758
Selim KA, Nagia MM, Ghwas DEE (2017) Endophytic fungi are multifunctional biosynthesizers: ecological role and chemical diversity. In: Endophytic fungi: diversity, characterization and biocontrol, vol 39. Nova Science Publisher's, Hauppauge
Senthilkumar N, Murugesan S, Mohan V, Muthumary J (2013) Taxol producing fungal endophyte, Colletotrichum gleospoiroides (Penz.) from Tectona grandis L. Curr Biotica 7:3–15
Shang NN, Zhang Z, Huang JP, Wang L, Luo J, Yang J et al (2018) Glycosylated piericidins from an endophytic streptomyces with cytotoxicity and antimicrobial activity. J Antibiot 71 (7):672–676
Shaw JJ, Spakowicz DJ, Dalal RS, Davis JH, Lehr NA, Dunican BF et al (2015) Biosynthesis and genomic analysis of medium-chain hydrocarbon production by the endophytic fungal isolate Nigrograna mackinnonii E5202H. Appl Microbiol Biotechnol 99(8):3715–3728

Shikano I (2017) Evolutionary ecology of multitrophic interactions between plants, insect herbivores and entomopathogens. J Chem Ecol 43(6):586–598
Shin E, Choi KM, Yoo HS, Lee CK, Hwang BY, Lee MK (2010) Inhibitory effects of coumarins from the stem barks of Fraxinus rhynchophylla on adipocyte differentiation in 3T3-L1 cells. Biol Pharm Bull 33(9):1610–1614
Siegel MR, Latch GCM, Bush LP, Fannin FF, Rowan DD, Tapper BA, Johnson MC (1990) Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. J Chem Ecol 16 (12):3301–3315.z
Silva GH, Teles HL, Zanardi LM, Young MCM, Eberlin MN, Hadad R, Araújo ÂR (2006) Cadinane sesquiterpenoids of *Phomopsis cassiae*, an endophytic fungus associated with *Cassia spectabilis* (Leguminosae). Phytochemistry 67(17):1964–1969
Silva GH, de Oliveira CM, Teles HL, Pauletti PM, Castro-Gamboa I, Silva DH, Berlinck RG (2010) Sesquiterpenes from *Xylaria* sp., an endophytic fungus associated with *Piper aduncum* (Piperaceae). Phytochem Lett 3(3):164–167
Soares MA, Li HY, Bergen M, Da Silva JM, Kowalski KP, White JF (2016) Functional role of an endophytic Bacillus amyloliquefaciens in enhancing growth and disease protection of invasive English ivy (*Hedera helix* L.). Plant Soil 405(1–2):107–123
Sommart U, Rukachaisirikul V, Tadpetch K, Sukpondma Y, Phongpaichit S, Hutadilok-Towatana-N, Sakayaroj J (2012) Modiolin and phthalide derivatives from the endophytic fungus Microsphaeropsis arundinis PSU-G18. Tetrahedron 68(48):10005–10010
Sonaimuthu V, Johnpaul M (2010) Taxol (anticancer drug) producing endophytic fungi: an overview. Int J Pharma Bio Sci 1(3):1–9
Stierle AA, Stierle DB (2000) Bioactive compounds from four endophytic Penicillium sp. of a Northwest Pacific yew tree. In: Studies in natural products chemistry, vol 24. Elsevier, pp 933–977
Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science 260(5105):214–216
Strobel G, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67(4):491–502
Strobel GA, Dirkse E, Sears J, Markworth C (2001) Volatile antimicrobials from Muscodor albus, a novel endophytic fungus. Microbiology 147(11):2943–2950
Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67(2):257–268
Sturz AV, Nowak J (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Appl Soil Ecol 15(2):183–190
Sun J, Awakawa T, Noguchi H, Abe I (2012) Induced production of mycotoxins in an endophytic fungus from the medicinal plant Datura stramonium L. Bioorg Med Chem Lett 22 (20):6397–6400
Taechowisan T, Lu C, Shen Y, Lumyong S (2005) Secondary metabolites from endophytic Streptomyces aureofaciens CMUAc130 and their antifungal activity. Microbiology 151 (5):1691–1695
Taechowisan T, Lu C, Shen Y, Lumyong S (2007) 4-arylcoumarin inhibits immediate-type allergy. Food Agric Immunol 18(3–4):203–211
Takahashi S, Kawakami S, Sugimoto S, Matsunami K, Otsuka H (2015) Lignan glycosides and phenolic compound glycosides from the branches of Tabebuia chrysotricha. Am J Plant Sci 6 (5):676
Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18 (4):448–459
Tang JW, Wang WG, Li A, Yan BC, Chen R, Li XN et al (2017) Polyketides from the endophytic fungus Phomopsis sp. sh917 by using the one strain/many compounds strategy. Tetrahedron 73 (26):3577–3584
Tang Z, Wang Y, Yang J, Xiao Y, Cai Y, Wan Y et al (2020) Isolation and identification of flavonoid-producing endophytic fungi from medicinal plant Conyza blinii H. Lév that exhibit higher antioxidant and antibacterial activities. PeerJ 8:e8978

Taylor LP, Grotewold E (2005) Flavonoids as developmental regulators. Curr Opin Plant Biol 8 (3):317–323
Tejesvi MV, Kini KR, Prakash HS, Subbiah V, Shetty HS (2007) Genetic diversity and antifungal activity of species of Pestalotiopsis isolated as endophytes from medicinal plants. Fungal Divers 24(3):1–18
Tejesvi MV, Kajula M, Mattila S, Pirttilä AM (2011) Bioactivity and genetic diversity of endophytic fungi in Rhododendron tomentosum Harmaja. Fungal Divers 47(1):97–107
Teles HL, Sordi R, Silva GH, Castro-Gamboa I, da Silva Bolzani V, Pfenning LH, Araújo ÂR (2006) Aromatic compounds produced by Periconia atropurpurea, an endophytic fungus associated with Xylopia aromatica. Phytochemistry 67(24):2686–2690
Terhonen E, Blumenstein K, Kovalchuk A, Asiegbu FO (2019) Forest tree microbiomes and associated fungal endophytes: functional roles and impact on forest health. Forests 10(1):42
Theantana T, Kanjanapothi D, Lumyong S (2012) In vitro inhibition of lipid peroxidation and the antioxidant system of endophytic fungi from Thai medicinal plants. Chiang Mai J Sci 39 (3):429–444
Tinikul R, Chenprakhon P, Maenpuen S, Chaiyen P (2018) Biotransformation of plant-derived phenolic acids. Biotechnol J 13(6):1700632
Tiwari P, Bae H (2020) Horizontal gene transfer and endophytes: an implication for the acquisition of novel traits. Plants 9(3):305
Umashankar T, Govindappa M, Ramachandra YL, Padmalatha Rai S, Channabasava (2015) Isolation and characterization of coumarin isolated from endophyte, alternaria species-1 of Crotalaria pallida and its apoptotic action on HeLa cancer cell line. Metabolomics 5 (158):2153–0769
Verma VC, Kharwar RN, Strobel GA (2009) Chemical and functional diversity of natural products from plant associated endophytic fungi. Nat Prod Commun 4(11):1934578X0900401114
Verma SK, Kingsley K, Irizarry I, Bergen M, Kharwar RN, White JF Jr (2017) Seed-vectored endophytic bacteria modulate development of rice seedlings. J Appl Microbiol 122 (6):1680–1691
Verma SK, Kingsley KL, Bergen MS, Kowalski KP, White JF (2018a) Fungal disease protection in rice (Oryza sativa) seedlings by growth promoting seed-associated endophytic bacteria from invasive Phragmites australis MDPI. Microorganisms 6:21
Verma SK, Kingsley K, Bergen M, English C, Elmore M, Kharwar RN, White JF (2018b) Bacterial endophytes from rice cut grass (Leersia oryzoides L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. Plant Soil 422 (1–2):223–238
Viiri H, Annila E, Kitunen V, Niemelä P (2001) Induced responses in stilbenes and terpenes in fertilized Norway spruce after inoculation with blue-stain fungus, Ceratocystis polonica. Trees 15(2):112–122
Walia A, Guleria S, Chauhan A, Mehta P (2017) Endophytic bacteria: role in phosphate solubilization, Endophytes: crop productivity and protection. Springer, Cham, pp 61–93
Wang Y, Dai CC (2011) Endophytes: a potential resource for biosynthesis, biotransformation, and biodegradation. Ann Microbiol 61(2):207–215
Wang LW, Zhang YL, Lin FC, Hu YZ, Zhang CL (2011a) Natural products with antitumor activity from endophytic fungi. Mini Rev Med Chem 11(12):1056–1074
Wang Y, Zeng QG, Zhang ZB, Yan RM, Wang LY, Zhu D (2011b) Isolation and characterization of endophytic huperzine A-producing fungi from Huperzia serrata. J Ind Microbiol Biotechnol 38(9):1267–1278
Wang Y, Xu L, Ren W, Zhao D, Zhu Y, Wu X (2012) Bioactive metabolites from Chaetomium globosum L18, an endophytic fungus in the medicinal plant Curcuma wenyujin. Phytomedicine 19(3–4):364–368
Wang J, Yao LY, Lu YH (2013) Ceriporia lacerata DMC1106, a new endophytic fungus: Isolation, identification, and optimal medium for 2′, 4′-dihydroxy-6′-methoxy-3′, 5′-dimethylchalcone production. Biotechnol Bioprocess Eng 18(4):669–678

Wang XJ, Min CL, Ge M, Zuo RH (2014) An endophytic sanguinarine-producing fungus from Macleaya cordata, Fusarium proliferatum BLH51. Curr Microbiol *68*(3):336–341

Wang JY, Liang YL, Hai MR, Chen JW, Gao ZJ, Hu QQ et al (2016) Genome-wide transcriptional excavation of Dipsacus asperoides unmasked both cryptic Asperosaponin biosynthetic genes and SSR markers. Front Plant Sci 7:339

White JF, Crawford H, Torres MS, Mattera R, Irizarry I, Bergen M (2012) A proposed mechanism for nitrogen acquisition by grass seedlings through oxidation of symbiotic bacteria. Symbiosis 57(3):161–171

White JF, Kingsley KI, Kowalski KP, Irizarry I, Micci A, Soares MA, Bergen MS (2018) Disease protection and allelopathic interactions of seed-transmitted endophytic pseudomonads of invasive reed grass (Phragmites australis). Plant Soil 422(1–2):195–208

Wibowo M, Prachyawarakorn V, Aree T, Mahidol C, Ruchirawat S, Kittakoop P (2016) Cytotoxic sesquiterpenes from the endophytic fungus Pseudolagarobasidium acaciicola. Phytochemistry 122:126–138

Wijeratne EK, Paranagama PA, Marron MT, Gunatilaka MK, Arnold AE, Gunatilaka AL (2008) Sesquiterpene quinones and related metabolites from Phyllosticta spinarum, a fungal strain endophytic in Platycladus orientalis of the Sonoran Desert. J Nat Prod 71(2):218–222

Wilson D (1995) Endophyte: the evolution of a term, and clarification of its use and definition. Oikos 73:274–276

Wilson D, Barr ME, Faeth SH (1997) Ecology and description of a new species of Ophiognomonia endophytic in the leaves of Quercus emoryi. Mycologia 89(4):537–546

Witaicenis A, Seito LN, Di Stasi LC (2010) Intestinal anti-inflammatory activity of esculetin and 4-methylesculetin in the trinitrobenzenesulphonic acid model of rat colitis. Chem Biol Interact 186(2):211–218

Wiyakrutta S, Sriubolmas N, Panphut W, Thongon N, Danwisetkanjana K, Ruangrungsi N, Meevootisom V (2004) Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. World J Microbiol Biotechnol 20(3):265–272

Wu LS, Hu CL, Han T, Zheng CJ, Ma XQ, Rahman K, Qin LP (2013a) Cytotoxic metabolites from Perenniporia tephropora, an endophytic fungus from Taxus chinensis var. mairei. Appl Microbiol Biotechnol 97(1):305–315

Wu SH, Huang R, Miao CP, Chen YW (2013b) Two new steroids from an endophytic fungus Phomopsis sp. Chem Biodivers 10(7):1276–1283

Xia Y, DeBolt S, Dreyer J, Scott D, Williams MA (2015) Characterization of culturable bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. Front Plant Sci 6:490

Xianzhi Y, Lingqi Z, Bo G, Shiping G (2004) Preliminary study of a vincristine-producing endophytic fungus isolated from leaves of Catharanthus roseus. Zhong Cao Yao= Chinese Traditional and Herbal Drugs 35(1):79–81

Xiao J, Lin LB, Hu JY, Duan DZ, Shi W, Zhang Q et al (2018) Pestalustaines A and B, unprecedented sesquiterpene and coumarin derivatives from endophytic fungus Pestalotiopsis adusta. Tetrahedron Lett 59(18):1772–1775

Xiaocheng Y, Yanbing L, Qiuhong L, Shengchao Y, Tao L (2018) Isolation and screening of endophytic fungi that might produce saponins in Paris polyphylla var. yunnanensis. Mol Plant Breed 2:52

Xu F, Zhang Y, Wang J, Pang J, Huang C, Wu X, Lin Y (2008) Benzofuran derivatives from the mangrove endophytic fungus Xylaria sp. J Nat Prod 71(7):1251–1253

Yadav AN, Kumar V, Dhaliwal HS, Prasad R, Saxena AK (2018) Microbiome in crops: diversity, distribution, and potential role in crop improvement. In: Crop improvement through microbial biotechnology. Elsevier, Amsterdam, pp 305–332

Yadav AN, Rastegari AA, Yadav N, Kour D (2020) Advances in plant microbiome and sustainable agriculture: diversity and biotechnological applications. Springer, Singapore

Yan C, Liu W, Li J, Deng Y, Chen S, Liu H (2018) Bioactive terpenoids from Santalum album derived endophytic fungus Fusarium sp. YD-2. RSC Adv 8(27):14823–14828

Yang NY, Jiang S, Shang EX, Tang YP, Duan JA (2012) A new phenylpentanamine alkaloid produced by an endophyte Bacillus subtilis isolated from Angelica sinensis. J Chem Res 36 (11):647

Yasser MM, Marzouk MA, El-Shafey NM, Shaban SA (2020) Diversity and antimicrobial activity of endophytic fungi from the medicinal plant Pelargonium graveolens (geranium) in Middle Egypt. Jordan J Biol Sci 13(2):197–205

Yin H, Sun YH (2011) Vincamine-producing endophytic fungus isolated from Vinca minor. Phytomedicine 18(8–9):802–805

Yin K, Zhang L, Chen D, Tian Y, Zhang F, Wen M, Yuan C (2016) Understory herb layer exerts strong controls on soil microbial communities in subtropical plantations. Sci Rep 6(1):1–8

Ying YM, Shan WG, Zhan ZJ (2014) Biotransformation of Huperzine A by a fungal endophyte of Huperzia serrata furnished sesquiterpenoid–alkaloid hybrids. J Nat Prod 77(9):2054–2059

Yuan L, Lin X, Zhao PJ, Ma J, Huang YJ, Shen YM (2009) New polyketides from endophytic diaporthe sp. XZ-07. Helv Chim Acta 92(6):1184–1190

Yun ES, Park SS, Shin HC, Choi YH, Kim WJ, Moon SK (2011) p38 MAPK activation is required for esculetin-induced inhibition of vascular smooth muscle cells proliferation. Toxicol In Vitro 25(7):1335–1342

Zhan J, Burns AM, Liu MX, Faeth SH, Gunatilaka AL (2007) Search for cell motility and angiogenesis inhibitors with potential anticancer activity: beauvericin and other constituents of two endophytic strains of Fusarium oxysporum. J Nat Prod 70(2):227–232

Zhang HW, Song YC, Tan RX (2006) Biology and chemistry of endophytes. Nat Prod Rep 23 (5):753–771

Zhang P, Zhou PP, Yu LJ (2009) An endophytic taxol-producing fungus from Taxus media, Cladosporium cladosporioides MD2. Curr Microbiol 59(3):227

Zhang Y, Han T, Ming Q, Wu L, Rahman K, Qin L (2012) Alkaloids produced by endophytic fungi: a review. Nat Prod Commun 7(7):1934578X1200700742

Zhang TY, Yu Y, Zhang MY, Cheng J, Chen ZJ, Zhang JY, Zhang YX (2018) Verruconis panacis sp. nov., an endophyte isolated from Panax notoginseng. Int J Syst Evol Microbiol 68 (8):2499–2503

Zhao JH, Zhang YL, Wang LW, Wang JY, Zhang CL (2012) Bioactive secondary metabolites from Nigrospora sp. LLGLM003, an endophytic fungus of the medicinal plant Moringa oleifera Lam. World J Microbiol Biotechnol 28(5):2107–2112

Zhao M, Yuan LY, Guo DL, Ye Y, Da-Wa ZM, Wang XL et al (2018) Bioactive halogenated dihydroisocoumarins produced by the endophytic fungus Lachnum palmae isolated from Przewalskia tangutica. Phytochemistry 148:97–103

Zhou J, Xu J (2018) Chemistry and biodiversity of rhizophora-derived endophytic fungi. In: Mangrove ecosystem ecology and function. IntechOpen, London

Zhou SL, Yang F, Lan SL, Xu N, Hong YH (2009) Huperzine A producing condition from endophytic fungus in SHB Huperzia serrata. J Microbiol 3:32–36

Zhou N, Zhao XL, Xie QW (2016) Content determination of total flavonoids in endophytic fungi Aspergillus Niger GZ-4 from sugarcane leaves. Chem Bioeng 1:19

Zhou W, Wheeler TA, Starr JL, Valencia CU, Sword GA (2018) A fungal endophyte defensive symbiosis affects plant-nematode interactions in cotton. Plant Soil 422(1–2):251–266

Zhou J, Liu Z, Wang S, Li J, Li Y, Chen WK, Wang R (2020) Fungal endophytes promote the accumulation of Amaryllidaceae alkaloids in Lycoris radiata. Environ Microbiol 22 (4):1421–1434

Zou WX, Meng JC, Lu H, Chen GX, Shi GX, Zhang TY, Tan RX (2000) Metabolites of Colletotrichum gloeosporioides, an endophytic fungus in Artemisia mongolica. J Nat Prod 63 (11):1529–1530

Application of Microbes in Synthesis of Electrode Materials for Supercapacitors

2

Dipanwita Majumdar

Abstract

The utilization of microorganisms for fabrication of different useful nanomaterials with precise control on their morphologies and micro-architectures has attracted substantial interest owing to their biocompatibility, renewability, sustainability, and cost-effectiveness. High-performance supercapacitors, one of the fast-developing sectors in electrochemical energy storage systems, essentially require active electrode materials with large specific surface area, interconnected uniform porous arrangement, outstanding electronic charge transport, and mechanical flexibility as prime features. In the present chapter, the state-of-the-art research developments in the exploitation of microbial generated materials comprising different carbon nanomaterials with vivid compositions along with their nanocomposites with various counter systems such as high costing synthetic nanocarbons, metallic compounds, conducting polymers, etc. that have been successfully applied as functional supercapacitor electrodes. These microbe-based systems have been mainly applied to fulfill the preferred purposes that include as (a) bio-templates; (b) mechanically flexible, supporting matrix to strongly anchor diverse electroactive materials; and (c) bio-carbonized substances that are highly porous, large surface-based functionalized carbons, which are subsequently also employed in formation of smart nanocomposites. Thus, amalgamation of bio-tools for materials designing in supercapacitor technology will surely open up innovative opportunities for extensive and scalable manufacture of highly proficient energy storage gadgets in the near future.

D. Majumdar (✉)
Department of Chemistry, Chandernagore College, Chandannagar, West Bengal, India

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Microbes · Specific capacitance · Supercapacitor · Electrochemical performances · Derived carbons · Bacterial cellulose · Carbon framework

2.1 Introduction

Highly undesirable and unfavorable ecological impacts imposed by random burning up of non-renewable fossil fuels have triggered serious concerns among scientists to focus on introducing novel, renewable, and sustainable modes of energy production and look over their efficient storage and inter-conversion processes (Larcher and Tarascon 2015; Wang et al. 2017a). This has resulted in fast progression in electrochemical energy storage (EES) devices such as capacitors, rechargeable batteries, supercapacitors, etc., in particular, to counter the sky-high growing demands of uninterrupted power supply (Sumboja et al. 2018; Winter and Brodd 2004). A comparative study on the relative performance of these EES systems has been outline in Table 2.1. Each of the devices has its own merits and limitations. Till date gigantic and voluminous batteries are considered as major power backup devices owing to their large values of specific energy (energy stored per unit mass) (30–300 Wh kg^{-1}) (Winter and Brodd 2004; Jayalakshmi and Balasubramanian 2008; Wang et al. 2017b). However, poor life span, inferior

Table 2.1 Comparative study of various EES devices

Property	Capacitors	Supercapacitors	Rechargeable batteries	Fuel cells
Capacitance	Below 10 mF	100 mF to 1500 F	–	–
Cell operating voltage	6–800 V	2–3 V/ cell	1.25–4.2 V/cell	0.6 V
Average life time	$>10^5$ cycles	$>5 \times 10^4$ cycles	150–1500 cycles	1500–10^4 cycles
Weight	1 g to 10 kg	1 g to 250 g	1 g to >10 kg	20 g to > 5 kg
Specific power	0.25–10,000 kW kg^{-1}	10–120 kW kg^{-1}	0.005–0.4 kW kg^{-1}	0.001–0.1 kW kg^{-1}
Specific energy	0.01–0.05 Wh kg^{-1}	1–10 Wh kg^{-1}	8–600 Wh kg^{-1}	300–3000 Wh kg^{-1}
Charging/ discharging duration	Pico-seconds to milliseconds	Milliseconds to seconds	1–10 h	10–300 h
Operating temperature	−20 to 100 °C	−40 to +85 °C	−20 to 65 °C	25–90 °C
Safety issues	Stable	Stable	Safety consideration required	Safety consideration required
Production cost	Low cost	High cost	Medium cost	High cost

specific power (energy delivered per unit time per unit mass), and environmental toxicity along with safety issues especially related to their recycling and disposal after its life termination are the foremost practical limitations. Nevertheless, lack of suitable substitutes has been the main ground for disregarding these drawbacks. Yet today, eco-friendly, high energy density fuel cells do have less significance for large quantity energy production sectors, primarily, because of their bulk size, large installation costs, and insufficient fuel storage capability (Santoro et al. 2017). Again, conventional capacitors though display inferior specific energy are capable of delivering energy at ultrafast rate along with long cycle life (Poonam et al. 2019; Kotz and Carlen 2000). The above points stimulated researchers to assemble and integrate, as far as possible, all the good qualities of each type of the above EES systems to fabricate novel device with enhanced energy delivering capabilities in the form of supercapacitors, also popularly named as electrochemical capacitors or ultracapacitors (Raza et al. 2018; Conway 1991; Burke 2000). However, both production cost and energy-power efficiency of first-generation supercapacitors are far below to the expectations, and thus, low-priced, handy, portable, miniatured, bendable yet robust, smart future-generation supercapacitors with large energy and power characteristics have been urgently demanded to replace the conventional voluminous, poor responsive, short-lasting batteries soon (Meng et al. 2013; Gidwani et al. 2014).

Rigorous researches have signalized scrupulous strategies to overcome the issues of poor specific energy of supercapacitors without surrendering their high-power performances and exceptional cyclic efficiency (Xue et al. 2017; Naoi and Simon 2008; González et al. 2016). Experts are targeting to harmonize the energy storing efficiency closer to that of popular rechargeable batteries (Yassine and Fabri 2017; Stoller and Ruoff 2010). On the contrary, extensive scientific explorations are being carried out to improve the power capacity of the prevailing batteries as well (Dupont and Donne 2016; Dong et al. 2016). This has commended in designing next-generation EES possessing elevated energy and power efficiency, strategically, either by (a) employing one of the electrodes as a composite of supercapacitor-type and battery-type ingredients or (b) by devising a setup in combination of supercapacitor electrode with a battery electrode. The so-configured devices with such hybrid electrode materials or electrodes configuration have been commonly termed as "supercapatteries" (Chen 2017; Majumdar et al. 2020; Chae et al. 2012). These devices have been sketched to possess the optimum properties of supercapacitors and rechargeable batteries, all together. A typical *Ragone plot* (as depicted schematically in Fig. 2.1a) is used to evaluate the relative device performances by considering the specific energies at different specific power values, expressed in logarithmic terms and then correlating them with the performances of other EES, existing in the scientific database (Christen and Carlen 2000; Mei et al. 2018). It clearly indicates the relative position of supercapatteries, bridging the void between the common capacitors and rechargeable batteries in terms of energy and power efficiencies. Although its current position is far to that of theoretically predicted energy-power efficiency of thermodynamically reversible heat engine, the plot signifies that there lies plenty of room for improvement in the supercapattery

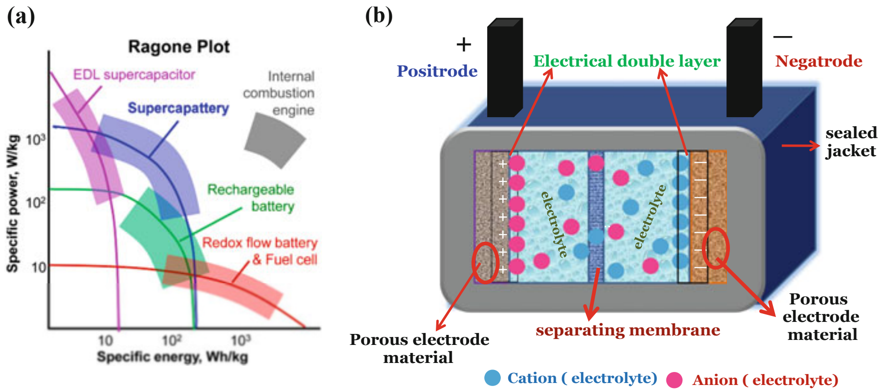

Fig. 2.1 (**a**) A characteristic Ragone plot showing relative performance of different energy storage devices (Chen 2017). (**b**) Schematic illustration of a characteristic supercapacitor device

technology in order to accomplish results that may be very close to the challenging one (Chen 2017; Majumdar et al. 2020).

2.1.1 Basics of Supercapacitors

Thus, before proceeding further, it is worth to have a basic understanding of the fundamental aspects of supercapacitors. Typically, a supercapacitor device (as shown in Fig. 2.1b) comprises the following sections: two electrodes, with positive and negative polarity, possessing large surface area as well as high porosity, connected ionically via electrolytes, partitioned by porous electrolyte-filled separating membrane. The efficiency of various electrochemical energy storage systems is designated by two important parameters, viz., specific energy and specific power, as mathematically expressed through the following Eqs. (2.1) and (2.2), respectively (Majumdar et al. 2020):

$$\text{Specific energy}(E) = \frac{1}{2} C_s (\Delta V)^2 \tag{2.1}$$

$$\text{Specific power } (P) = (E/\Delta t) \tag{2.2}$$

The term C_s stands for the *specific or gravimetric capacitance*, ΔV corresponds to working potential range, and Δt represents the discharging time period, respectively. Usually, large magnitudes of E and P are ideal for high-performing supercapacitors. The values of power and energy densities can be achieved by considering the volume of the electrode material instead of its mass while computing the capacitances, as applied in the above-stated equations (Zuo et al. 2017a; Akinwolemiwa and Chen 2018). Moreover, some additional crucial parameters also need essential consideration and have been mentioned as follows: *Rate capability or capacity*, variation of

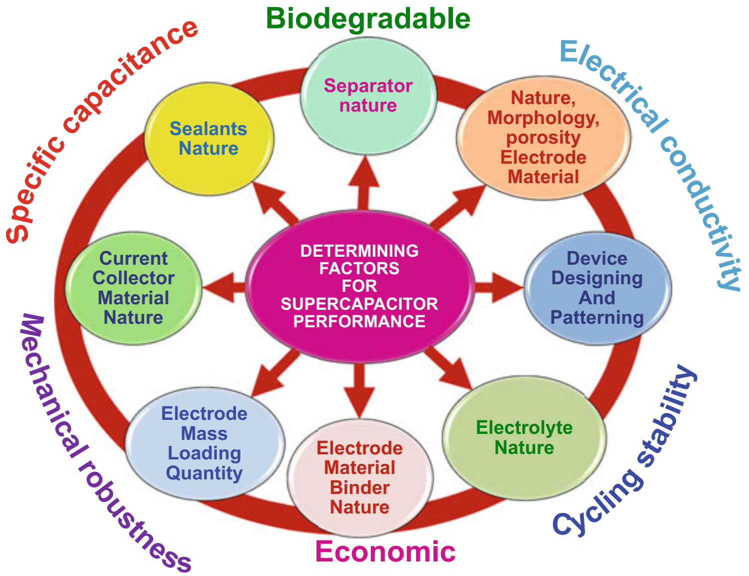

Fig. 2.2 Outline of different parameters that controls the performance of supercapacitors

capacitance at different current densities or at varying voltage sweep rates. It judges the ability of the device to generate large power with minimum loss in voltage even at high applied currents. *Electrochemical reversibility* involves measurement of rates of charge transfer process occurring at the electrode/electrolyte interfaces. *Electrochemical window:* voltage range within which the system is neither oxidized nor reduced. The electrode as well as electrolyte's ability to avoid electrochemical decompositions in a given potential range determines its electrochemical stability window. *Electrochemical stability*: the reversibility in charge storing capacity (capacitance) of the device in a given potential range under constant current or potential scans rates determines its electrochemical stability. It is indicated by high specific capacitance retention efficiency of the device for large galvanostatic charging/discharging cycle numbers. In addition, factors such as mechanical flexibility, environmental stability, and operational temperature range are also analyzed (Afif et al. 2019; Miller et al. 2018; Shi et al. 2013). A number of factors control the electrochemical efficiency of supercapacitor devices, namely, (a) electrode materials, nature, porosity, and morphology, (b) electrolyte ions and their nature, (c) electrode materials' binder, (d) quantity of electrode material loading, (e) nature of current collector used to hold electroactive materials, (f) separator membrane, (g) sealants used, (h) devise patterning and designing, etc. have been outlined in Fig. 2.2. Each of the components are vital; however detailed discussions on them are beyond the scope of the chapter. The readers can go through the literature for obtaining more information and knowledge about them (Majumdar et al. 2020).

Electrochemical performances of electrodes in these devices are usually investigated using cyclic voltammetric (CV) analysis, galvanostatic charging-discharging cycling tests (GCD), and electrochemical impedance spectroscopy (EIS) (Majumdar et al. 2020; Wang et al. 2017b).

Specific or gravimetric capacitance (C_s) of electrode materials is commonly calculated using the following equations from the curves and parameters derived from CV or GCD experiments as shown in Eqs. (2.3) and (2.4), respectively (Majumdar et al. 2020):

$$C_s = \left(\frac{1}{ms}\right)(\Delta V)\int_{V1}^{Vn} i\mathrm{d}V \tag{2.3}$$

$$C_s = \frac{I}{m\,(\Delta V/\Delta t)} \tag{2.4}$$

where the integration $\int(i\mathrm{d}V)$ gives the integrated area under the CV voltammogram curve, s stands for voltage sweep rate, ΔV is the potential range, V_n and V_1 are the terminal voltage limits of voltage scans, and "m" corresponds to the mass of the electrode materials, respectively. I indicates the steady current used for the GCD test. The popular unit for specific or gravimetric capacitance is "F g^{-1}", while that of areal and volumetric capacitances are "F cm^{-2}" and "F cm^{-3}", respectively (Wang et al. 2014a).

Electrochemical impedance spectroscopy (EIS) is used to characterize the resistive as well as capacitive nature of the electrode materials (Majumdar et al. 2020; Obreja 2008).

2.1.2 Electrode Materials for Supercapacitors

Among the above factors, the most decisive one is unambiguously the nature and morphology of electrode materials employed for devising supercapacitors (Iro et al. 2016). The fundamental operational principle for supercapacitors in contrast to batteries encourages judicious designing of electrode materials and electrolyte to undergo faster electron/ions transport in the bulk electrode with special stress on tuning material dimension, surface nature, crystal forms, and electrode/electrolyte interfacial chemistry, respectively, to recognize exceptional capacitances, energy, and power densities (Xiea et al. 2018). Thus, this is certainly a serious and urgent assignment to materialize "smart and efficient" supercapacitor electrodes with outstanding electrochemical features.

Energy storage mechanism in supercapacitors is usually guided by two types of charge storage phenomena exhibited by the electrode materials, specifically, electrical double layer capacitors (EDLCs) and pseudocapacitors, respectively (Simon and Gogotsi 2008; An et al. 2019; Le Fevre et al. 2019; Borenstein et al. 2017; Wang et al. 2015a). EDLC-type electrode materials accumulate charge via rapid ion-adsorption/desorption processes at the electrolyte/electrode boundary during

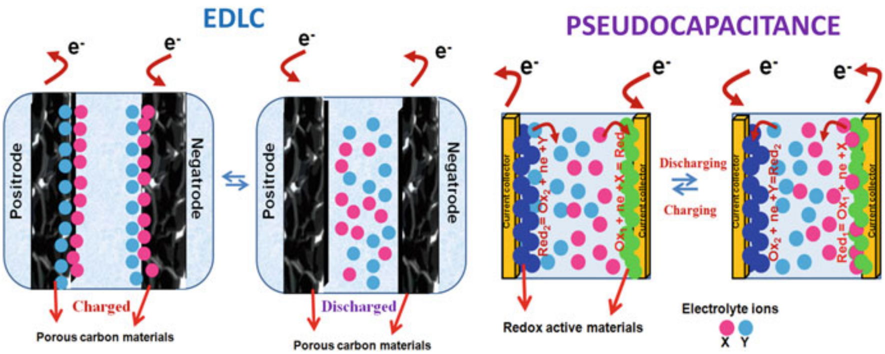

Fig. 2.3 Illustration of charging/discharging processes in electrical double-layer capacitor (EDLC) and pseudocapacitor systems

charging/discharging courses as demonstrated schematically in Fig. 2.3. High surface area-containing organic-based materials such as active-carbons, mesoporous carbons, carbon nanotubes/nanofibers, graphenes, carbon foams, carbon aerogels, etc. with stable electrochemical behavior are usually categorized under EDLC materials (Najib and Erdem 2019). EDLC-type materials generally demonstrate low capacitance although survive high figure of fast and stable charging/discharging cycles efficiently (Guan et al. 2016). Pseudocapacitors, on the other hand, illustrate better capacitance values compared to EDLCs but poor charging/discharging efficacy (Wang et al. 2017c; Jiang and Liu 2019; Augustyn et al. 2014). They include mostly transition metal oxides and chalcogenides, and derived compounds along with conducting polymer derivatives involve slow, diffusion-controlled movement of ions to achieve this charge storing capacity, as depicted in Fig. 2.3 (Brousse et al. 2015; Majumdar 2016; Zhan et al. 2018; Majumdar and Bhattacharya 2017). Capacitive faradaic process in these semiconducting materials typically involves incessant electron transfer over comparatively wide potential range owing to activation of delocalized electrons dynamically associated with several redox-active sites thereby establishing groups of energy states (Guan et al. 2016).

It has been accomplished that hybrids/composite materials are beneficial as they successfully eliminate the individual shortcomings of the components (Majumdar et al. 2019a, 2019b; Majumdar 2018, 2019a, b). In order to devise supercapattery devices, such hybrids materials are fabricated with organic/inorganic nanomaterials with different charge storage mechanisms covering both pseudocapacitance and batteries features that are essential to reach the desired energy efficiency (Chen 2017; Majumdar et al. 2020; Chae et al. 2012). Accordingly, various nanocarbons such as graphene, carbon foams/cloths, etc. have been blended with metal-based compounds, conducting polymers, etc. to obtain electrodes with enhanced specific surface area, high and uniform porosity, as well as improved electrical conductivity so as to overcome the problems of inferior energy density, low cycling rates, and mechanical instability (Majumdar 2019a, 2019b; Chen et al. 2017; Dubey and Guruviah 2019).

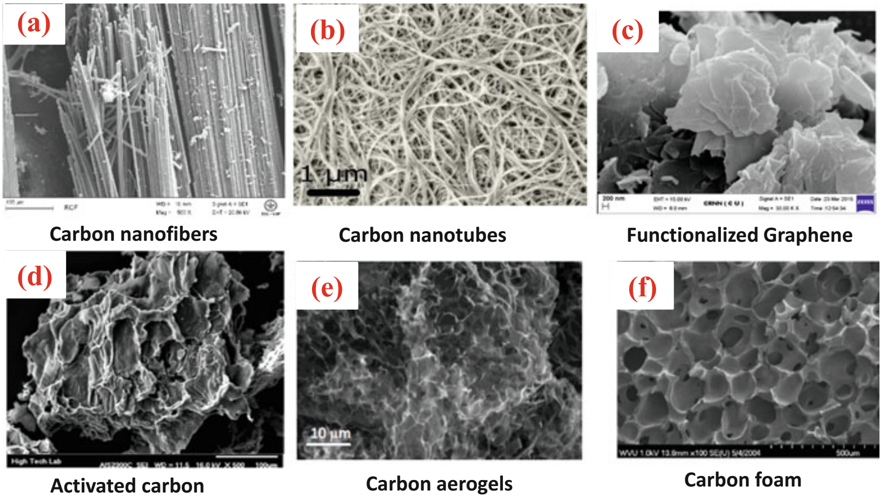

Fig. 2.4 Different synthetic carbons such as (**a**) carbon nanofibers (Hirayama et al. 2017), (**b**) carbon nanotubes (File:CNTSEM.JPG n.d.), (**c**) functionalized graphenes, (**d**) activated carbon (Omidi-Khaniabadi et al. 2015), (**e**) carbon aerogels (Ding et al. 2018), (**f**) carbon foams employed in energy storage applications (Chen et al. 2006)

In this aspect, it has been spotted that the carbon materials used in designing such composites must satisfy some essential features: (a) easy fabrication from natural abundant resources so as to minimize production cost; (b) large surface area with tunable porosity; (c) easy assembling to form hierarchical three dimensional network structure to facilitate faster transport of ions/electrons; (d) containing adequate surface functionalities to strongly anchor various nanomaterials on their surfaces, thus ensuring large mass loading of electroactive materials as well as inhibit their agglomeration and easy detachment during large charging/discharging cycles; and (e) mechanically flexible with high tensile strength so that they can form efficient matrix for manufacturing flexible energy storage devices (Zhang and Zhao 2009; Borenstein et al. 2017; He et al. 2013).

The synthetic carbons like graphenes, carbon nanotube, carbon foams, activated carbons, carbon aerogels, etc. as illustrated in Fig. 2.4 often require costly instrumentations and rigorous post-synthetic treatments with limited control on surface area, porosity, and electrical conductivity (Hirayama et al. 2017; File: CNTSEM.JPG n.d.; Omidi-Khaniabadi et al. 2015; Ding et al. 2018; Chen et al. 2006). Moreover, they are often susceptible toward agglomeration and restacking that drastically reduces their effective surface area. Again, optimizing of conductivity with functionalization and uniform doping are essentially required as too much surface modification considerably lowers their electronic conductivity (Li and Wei 2013). Such optimization urges several hurdle synthetic steps that considerably limit their practical applications.

Thus, scientists worldwide have devoted their research in designing electrode materials based on carbons gathered not only from natural resources but successfully able to satisfy all the requisite criteria discussed above (Li and Wei 2013). As an essential aspect of green technology and to save our environment, various bio-masses/bio-wastes as well as microorganisms that are widely available in the nature have been meticulously channelized as effective carbon sources for designing advantageous materials in several technological domains (Yang et al. 2019; Bi et al. 2019; Lyu et al. 2019; Correa and Kruse 2018; Lakshmi et al. 2018).

This book chapter intends to discuss on the application of different microbes in designing and fabrication of electrode materials for flexible supercapacitors/ supercapatteries—their current progress and advancements with special emphasis on the role of such microorganisms in structure-designing diversities that led to effective functional modifications so as to improve the overall electrochemical efficiency in these energy storage devices.

2.1.3 Why Microbes in Energy Storage Devices?

Microbes or microorganisms are minute living organisms existing everywhere in the environment that can be detected only under microscopic devices. Microorganisms have been chiefly categorized into different classes such as bacteria, fungi, archaea, algae, virus, etc. (Windt et al. 2005; Shen et al. 2019). The invention and subsequent up-gradations of microscopes have travelled through the skilled hands of the several technologists that have enormously helped the scientific community to recognize and identify the existence of the vast microorganism world. They play crucial role as major foodstuff in the food chain of our ecosystem as well as decomposers in the disintegration of dead organisms that facilitate sustaining of cycle of life (Klaus et al. 1999). With intensive studies, their industrial applications in various important sectors, such as nutrition, pharmaceutical, metallurgy, fuels, chemicals, etc., got revealed (Lahoz and Ibeas 1968; Tasaki et al. 2017; Higgins and Dworkin 2012; Jang et al. 2017). Although many of them are susceptible toward causing diseases to human beings and animals, plenty of their merits that include speedy reproduction rate, biomineralization, genetic revision, self-assembly, variety, and appreciable adaptableness to extreme situations have forced the scientists to show tremendous interest in the past decades (Liu et al. 2016a; Wu et al. 2013; Chen et al. 2010; de Petris 1967; Nam et al. 2006). Some naturally occurring microbes are skilled of fabricating nanoparticles under ambient state without requiring supplementary chemical reagents or physical conditions that have fascinated extensively in promoting green technology over the last few decades (Reverberi et al. 2016; Gopinath et al. 2017; Zhang et al. 2019; Fang et al. 2019). Microbes have proved themselves as efficient precursors for preparing various mesoporous carbons used for various technological applications in the recent past (Deng et al. 2019a). They often possess large surface area-based cellular structures that can result in highly urged porous and large surface-based carbon materials (Dong et al. 2013; Moradi et al. 2015; Gerasopoulos et al. 2012). They can be produced on large industrial scales using mild preparation conditions for serving various purposes from a range of bio-wastes that may otherwise create huge ecological pollutions (Yang et al. 2016; Wang et al. 2015b; Divyashree and Hegde 2015). Further, they possess diverse morphologies which have fruitful implications in natural processes that can be effectively channelized to function analogously in smart energy storage devices (Shen et al. 2019). Thus, microbes can be very effective as bio-templates for controlling morphology during synthesis of nanoscale materials. Again, they can provide efficient matrix for high-density electroactive materials mass loadings; especially their hierarchical three-dimensional interlinked structures promote electronic conductive network pathways along with porous channels that facilitates easy intercalation and approachability of electrolyte ions to the electroactive sites. Their morphology often imparts attractive mechanical tenacity for designing flexible devices. Moreover, these systems can act as precursors of various derived carbons with tunable porosity and surface functionalities. They are also the sources of other elements like nitrogen, phosphorous, and sulfur with trace element amounts that may assist in uniform doping of these derived carbons that may promote adequate density of electrochemical activity sites, which is otherwise difficult to achieve in synthetic carbon analogues. All these unique features have made microbes highly important and indispensable materials in energy storage applications as highlighted in Fig. 2.5 (Ghosh et al. 2012; Pomerantseva et al. 2012).

2.2 Different Microbes Commonly Used in EES

In the recent years, varieties of bacteria, viruses, and fungi have been employed as precursor for the synthesis of smart electrode materials for high energy storage applications (Table 2.1). This may be in the form of bio-templates or as supporting matrix to hold various electroactive materials or as precursors of various neat as well as heteroatom doped to form a variety of carbon-based composites. Some essential information about a number of popular microbes such as bacteria, fungi, and viruses commonly employed in the preparation of electrodes for flexible supercapacitors have been outlined in Table 2.2, and corresponding images of those microorganisms have been shown in Fig. 2.6 (Micrococcus n.d.; Deinococcus_radiodurans n.d.; Bacillus subtilis n.d.; Geobacter sulfurreducens n.d.; Neurospora crassa n.d.; Cladosporium cladosporioides n.d.; Tobacco mosaic virus n.d.; M13 bacteriophage n.d.; Lee et al. 2014; File:Pseudomonas aeruginosa 01.jpg n.d.; Rhizobia n.d.; Sarcina (bacterium) n.d.; Achromobacter n.d.; Enterobacter n.d.; Escherichia coli n.d.; Dickeya dadantii n.d.; Agaricus n.d.).

2.2.1 Bacteria

Bacteria are popularly regarded as the largest variety of living microorganisms that participates actively in material cycling in nature. They display wide range of morphologies, besides being economic, scalable with biomineralization ability and

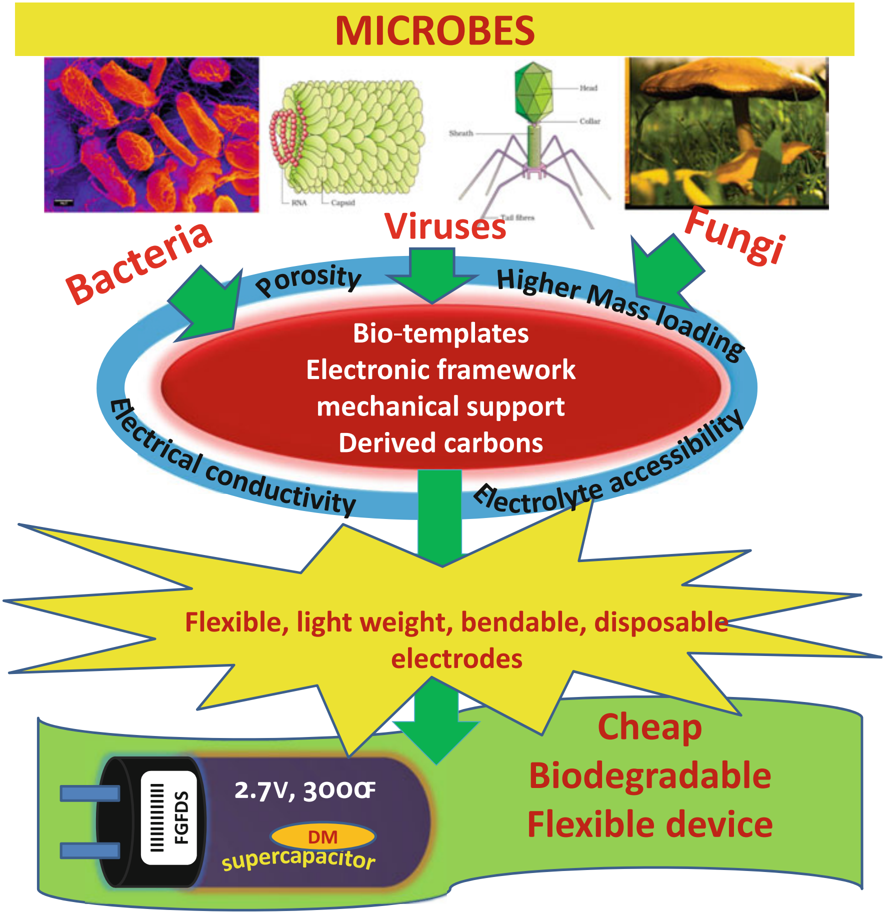

Fig. 2.5 Role of microbes in fabrication of energy storage devices

outstanding physicochemical properties. They also exhibit unique electrochemical activity that makes them high-potential candidates in energy-related disciplines (Wilkinson 1963). They can be employed as proficient precursor materials and dopant resources to prepare uniform, in situ singly, as well as multi-element-doped pyrolytically derived carbon nanomaterials after anaerobic thermal decomposition of the proteins, phosphor-lipids, and metal salts in their structure (Shen et al. 2019; Tshikantwa et al. 2018). Remarkable features of such derived carbons along with their nanocomposites have been discussed in subsequent sections. Even one of their synthesized biodegradable, natural cellulose called bacterial cellulose has been effectively employed as well-accepted and efficient flexible electrode material components in high energy supercapacitors for sustaining uninterrupted power in smart wearable electronics (Esa et al. 2014).

Table 2.2 Functional roles of some important microbes in energy storage applications

Microbes as bio-templates			
Bacteria	*Bacteria*	*Bacteria*	*Bacteria*
Micrococcus mucilaginosis are aerobic gram-positive bacteria, having normal habitat in the skin, dust, and water. It grows in various forms such as tetrads, irregular clusters, cubical packets, as well as colonies. They are strictly aerobic (Micrococcus n.d.).	***Deinococcus radiodurans*** are gram positive, extremophilic bacteria that can survive in extreme environment conditions such as cold, dehydration, vacuum, and acid, etc. It is a popular radiation resistant organism known (Deinococcus_radiodurans n.d.)	***Bacillus subtilis*** (hay bacillus or grass bacillus) are gram-positive bacteria, found in soil and the gastrointestinal tract of animals and humans. They are popular fungicides used for protecting vegetable and soybean plants (Bacillus subtilis n. d.)	***Geobacter sulfurreducens*** rod-shaped microbe with a gram-negative cell wall. Geobacter is known as a type of bacteria that conducts electricity, It is also used to convert U (VI) to U (IV) (Geobacter sulfurreducens n. d.).
Fungi	***Fungi***	***Virus***	***Virus***
Neurospora crassa are a type of red bread molds found mostly in tropical and subtropical regions of the world. It can be found growing on dead plant matter especially after fires (Neurospora crassa n.d.)	***Cladosporium cladosporioides*** are darkly pigmented molds found worldwide both outdoors and indoors locations. Its spores cause seasonal allergic disease to animals and also cause diseases in plants. It can survive even under dry environment and at very low temperatures (Cladosporium cladosporioides n.d.)	***Tobacco mosaic virus*** (TMV) is a single-stranded RNA virus species that infects plants, belonging to Solanaceae family of plants *such as* tobacco. The infection leads to typical "mosaic"-like patterns, along with mottling and leaves discoloration (Tobacco mosaic virus n.d.)	***M13 bacteriophages*** are filamentous bacteriophage that mainly infects *E. coli* host. They remain encapsulated in 2700 copies of the major coat with P8-protein, capped by five sets of different minor coat proteins like P9, P6, and P3. The minor P3 anchors to the receptor at the tip of the bacteria host (M13 bacteriophage n.d.)
Microbes as supporting matrix			
Microbes producing Bacterial cellulose			
Bacteria	***Bacteria***	***Bacteria***	***Bacteria***
Acetobacter xylinum is a common non-pathogenic mesophile recognized by A.J Brown in 1886 due to its ability to produce cellulose	***Pseudomonas*** is a popular genus of gram-negative bacteria, causing certain infections in the body under circumstances but curable by antibiotics. They are cultivated in wastewater and thus may be applied for	***Rhizobia*** are gram-negative, diazotrophic (capable of atmospheric nitrogen fixation) bacteria found inside the root nodules of legumes	***Sarcina ventriculi*** is a gram-positive coccus bacteria belonging to the *Clostridiaceae* family. They are found mostly in the animal skin and large intestine. It

(continued)

Table 2.2 (continued)

Microbes as bio-templates			
Bacteria	*Bacteria*	*Bacteria*	*Bacteria*
pellicles. In nature, it is exists in soil and on decaying fruits. It is used in food packaging for keeping food fresh, making papers harder than woods, etc. (Lee et al. 2014)	bio-remediation, in the production of polymers, low molecular weight compounds, etc. It mainly yields indistinct bacterial cellulose (File: Pseudomonas aeruginosa 01.jpg n.d.)	(Fabaceae). However, they need to involve a plant host to express genes for nitrogen fixation. They look like non-sporulating rods. They mostly generate fibrous bacterial cellulose (Rhizobia n.d.)	yields amorphous type of bacterial cellulose. The cellulose thus produced remains intimately associated with the bacterial cell wall and functions in tight binding of the cells into large packets (Sarcina (bacterium) n.d.)
Bacteria	***Bacteria***	***Bacteria***	***Bacteria***
Achromobacter belongs to gram-negative bacteria with straight rods and can move by using their peritrichous flagella. They are strictly aerobic and are found in water and soils. They lead to ribbon-like bacterial cellulose (Achromobacter n.d.)	***Enterobacter*** are gram-negative bacteria that are facultatively anaerobic, being able to produce ATP by aerobic respiration in presence of oxygen, but can switch over to fermentation in scarcity of air. They are rod-shaped, non-porous bacteria. They yield fibrillar bacterial cellulose (Enterobacter n.d.)	***Escherichia coli*** are gram-negative, facultative anaerobic, rod-shaped, coliform bacterium found in the intestine of warm-blooded organisms. Most *E. coli* are harmless in nature. It produces bacterial cellulose fibrils (Escherichia coli n.d.)	***Dickeya dadantii*** are plant pathogens that produce cellulose-containing biofilms, called pellicles, at the air-liquid interface of liquid cultures. They are also facultative anaerobes that can ferment sugar molecules to lactic acid. They mainly produce bacterial cellulose pellicles (Dickeya dadantii n.d.)
Microbes for derived carbons			
Bacterial cellulose materials as source for derived carbons			
Fungi	***Fungi***	***Fungi***	***Fungi***
Agaricus have different mushroom varieties that are both edible and poisonous species. They are used in treatment of used for cancers, diabetes mellitus, cholesterol, arteriosclerosis,	***Saccharomyces cerevisiae*** are yeasts used commonly in making of wines, baking foods, and brewing beers since long past. It is believed to have been originally isolated from the skin of grapes (Saccharomyces cerevisiae n.d.)	***Ganoderma lucidum*** are polypore fungi that have red-varnished appearance, generally kidney-shaped, capped with fan-like appearance. They are used in control of blood glucose levels, modulation	***Calocybe indica*** commonly known as the milky white mushroom is a species of edible mushroom found widely in India. They mostly appear in summer after rainfall in crop fields and on road

(continued)

Table 2.2 (continued)

Microbes as bio-templates			
Bacteria	*Bacteria*	*Bacteria*	*Bacteria*
liver disease, ulcers, etc. (Agaricus n.d.)		of immune system (Lingzhi (mushroom) n.d.)	verges (Subbiah and Balan 2015)
Fungi	***Fungi***	***Fungi***	***Fungi***
Tremella fuciformis are white, frond-type gelatinous fungi widely found in tropical regions and grow on the dead branches of broad-leafed trees. They are edible and used as medicines as well (Tremella fuciformis n.d.)	***Phallus indusiatus*** (**bamboo fungus**) are long, net-like fungus growing on well-rotted woody materials and found mostly in tropical areas of Southern Asia, Africa, America, and Australia. They are used in the treatment of neural diseases (Phallus indusiatus n.d.)	***Xylaria*** are commonly found growing on dead wood. ***Xylaria polymorpha***, named as dead man's fingers, often grows as clusters just below ground level. They are mostly used in the spalting of sugar maples (Xylaria n.d.)	***Auricularia*** are jelly-like fungi and mostly are edible. *Auricularia* species are widely distributed in Kerala's Western Ghats, India, mostly used in the treatment of cardiovascular problems (File: Hirneola_auricula-judae_(xndr).jpg n.d.)

What so Special About Bacterial Cellulose?

Bacterial cellulose is BC is a special variety of nontoxic, cellulose materials composed of polysaccharides having general chemical formula which is $(C_6H_{10}O_5)_n$, containing β-1,4-glycosidic linkages, as indicated in Fig. 2.7a, b (Ma et al. 2016a). It is mostly produced in large scale economically by glucose or hexose analogues and fermentation process via several microbes such as *Acetobacter*, *Rhizobium*, *Pseudomonas*, *E. coli*, etc. as highlighted in Table 2.2. The synthesis of bacterial cellulose involves multistep procedures by means of formation of "uridine diphosphoglucose" from catalytic phosphorylation of hexoses or similar carbon sources followed by isomerization and polymerization steps to form long and un-branched β-1→4 glucan chains by cellulose synthase (Ma et al. 2020). Since the last two decades, BC-based technology has become a fast-developing sector with their extensive usage in biomedical applications, including bio-sensing, biomedical, and tissue-engineering fields, high-quality paper-making industries in addition to the domains of acoustics, optoelectronic usages, food industry, and so on (Lin et al. 2013; Picheth et al. 2017; Stumpf et al. 2018). BC possesses unique properties that are highly urged for manufacturing smart materials for energy applications. These include:

- Simple and cost-effective synthesis as resourced from bio-renewable materials (Luo et al. 2014, 2017).
- Specific ultrafine interconnected networks of bacterial cellulose nanofibers with adequate pore density with high water retention capability (Yano et al. 2005; Li et al. 2014a).

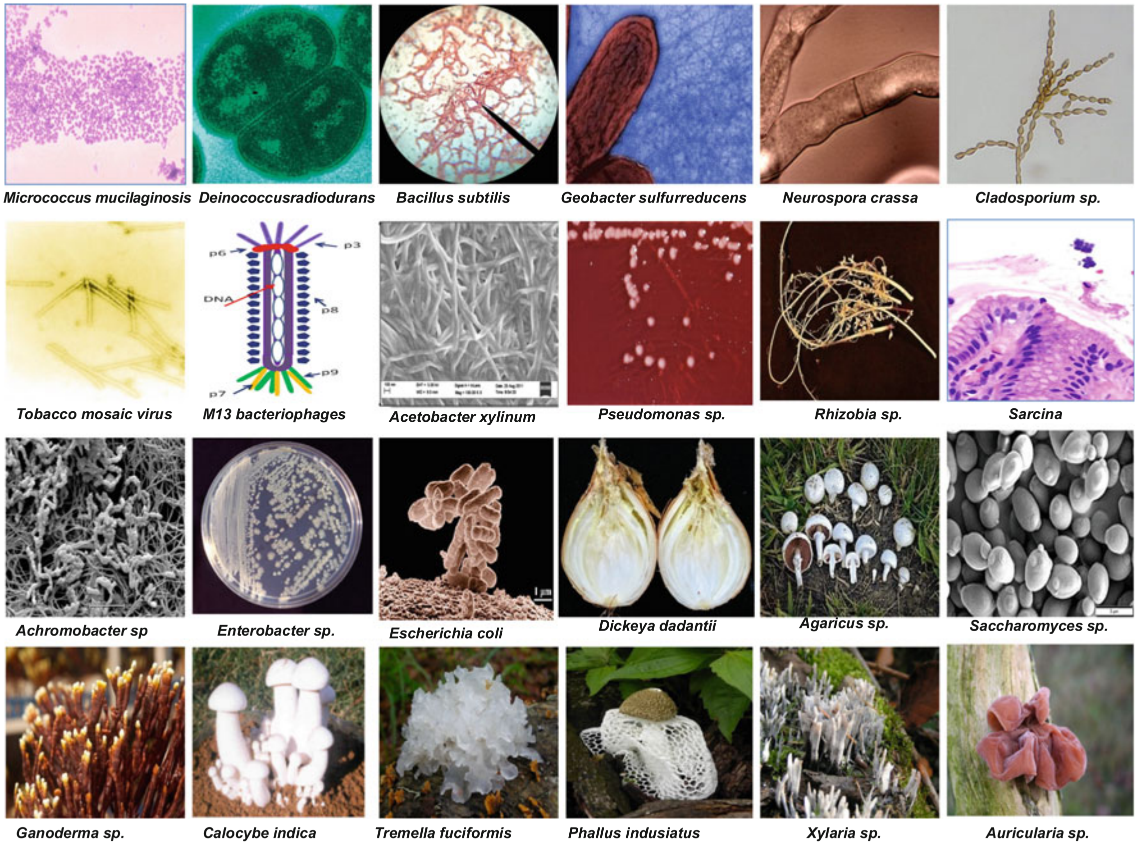

Fig. 2.6 Various microbes used in energy storage applications (Micrococcus n.d.; Deinococcus_radiodurans n.d.; Bacillus subtilis n.d.; Geobacter sulfurreducens n.d.; Neurospora crassa n.d.; Cladosporium cladosporioides n.d.; Tobacco mosaic virus n.d.; M13 bacteriophage n.d.; Lee et al. 2014; File:

- Good biodegradability with no toxic products (Wang et al. 2016a).
- Flexible, with substantial elastic stretching and bending features along with high tensile strength (Young's modulus ~138 GPa and tensile strength >2 GPa) in addition to biocompatibility, renewability, and hydrophilicity (Klemm et al. 2011).
- BC fibers being smaller and have distinctive structure than conventional plant cellulose fibers as indicated in Fig. 2.7c, d, the former being more capable of forming smooth and versatile paper-type electrodes for flexible energy storage devices (Ma et al. 2016a).
- Further, dry BC aerogels have self-assembled interlinked nanofibrillar morphology that can be subjected to pyrolysis to achieve 3D carbon-based aerogels that find useful applications in energy storage devices, artificial body parts, sensors, etc. because of light weight, porous nature, enhanced surface-active area, and boosted electrical conductivity along with improved structural flexibility (Ma et al. 2020).
- BC pellicles (films/membranes) can be employed as starting materials for fabricating stretchable conducting systems with amazing electromechanical stability, even on exposure to high stretching and bending conditions (Liang et al. 2012).
- BC gel electrolytes having adequate porosity and hygroscopic and hydrophilic nature can significantly improve electrolyte-ion mobility in high-performance supercapacitors (Zhao et al. 2019).
- High light transparency properties enable their usage in optically visible displays (Ummartyotin et al. 2012).
- Very low coefficient of thermal expansion along the axis, with tunable characteristics, making it eligible for transparent electronic device designs (Yano et al. 2005).

2.2.2 Viruses

Viruses such as M13 bacteriophages and *Tobacco mosaic virus* (TMV) differ from bacteria and fungi class largely because of their nanoscale dimensions, single DNA or RNA, as well as parasitic behavior (Dong et al. 2013; Gerasopoulos et al. 2012). DNA or RNA genetic modification of such viruses opens up several functional groups (e.g., –COOH, –OH, NH_2, –SH residues) that can effectively anchor various ions as well as nanomaterials (Pires et al. 2016; Heinemann and Walker 2019; Cleaves et al. 2019). Further, they can undergo continuous interlocking web

Fig. 2.6 (continued) Pseudomonas aeruginosa 01.jpg n.d.; Rhizobia n.d.; Sarcina (bacterium) n.d.; Achromobacter n.d.; Enterobacter n.d.; Escherichia coli n.d.; Dickeya dadantii n.d.; Agaricus n.d.; Saccharomyces cerevisiae n.d.; Lingzhi (mushroom) n.d.; Subbiah and Balan 2015; Tremella fuciformis n.d.; Phallus indusiatus n.d.; Xylaria n.d.; File:Hirneola_auricula-judae_(xndr).jpg n.d.)

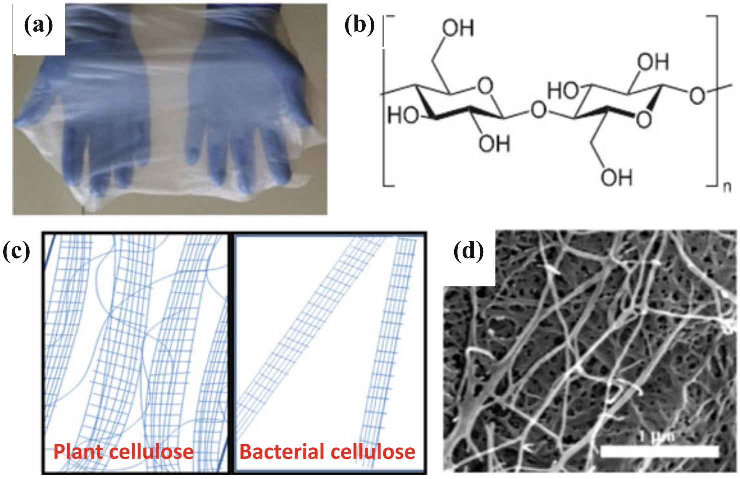

Fig. 2.7 (**a**) Snap-shot of BC slice held in hand. (**b**) Chemical structure of bacterial cellulose (BC). (**c**) Schematic comparative presentation of plant cellulose fibrils (left) with BC microfibers (right). (**d**) SEM image of the BC fibers (Ma et al. 2016a)

structure or form smooth uniform films by assembling vertically on substrates that serve as effective binder-free building units. Such successful attempts have already been reported for various electronic devices such as micro-batteries, light harvesting systems, etc. (Chaturvedi and Shrivastava 2005; Miller et al. 2007; Tarascon 2009). M13 viruses are filamentous, single circular DNA-containing microorganisms. They only contaminate *E. coli* bacteria with F+ (F-plasmid) and replicate therein (Lee et al. 2009). TMVs, on the other hand, are cylindrical, rod-type, RNA viruses that predominantly infect specific plants, like tobacco and other nightshade herbs/shrubs, and are therefore considered as pathogens of tobacco mosaic disease (Fan et al. 2013). TMV undergo reproduction at temperatures >60 °C and in pH range of 2–10. However, they are generally nontoxic and, so far, caused no harm for humans and thus can be effective in producing beneficial materials for various technological applications (Ren et al. 2010).

2.2.3 Fungi

Fungi are heterotrophic microorganisms and cannot produce own food by carbon fixation or similar process. They show vast possibilities in energy storage domains in the past few years owing to their high reproductive efficacy, scalability, and varieties (Bhattacharya and Raha 2002; Wang et al. 2014b). Chitin, the chief constituent of the fungi cell wall, is higher polysaccharide macromolecule, containing acetamide functionalities unlike that of plant cellulose with hydroxyl groups. Some of them are toxic as well. Fungi of different types, such as molds, yeasts, mushrooms, etc., have been employed in energy fields (Chang et al. 2010; Krishnan et al. 2009; Li et al.

2019; Campbell et al. 2016). Molds are hyphae-type fungi with branched, filamentous structure, while yeasts possess various shapes such as spherical, oval, ellipsoid, and rod types that can survive in both aerobic and anaerobic environments (Ni et al. 2010). Mushrooms, on the other hand, display various shapes, and often their high porous structure can be useful for energy storage and transfer. Recent research reveals that mushrooms with large K^+ ion concentration can activate and enhance battery storage capacity considerably (Campbell et al. 2015).

In the following section, the author highlights on the different naturally occurring microbes that have been employed in devising electrode materials in energy storage applications. Such microbes essentially fulfill three essential prospects of material preparation, namely, bio-templates, supporting matrix, and source/precursor for derived porous carbons with superior physicochemical properties.

2.3 Microbes as Bio-templates for Energy Storage Materials

Template-driven strategy has been among the most extensively employed approach for synthesizing wide range of inorganic nanomaterials (Liu et al. 2013). Therefore, in the recent time, there has been an enormous impulse for developing useful, productive, and green methodologies in nanomaterial production using biological systems. Bio-templates provide nanoscale control of synthesis of nanoscale materials similar to that of the existing in natural systems (Singh and Chakarvarti 2016). They also serve as stabilizers and promote uniform dispersion of structures. They provide mild synthetic conditions for synthesis of materials with formation of microscopic to macroscopic hierarchical structures with nanoscale building units and hence open up greater possibilities for effective control on morphology. Large replication rate, self-assembly as well as possess adequate surface charge that behave as nucleation sites for resting particles to form various morphology-based systems, through aid of medium pH and ionic strength maintenance (Stephanopoulos et al. 2013).

2.3.1 Bacteria as Bio-templates

Self-assembled bacterial nanostructures can serve as effective templates using their surface proteins (S-layer proteins) for natural mineralization leading to the production of high-quality nanomaterials as illustrated schematically in Fig. 2.8a (Shim et al. 2013). Several instances have come up that shows that such templates promote porosity and better loading of electroactive inorganic materials thus promoting better capacitive features. For example, Shim et al. fabricated three-dimensional hierarchical porous flower-like Co_3O_4 systems by means of *Micrococcus lylae* protein serving as bio-template as shown in Fig. 2.8b. The sample electrode showed good capacitive behavior as observed from Fig. 2.8c with good rate capacity and also recorded improved pseudocapacitance of 214 F g^{-1} (2.04 F cm^{-2}) @ 2 Ag^{-1} (19.02 mA cm^{-2}) superior to many reports available in the scientific database,

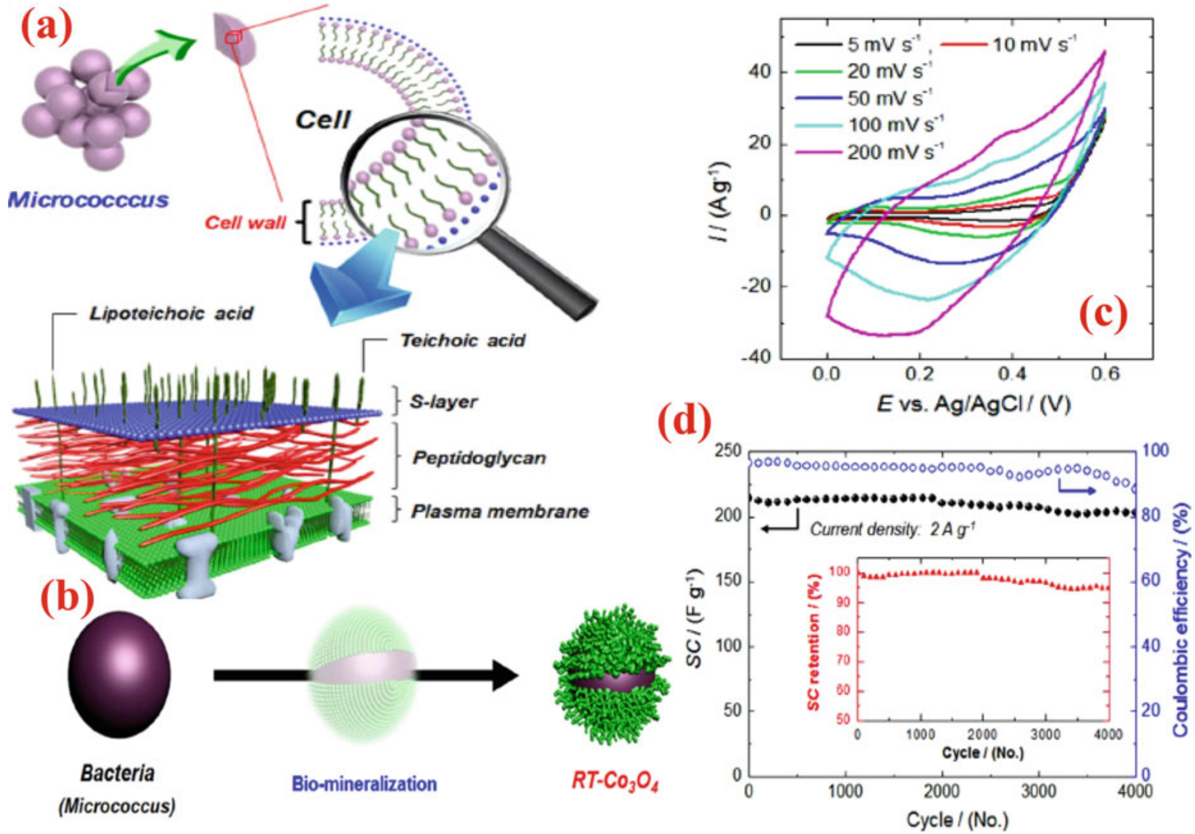

Fig. 2.8 (**a**) Schematically shown detailed description of the *Micrococcus* cell envelope containing thick peptide-glycan, S-layer, and teichoic acid glycopolymers that bind the metal ions. (**b**) Schematic presentation of the process of single-step synthesis of 3D hierarchical Co_3O_4 nanostructures via

mostly owing to high specific mass loading (≈10 mg cm^{-2}). Further, it displayed >95% coulombic efficiency and electrochemical stability as described in Fig. 2.8d (95% specific capacitance retention over 4000 GCD cycles, attributed to the capability of the scaffolds to successfully withstand structural fluctuations during fast and large GCD cycles (Shim et al. 2013).

Another report was based on the fabrication of mesoporous NiO micro-ellipsoids obtained in aid of elliptical-shaped *Deinococcus radiodurans* bacteria as bio-templates, under ordinary reaction conditions, that recorded noticeable gravimetric capacitance of 237 F g^{-1} @ 0.8 A g^{-1} specific current in 6 M aqueous KOH electrolyte (Atalay et al. 2015). Bacteria, of the type *Bacillus subtilis* that are devoid of such S-layer proteins, use peptidoglycans and teichoic acid moieties as metal binding sites on their surfaces (Allred et al. 2005). Lately, rod-type cobalt oxide were obtained using *Bacillus subtilis* as soft templates under ambient conditions. Furthermore, porous Co_3O_4 hollow rods were produced on annealing at 300 °C that showed exceptional Li storage capability (Shim et al. 2011) .

Bacteria may as well be utilized as bio-templates for fabricating highly porous, large surface-based carbon materials for supercapacitors. Hierarchical porous carbons were produced via freeze-casting technique by assembling graphene oxide on the surface of *Escherichia coli* (Sun et al. 2012). The so-obtained sample possessed large surface area and porosity and accordingly delivered improved gravimetric capacitance of 327 F g^{-1} @ 1 A g^{-1} current density with adequate surface functionalities that promoted sufficient pseudocapacitive contributions besides electrical double layer capacitance. The material also displayed better electrochemical response in aqueous electrolyte in comparison to other popular carbon systems (Zhu et al. 2011). The microorganism *Geobacter sulfurreducens* containing high number of c-type cytochromes genes coding mainly survives by reducing metals. These c-type cytochromes can serve as effective electron reservoirs and, hence, perform as capacitors. They exhibit an elemental idea for the development of novel methods of energy generation in nature. For instance, it has been suggested that the capacitor behavior plays an important role for bioremediation of uranium (Malvankar et al. 2012).

2.3.2 Fungi as Bio-templates

Fungi like bacteria also demonstrate another effective source of bio-templates owing to their extreme metal bio-accumulation efficiency (Selvakumar et al. 2014). Fungi

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Fig. 2.8 (continued) assembling of cobalt oxides (green) by the bio-sorption of Co^{2+} directly onto bacterial surface at room temperature followed by subsequent redox reactions. (**c**) Cyclic voltammograms for the above material recorded at various voltage sweep rates in 3 M KOH at room temperature. (**d**) Cycling performance of the sample electrode in terms of specific capacitance (SC) and Coulombic efficiency while the inset shows variation of SC retention capacity after 4000 consecutive GCD cycling tests (Shim et al. 2013) display a variety of biomineralization effects as well as their filamentous mycelium offering mechanical substructure for efficient mineral loading. For example, the mold *Neurospora crassa* has been often employed as an effective bio-template for the production of mineral-based composites with carbonized fungal biomass, possessing large charge storing capacity (Zhang et al. 2017). Fungal Mn biomineralization with high gravimetric capacitance >350 F g^{-1} and good cycling stability has been reported (Li et al. 2016). Further, porous, nickel oxide nano-tubular structures were obtained using cylindrical-shaped *Cladosporium cladosporioides* fungi as bio-templates via chemical precipitation technique. The material delivered high capacitance value of 334 F g^{-1} @ 0.8 A g^{-1} current density, with 95% capacitance retaining efficacy even after 1000 GCD cycles tests (Atalay et al. 2016). In a recent study, La-based nanostructured materials were obtained via chemical precipitation technique using *Cladosporium cladosporioides* hyphae as bio-template which was then annealed at high temperatures. The resultant porous material displayed good capacitive response recording and very large gravimetric capacitance of 2190 F g^{-1} @ 2 mV s^{-1} voltage sweep rates, in 0.5 M Na_2SO_4 aqueous medium (Atalay et al. 2017).

2.3.3 Viruses as Bio-templates

Viruses comprise two chief constituents—core with genetic information and protective shell made of mostly protein moieties (Vilona et al. 2015). Their protein shell often assists biomineralization/bio-metallization processes as the amino acids show large metal ions affinity (Fischlechner and Donath 2007). Thus, tobacco mosaic virus (TMV), M13 bacteriophage, cowpea mosaic virus, etc. because of their non-pathogenic behavior toward humans and other living systems have been widely employed for the purpose (Selvakumar et al. 2014; Douglas and Young 2006). Three-dimensional hierarchical Ni/NiO electrodes were obtained via bio-template mediated electro-less synthesis of Ni-coated TMVs as nano-3D-current collectors, self-assembled on gold-coated Si-micro-pillar arrays as shown in Fig. 2.9a. The large aspect ratio-based morphology confers enhanced surface area, depending on density and height of the columnar arrays. Such unique geometry enabled better mass loading of the electroactive materials as a result of which the rationally designed electrode reported about 32.6 times increase in areal charge capacity in contrast to pure-planar electrode, as depicted in Fig. 2.9b, thus indicating crucial role of surface area and mass loading capacity in promoting electrochemical efficiency (Chu et al. 2016).

Tobacco mosaic virus (TMV) macromolecules show facile behavior of forming intense bio-nano-scaffolds layers within very short time period (Vilona et al. 2015; Lomonossoff and Wege 2018). Thus, with the aid of electro-less plating and thermal oxidation processes, the obtained large surface-based nano-NiO electrodes demonstrated 3.6-fold rise in areal capacitance in comparison to simple NiO planar structures. Hence, easy photolithography and self-assembly techniques stand fine to absolutely reduce the necessity of high costing sophisticated deposition procedures

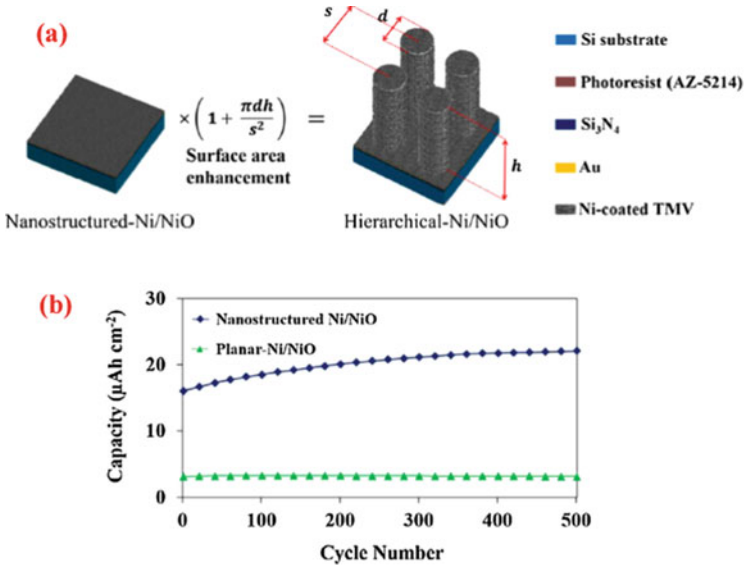

Fig. 2.9 (**a**) Schematic indication of enhanced surface area for the Si-micropillar array structure over planar one. (**b**) Relative variation of areal discharge capacity for the initial 500 cycles for both the nanostructured—Ni/NiO and planar—Ni/NiO electrodes, respectively (Chu et al. 2016)

(Zang et al. 2017). Atomic layer deposition of polycrystalline RuO_2 was carried out on TiN/Ni/TMV hetero-structures using genetically modified TMV as bio-template (Gnerlich et al. 2013). The so-obtained composite in combination with nafion as solid proton-conducting electrolyte recorded appreciable charge storage capacity with high capacitance retaining efficacy of 80% yet after undergoing continuous 25,000 GCD cyclic tests (Gnerlich et al. 2015). Genetically engineered M13 bacteriophages have become one of the useful toolkits in fabricating several nano-hybrid materials for EES devices. For example, non-covalently bonded engineered M13 virus and graphene were found to enhance the dispersion character of the graphene at low pH and high ionic strength medium (Oh et al. 2012). Additionally, inorganic materials anchored on such modified graphene sheets increased the overall conductivity and stability of the material considerably. Thus, M13 virus stabilized-graphene with bismuth oxy-fluoride nanocomposites demonstrated superior specific capacity of 131 mA h g^{-1} @ high current density of 300 mA g^{-1} (Oh et al. 2012).

2.4 Microbe-Based Carbon Materials as Supporting Matrix

The key issue for devising high-performing supercapacitors is to design flexible electrodes that guarantee outstanding electrochemical features along with mechanical/environmental stability (Wang et al. 2009; Kim et al. 2015; Chen et al. 2013a; Gwon et al. 2011; Maiti et al. 2014). The principal objective lies in optimizing the various determining factors such as reducing nanomaterial agglomeration, minimizing interfacial resistance, promoting faster electron transport, as well as augmenting charge diffusion kinetics along empty, porous channels which may boost the capacitance value closer to the theoretically estimated, besides imparting higher flexibility, robustness, and environmental stability keeping in view of practical usage in portable and wearable electronics, space, defense, as well as biomedical applications (Hyun et al. 2013; Tolle et al. 2012). In the recent past, several carbon nanocomposite materials based on functionalized graphenes and carbon nanotube (CNT) composites film have illustrated remarkable mechanical flexibility for designing robust energy storage devices that are well documented in the literature (Du et al. 2014; Kim et al. 2013). Despite the striking electronic conductivity features in these nanocarbons, their practical usage is limited by high fabrication cost, poor mass loading efficiency, and irretrievable agglomeration issues that results in inferior areal capacitances as well as shortened cycling life span (Long et al. 2014). Additionally, poor scalability and rigorous post-synthetic processes of such nanocarbon systems have largely restricted their commercialization. Thus, scientists have been endlessly devoting their efforts to find out alternative porous, conducting, and flexible carbon materials that would fulfill the criteria of abundant resources, scalability, easy fabrication, reproducibility, and environmentally compatible characteristics. Currently, microbe-based carbon materials have successfully satisfied the above criteria and accordingly have captivated many academicians in this domain of research (Zhou et al. 2012).

Thus, in this context, bacterial cellulose (BC) systems have gained considerable popularity as they offer excellent scaffolds for tailoring hybrid nanomaterials. The inherent surface hydrophilic functional groups such as –OH and –COOH facilitate hydrophilicity and high mass loading along with strong integration with electroactive materials like conductive polymers, metal oxides, and other semiconductors which strengthens their anchoring to the substrate (Kaewnopparat et al. 2008; Li et al. 2014b; Gao et al. 2013; Tang et al. 2015; Jana et al. 2017). Moreover, 2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPO) radical-mediated oxidation of bacterial cellulose fibers has attracted great attention as the obtained products display superior aspect ratio and higher elastic modulus (Isogai et al. 2018). Further, hierarchical morphology facilitates easy accessibility of electrolyte ions to the reactive sites, thereby effectively speeding up diffusion-controlled processes. Compared with other substrates such as graphene paper or CNT films, BC paper provides additional yet sufficient void space and hence enables high electrolyte mass transfer (Deng et al. 2019b). However, BC membrane often possesses relatively low intrinsic electrical conductivity owing to absence of suitable charge carriers in their structure (Hu et al. 2011). To deal with the low conducting and capacitive behavior of BC, they are intimately blended with other conductive carbons such as doped activated carbons, CNTs, graphene nanosheets, as well as pseudocapacitive materials conducting polymers, metal oxides, etc. that considerably accelerate the charge transport in the composites and improve the electrochemical utilization of porous carbon materials (Dutta et al. 2017).

Rigorous investigations have frequently highlighted that conducting polymers though potentially display high pseudocapacitive performance but limited by poor electrochemical stability and structural instability, especially during high current rates and fast voltage scans which can be considerably improved by blending with bacterial cellulose (Meng et al. 2017). Studies reveal that one of the main reasons for such popularization of BC/conducting polymer nanocomposites has been its simplistic synthetic steps as schematically outlined in Fig. 2.10a, which also provides sufficient regulating reaction parameters to tailor diverse morphologies for improved capacitive performances as well as mechanical versatility.

Such binary nanocomposites have been successfully employed in fabricating binder-free, additive-free, current collector-free, flexible paper like supercapacitor electrodes for energy support to various wearable electronic devices (Luo et al. 2019). Further systematic explorations related to charge transport enhancement supported the fact that core-shell morphology have significant impacts on conductivity improvement in these composites. Accordingly, BC/PPY core-shell nanocomposites were fabricated by in situ oxidative polymerization of self-assembled pyrrole on BC nanofibers surface in dimethylformamide-water medium. The optimum composition of the nanocomposite exhibited appreciable improvement in electrical conductivity and boosted specific capacitance of 316 F g^{-1} recorded @ 0.2 A g^{-1}current density, the value being much superior to that of plant cellulose-nanocrystal/PPy porous composites. Moreover, the electrochemical stability of the system showed only 10.8% of initial capacitance loss even after completing 1000 GCD cycles. It was concluded that the unique structure promoted better porosity features as well as favored larger active mass loading that resulted in such enhanced electrochemical performance in this nanocomposite (Wang et al. 2013).

Similar strategies were applied to design flexible electrode materials composites with widely explored 2D-graphene systems (Fang et al. 2016). Flexible supercapacitors were designed using combinations of holey reduced graphene oxide (rGO) and BC films by biosynthesis process that produced interesting results. A compact, regular, and aligned honeycomb inter-linked framework of the composite was generated on bacterial culture of the scattered functionalized graphene sheets attached to the BC nanofibers as shown in Fig. 2.10b (Guan et al. 2018). Different HGO concentrations such as 0.8, 1.0, and 2.0 mg mL^{-1} were mixed with constant BC proportions to prepare the desired nanocomposite named as 0.8-HGO/BC, 1.0-HGO/BC, and 2.0-HGO/BC, respectively. The optimum composition 1.0-HGO/BC sample showed amazing tensile strength under various strained conditions as indicated in Fig. 2.10c, making the nanocomposite highly suitable for powering foldable electronics (Guan et al. 2018). Few more of such interesting results for various BC-based nanocomposites have been demonstrated in Table 2.3 (Bu et al. 2018; Li et al. 2014a, 2017; Cai et al. 2019; Liu et al. 2015a; Wang et al. 2012, 2016b; Xu et al. 2013, 2016).

Further, to pick up the electrochemical efficiency of the BC-based binary nanocomposites, corresponding ternary and quaternary nanocomposites comprising hybrids of bacterial cellulose, conducting polymer, various nanocarbons, and pseudocapacitive metal compounds like oxides, sulfides, etc. with different

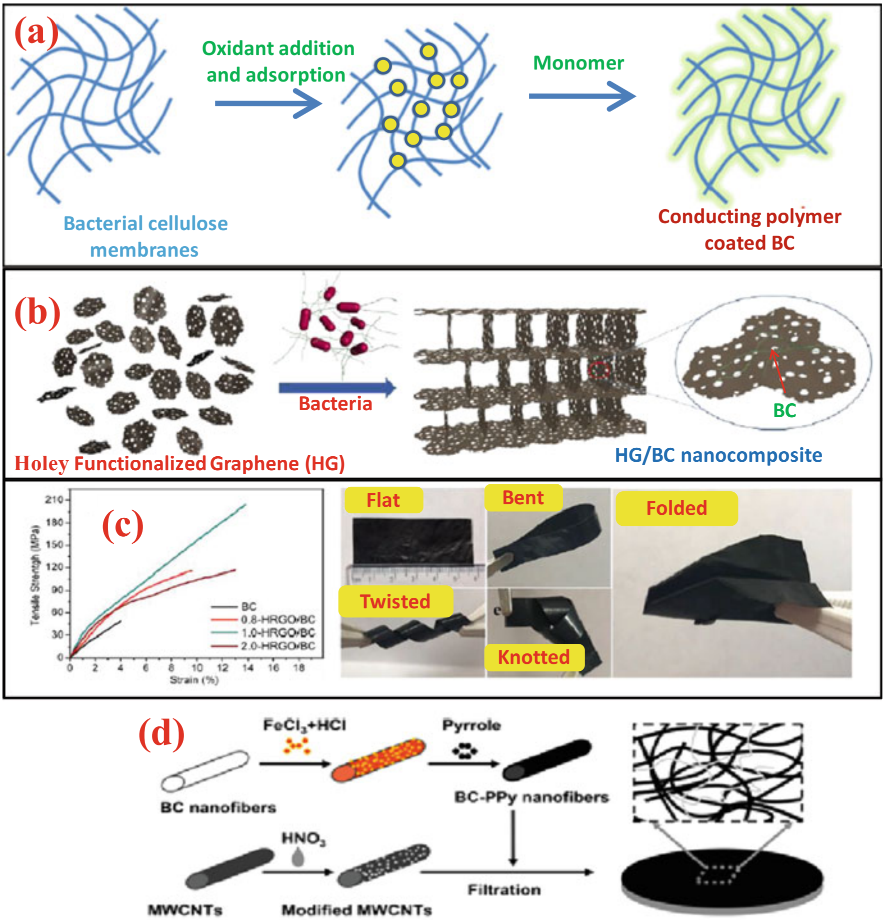

Fig. 2.10 (**a**) Diagrammatic illustration of generalized fabrication strategy of conducting polymer-based BC binary nanocomposites. (**b**) Schematic outline of synthesis of 3D inter-connected holey graphene (HRGO)/BC nanocomposite electrodes. (**c**) Comparative study of tensile strength versus strain for HRGO/BC and pristine-BC air-dried films along with snap shots of 1.0-HRGO/BC composite films at different strained postures. (Reproduced on permission from Guan et al. 2018). (**d**) Schematically illustrated procedure of fabrication of bacterial cellulose/polypyrrole nanofiber/multi-walled carbon nanotubes ternary composite membrane (Li et al. 2014a)

morphologies were fabricated successfully, some of which have been illustrated in Table 2.3 as well for comparison (Jiao et al. 2019; Zhang et al. 2018; Li et al. 2017; Yao et al. 2018; Liu et al. 2015b, 2016b, c, 2017; Ma et al. 2016b, c; Peng et al. 2016, 2017; Wu et al. 2018; Yuan et al. 2018; Jiajia et al. 2020). Figure 2.10d represents schematically the common strategy for synthesizing ternary composites with BC, conducting polymer, and CNTs, which is rather simple, productive, as well as reproducible with minimum post synthetic hurdles (Li et al. 2014a). The same

Table 2.3 Various BC-based nanocomposites for energy storage applications

Composition of the composite	Methodology of synthesis of electrode materials	Electrolyte used	Operating voltage window	Specific capacitance @ current density/voltage scan rate	Charging/discharging cyclic stability	Reference
Binary nanocomposites						
Ultrathin bacterial cellulose/poly(ethylene-dioxy-thiophene) nanofibers paper electrodes	Chemical polymerization	PVA-H_2SO_4 gel electrolyte	−0.2 to +0.8 V	106.3 F cm^{-3} @ 0.83 A cm^{-3}	~100% after 3000 cycles	Bu et al. (2018)
Bacterial cellulose/ polypyrrole membranes	Chemical polymerization	Aqueous NaCl	−0.9 to +0.9 V	191.94 F g^{-1} @ 5 mV s^{-1}	–	Li et al. (2017)
Bacterial cellulose @ Ni $(OH)_2$ paper	Biosynthesis and chemical precipitation technique	6.0 M KOH aqueous solution	0–0.6 V	2047 mF cm^{-2} @ 5 mA cm^{-2}	94% after 5000 cycles	Cai et al. (2019)
Free-standing bacterial cellulose-polypyrrole nanofibers paper electrodes	Mixing and vacuum filtration	2.0 M LiCl aqueous solution	−0.2 to 0.6 V	2.43 F cm^{-2}	94.5% after 5000 cycles	Li et al. 2014a
Graphene oxide with bacterial cellulose fibers	One-step esterification	1 M H_2SO_4 aqueous solution	−0.2 to 0.8 V	160 F g^{-1} @ 0.4 A g^{-1}	90.3% over 2000 recycles	Liu et al. (2015a)
Bacterial cellulose nanofiber-supported polyaniline nanocomposites	In situ polymerization of aniline onto BC nanofibers scaffolds	1 M H_2SO_4 solution	+0.2 to +0.8 V	273 F g^{-1} @ 0.2 A g^{-1}	94.3% after 1000 cycles	Wang et al. (2012)
Bendable and flexible supercapacitor based on polypyrrole-coated bacterial cellulose core-shell composite network	In situ oxidative polymerization	1 M $EMIMBF_4$ solution	−0.5 V to +0.5 V	153 F g^{-1} @ 0.2 Ag^{-1}	~93% after 100 cycles	Wang et al. (2016b)

Conductive polypyrrole-bacterial cellulose nanocomposite	In situ oxidative polymerization	2.0 M NaCl solution	−0.9 to +0.9 V	101.9 mA h g^{-1} (corresponding to 459.5 F g^{-1}) @ 0.16 A g^{-1}	70.3% after 50 cycles	Xu et al. (2013)
Hierarchically structured cotton yarns coated by bacterial cellulose nanofibers	Surfactant assisted in situ polymerization	PVA/ H_2SO_4 gel electrolyte	−0.9 to +0.9 V	76.6 mF cm^{-2} @ 0.42 mA cm^{-2}	Same ~100% for 250 cycles	Xu et al. (2016)
MXenes/bacterial cellulose composite paper	Laser-cutting kirigami patterning process	PVA/ H_2SO_4 gel electrolyte	0–0.6 V	111.5 F cm^{-2} @ 2.0 mA cm^{-2}	72.2% after 5000 cycles under repeated tensile deformation: 0–100% elongation	Jiao et al. (2019)
BC supported ultrathin K-birnessite MnO_2 nanosheets	Hydrothermal method	1.0 M Na_2SO_4 electrolyte	0–0.8 V	328.2 F g^{-1} @ 0.2 A g^{-1}	91.6% after 2000 cycles	Zhang et al. (2018)
Ternary nanocomposites						
N-doped activated shaddock peel carbon/ graphene/bacterial cellulose hybrid paper electrode	Vacuum filtration and film coating techniques	Na_2SO_4 electrolyte aqueous solution	1.4 V	250.5 F g^{-1} (areal capacitance of 2004 mF cm^{-2}) @ 2 mA cm^{-2}	97% after 10,000 cycles	Li et al. (2017)
Hierarchical core-sheath polypyrrole @ carbon nanotube/bacterial cellulose macrofibers	Blending and in situ polymerization	1 M Na_2SO_4 aqueous solution	−0.2 to +0.6 V	258 F g^{-1} (223 F cm^{-3}) @ 0.5 A g^{-1}	10% over 6000 cycles	Yao et al. (2018)
Polypyrrole/bacterial cellulose/graphene composites	Chemical method	1 M H_2SO_4 aqueous solution	−0.2 to +1.2 V	278 F cm^{-3} (Volumetric capacitance)	95.2% over 5000 cycles	Liu et al. (2015b)
				6.15 F cm^{-2} @ 1 mA cm^{-2}		

(continued)

Table 2.3 (continued)

Composition of the composite	Methodology of synthesis of electrode materials	Electrolyte used	Operating voltage window	Specific capacitance @ current density/voltage scan rate	Charging/discharging cyclic stability	Reference
Polyaniline/bacterial cellulose/graphene film	Simple filtering method	1 M H_2SO_4 aqueous solution	−0.1 to +0.7 V		53.6% over 5000 cycles	Liu et al. (2016b)
Cobalt oxide/graphene/bacterial cellulose	Hydrothermal and filtering method	2 M KOH solution	0.1 to +0.6 V	Areal capacitance = 12.25 F cm^{-2} Gravimetric capacitance = 1274.2 F g^{-1}	96.4% after 20,000 cycles	Liu et al. (2016c)
Polyaniline/graphene/bacterial cellulose	Facile chemical polymerization and filtering method	1 M H_2SO_4 aqueous solution	−0.1 to +0.8 V	Areal capacitance = 4.16 F cm^{-2}	91.5% after 2000 cycles	Liu et al. (2017)
Polypyrrole/bacterial cellulose/graphene paper	In situ polymerization and filtering method	1.0 M $NaNO_3$ aqueous solution	−0.4 to +0.6 V	Areal capacitance = 3.66 F cm^{-2} @1 $mAcm^{-2}$	73.5% after 8000 cycles	Ma et al. (2016b)
Nitrogen-doped carbon networks/graphene/bacterial cellulose	Blending + single-step carbonization treatment	Both in KOH and also H_2SO_4 aqueous solution	−0.8 to +0.2 V in aqueous KOH 0–1.5 V in aqueous H_2SO_4	Areal capacitance = 2106 mF cm^{-2} (263 F g^{-1}) in KOH electrolyte 2544 mF cm^{-2} (318 F g^{-1}) in H_2SO_4 electrolyte	~100% retentions after 20,000 cycles for the symmetric supercapacitor in acid medium	Ma et al. (2016c)
Polypyrrole/cobalt sulfide/bacterial cellulose composite membranes	Mixing, in situ oxidative polymerization	2.0 M NaCl aqueous solution	0–0.8 V	614 F g^{-1} @ 0.8 mA cm^{-2} (0.70 Ag^{-1})	62.4% after 300 cycles	Peng et al. (2016)
Polypyrrole/copper sulfide/bacterial cellulose	Deposition + in situ polymerization	2.0 M NaCl aqueous solution	−0.9 to +0.9 V	580 F g^{-1} @ 0.8 mA cm^{-2}	73% after 300 cycles	Peng et al. (2017)

nano-fibrous composite membranes						
Ni-Co layered double hydroxide/polyaniline/bacterial cellulose	Successive coating PANI and NiCo-LDH on BC	KOH-PVA gel	0–1.6 V	1690 F g^{-1} @ 1 A g^{-1}	91.4% after 3000 cycles (asymmetric cell with N-doped carbonized BC/carbon cloth as the negative electrode)	Wu et al. (2018)
Ni-Mn layered double hydroxide and polypyrrole on bacterial cellulose nanofibers	Successive layer assembly of polypyrrole and bimetallic hydroxide	2 M KOH electrolyte	0.0–0.5 V	653.1 C g^{-1}@ 1.0 A g^{-1}	66.75% after 2000 cycles	Yuan et al. (2018)
Polyindole/carbon nanotube/bacterial cellulose (PIn/CNT/BC) nanofiber nonwoven electrode	Combination of “electrospinning and electro-spray” process and potentiostatic polymerization	D-tartaric acid (1 M)	0–1.0 V	552.6 F g^{-1}	95.6% capacitance retention after 5000 cycles, 96.4% after 1500 bending cycles	Jiajia et al. (2020)
Quaternary nanocomposite						
PEDOT:PSS/SnO_2/rGO/BNC (symmetric solid state supercapacitor)	Bacteria-mediated synthesis	PVA-H_2SO_4 electrolyte	0–1 V	445 F g^{-1} at 2 A g^{-1}	84.1% after 2500 cycles	Liu et al. (2018a)

preparation scheme can be generalized for fabricating several other BC-based ternary nanocomposites too. In most cases, BC serves as porous carbon network matrix with heteroatoms functionalized surfaces that promote easy anchoring of graphenes and conducting polymers to form flexible composites with advanced electrochemical features. Various metal compound-based ternary BC composites have also designed. Especially the metal chalcogenides containing nanocomposites of the composition such as cobalt oxide/graphene/bacterial cellulose and polypyrrole/cobalt sulfide/bacterial cellulose have showed excellent areal capacitances than previous reports with artificially carbons composites (Liu et al. 2015b, 2016b). Herein as expected BC offers highly flexible supporting matrix with large surface area and adequate porosity to hold greater proportions of functionalized materials on its surface and also serve as effective scaffolds to form diversified nanostructures.

In the recent past, a quaternary nanocomposite based on PPy/RGO/CNT/BC was fabricated for symmetric flexible supercapacitor applications. The resultant optimized nanocomposite (PPy/RGO/CNT/BC_{20})-based symmetric cell indicated superior capacitive signatures recorded at different potential sweep rates and varying current densities as indicated from CV and GCD profiles in Fig. 2.11a, b, respectively. In addition, it also responded to outstanding electrochemical reversibility, achieving ~83% capacitance retention efficiency even after 5000 cycling tests, as well as achieved noticeable areal energy densities at different power densities as specified in Fig. 2.11c, d, respectively. To investigate the mechanical stability of the resultant device, capacitance measurement was carried out at various bending frequencies that indicated a mere loss of 4.6% of original capacitance even after 800 bending cycles (Fig. 2.11e). Even the capacitance loss was negligible for various bending positions (Fig. 2.11f), signifying that the device works with similar efficiency even under deformation and thus ideally suitable for flexible energy storage usage (Bai et al. 2018a).

2.5 Microbe-Derived Carbons for Energy Storage Applications

Popular nanocarbons such as fullerenes, carbon nanotubes, graphenes, etc. have drawn remarkable recognition in energy storage applications because of their exceptional physicochemical characteristics of extensive surface area, advanced electronic charge transport, and outstanding mechanical flexibility (Obreja 2008; Borenstein et al. 2017; Chen et al. 2017; Dubey and Guruviah 2019; Zhang and Zhao 2009). These EDLC-based materials usually display stable and uniform charge-discharge rates, large cycling efficiency, but poor energy storing response owing to rapid agglomeration, layer restacking, and improper pore size distribution (Dubey and Guruviah 2019). Thus, they need to assemble with other suitable pseudocapacitive materials for better device efficiency (Zhang and Zhao 2009). Moreover, working with these materials often experiences low processability issues, high manufacturing costs, and rigorous/inhomogeneous functionalization steps which are mandatory to modify their properties conducive for advanced applications.

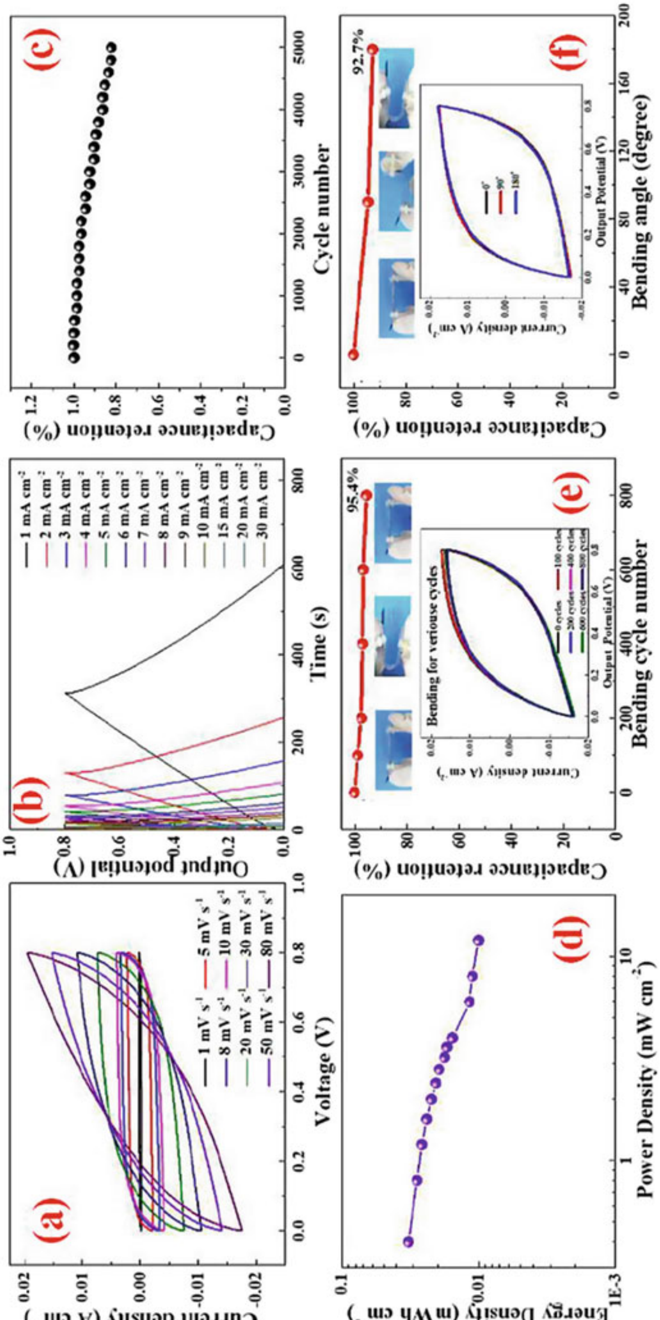

Fig. 2.11 (**a**) Cyclic Voltammograms of PPy/RGO/CNT/BC_{20} symmetric supercapacitor at various voltage sweep rates. (**b**) GCD profiles of PPy/RGO/CNT/BC_{20} symmetric supercapacitor at varying current densities. (**c**) Cycle stability of PPy/RGO/CNT/BC_{20} symmetric supercapacitor at 1 mA cm^{-2} at room temperature. (**d**) Ragone plot for the above sample at various areal energy-power densities. (**e**, **f**) Variation of capacitance retention efficiency and inset showing CV profiles of above device after repeated bent cycles (0th, 100th, 200th, 400th, 600th, and 800th) and at different bending angles (0°, 90°, and 180°), respectively (Bai et al. 2018a)

Compared to chemical methods, biological methods of synthesis are always preferred owing to their clean, green, and mild behavior along with high yield and reproducibility with special control on the structural and morphological aspects at molecular level (Enock et al. 2017). Therefore, biologically derived carbons using waste-biomasses, microbe-derived ones, etc. are getting importance as fantastic active host materials by virtue of their scalable production, morphological variety, and in situ heteroatoms doping advantages that would promote the material electronic conductivity, chemical adsorption efficiency, wettability, reaction activities, and electrochemical kinetics largely (Shang et al. 2020; Liu et al. 2018b).

One of the most common methods of fabrication of such bio-derived carbons includes biomass carbonization technology that involves thermo-chemical transformation of bio-materials at elevated temperatures under inert atmosphere such that mainly the carbon skeletons are retained while most of other unstable components getting eliminated (Biswal et al. 2013). It is to note that sp^2 carbon-based structures are advantageous for enhancement of electrical conductivity of the carbon materials. Hence, in this regard, the number of crucial production parameters such as selection of appropriate bio-source with high thermal stability, carbonization temperature and duration, heating rate, etc. along with other factors related to porosity control is important to control in order to derive required micro/nano-structure for desired applications.

Thus to obtain microbe-derived carbons, initially, the microbial cells grown in aqueous culture media are allowed to attain their optimum production yield (Nurfarahin et al. 2018). Then, they are normally harvested via centrifugation and washed thoroughly to eliminate culture medium residues and unwanted by-products formed at some stages in growth process. Subsequently, they are dried under oven-drying or freeze-drying to minimize the water content. The dried cells are then subjected to carbonization under optimum conditions. Often, additional chemical activation steps that are such as treatment with suitable reagents such as steam, supercritical fluids, etc. to introduce porosity as well as H_3PO_4, KOH, etc. to promote exfoliation and surface functionalities of the final products (Ukanwa et al. 2019; Wang and Kaskel 2012). However, chemical complexity of microbial cells limits understanding of detailed mechanisms of such activation procedures, but experimental results suggest that the pore size distribution and carbon yield can be systematically controlled by tuning activation and carbonization parameters. The following section highlights some of the interesting results related to microbe-derived carbons achieved mainly from bacteria and fungi sources that have been fruitfully applied as supercapacitor electrode materials. It is further to note that virus being much smaller in dimensions, very low content of carbon can be derived from them which may be one of the main causes of why virus derived carbons are rare in the scientific literature (Wei et al. 2016).

2.5.1 Bacteria-Derived Carbons for Energy storage applications

There are two varieties of bacterial cellulose (obtained from *nata de coco*)—one with loose fibrous morphology (freeze-dried) and the other dense paper type on pyrolysis at 950 °C followed by CO_2 activation (Lee et al. 2013). The loose fibrous form resulted in carbon-nanofiber material with too low carbon yield for further activation while the paper-like produced was activated successfully that resulted in activated graphitic carbon as evident from Raman spectroscopy, with peak intensity ratio of the characteristic D- to G-bands in the range of 2.2—2.8, comparable to commercial carbon fibers. The latter material with high surface area demonstrated good EDLC behavior in aqueous K_2SO_4 solution, in the voltage range of −0.2 to +0.2 V, recording gravimetric capacitance of 42 F g^{-1} and large areal capacitance of 1617 F cm^{-2}, almost fourfold rise in capacitance than that of commercial carbon nanofibers (365 F cm^{-2}), respectively (Lee et al. 2013). In another approach, composites of bacterial cellulose with varying sodium alginate were calcined at 700 °C followed by KOH activation. The resultant three-dimensional interconnected sheet-like hierarchical porous carbon nanomaterial was enriched with oxygen functionalities displaying high percentage of sp^2 carbons with good electrical conductivity. Systematic analysis revealed that KOH activation was essentially important for upgrading the capacitive behavior in these materials. The pseudo-rectangular CV profiles and very-triangular GCD curves of the optimized composition indicated ideal capacitive response in these activated derived carbons. The optimal composition delivered appreciable gravimetric capacitance of 302 F g^{-1} @ 0.5 A g^{-1} current density and high rate capacity of 75.2% recorded at high current density of 10 A g^{-1}, along with outstanding capacitance retaining efficiency of 93.8% beyond 10,000 GCD cycles in 6 M aqueous KOH electrolyte, in the voltage range of −1.0 to 0.0 V (Bai et al. 2018b).

High demands for miniatured kilohertz high-frequency electrochemical capacitors in support of filtering of ripple-current for AC/DC conversions as well as harvesting of natural vibration energy have urged cross-linked carbon nanofiber aero-gel fabrication obtained via fast microwave plasma pyrolysis of bacterial cellulose. To combat the small areal density of previously demonstrated electrodes at 120 Hz owing to thick electrodes, the as-prepared carbon nanofiber aero-gel film electrodes demonstrated appreciable areal capacitance of 4.5 mF cm^{-2} at 120 Hz in an aqueous electrolyte. The electrode also showed widespread potential range of greater than 3 V in non-aqueous electrolyte as well (Islam et al. 2017, 2018).

Generally, organic resorcinol-formaldehyde or lignin-resorcinol-formaldehyde aerogels are delicate and brittle, and so they are now being replaced by carbon aerogels made with high aspect ratio carbon nanofibers obtained from carbonization of bacterial cellulose composites. These materials show larger surface area, better crystalline nature, advanced surface functionalities, mechanical flexibility, and charge transport features for superior adsorption and energy storage utilities. Exceedingly graphitized carbon aerogels obtained from BC nanofibers and lignin-resorcinol-formaldehyde polymer composite deliver outstanding areal capacitance with large mesoporous scaffold for electrolyte-ion transportation of ions and reversible deformation due to the interpenetrated networks. Hence, they report themselves as well potential candidates for flexible solid-state energy storage systems (Xu et al. 2015).

It has been well-recognized that heteroatom doping with oxygen, nitrogen, phosphorus, sulfur, etc. in nanocarbon materials has led to exceptional improvement in electronic properties that have urged their impeccable applications in various technological fields (Abbas et al. 2019). However, preparation of 3D porous carbons with uniformly doped heteroatoms is a huge challenge due to lengthy, complicacy, and expensive instrumentations as well as involvement of hazardous and toxic chemicals that acutely constrained their practical applications. Thus, methods involving easy, scalable, green, multifunctional, common strategies to design 3D heteroatom-doped nanocarbons are in the pursue. Chen et al. reported a facile, environmental benign, scalable procedure of fabricating three-dimensional (3D) phosphorus-doped; nitrogen, phosphorus co-doped and boron, phosphorus-co-doped carbon nanofiber networks via pyrolysis of bacterial cellulose treated with aqueous ortho-phosphoric acid (H_3PO_4), ammonium dihydrogen phosphate ($NH_4H_2PO_4$), and ortho-boric acid/ ortho-phosphoric acid mixture (H_3BO_3/ H_3PO_4), respectively. Among them, the P and N-co-doped carbon nanofibers recorded better capacitive signature with notable gravimetric capacitance of 204.9 F g^{-1} @ 1.0 A g^{-1} current density in 2 M aqueous H_2SO_4 electrolyte (Chen et al. 2014). Similarly, polypyrrole-coated bacterial cellulose composites on carbonization led to formation of 3D inter-linked large surface area-based N-doped carbon nanofiber networks that illustrated impressive results as both supercapacitor and Li-ion battery electrodes. The unique structure promoted smooth and adequate electrode/electrolyte interface areas, for faster charge transport pathways as well as high electronic conductivity (Lei et al. 2016). In an attempt to design free-standing, N-doped carbon material interlinked frameworks for supercapacitor applications, reduced graphene oxide-embedded bacterial cellulose was pyrolyzed and subsequently treated with urea as nitrogen doping agent. The engineered electrode material recorded utmost capacitance of 216 F g^{-1} recorded @ current density of 1 A g^{-1}, showing ultimate capacitance loss of only 17% even after 10,000 GCD cycles tests (Chang et al. 2017). Hu et al. also employed $NH_4H_2PO_4$ impregnated BC pellicles to derive 3D N, P-co-doped porous carbon nanowires network structure via carbonization procedure. The resultant material with synergic doping of N and P heteroatoms reported capacitance of 258 F g^{-1} recorded at current density of 1 A g^{-1} with admirable electrochemical reversibility beyond 30,000 cycles. The symmetric supercapacitor with the sample displayed specific energy of 5.4 Wh kg^{-1} at a specific power of 200 W kg^{-1} and cyclic stability of 87% after 6000 GCD cycles (Hu et al. 2016). In another attempt, Zhu group demonstrated doping with various salts for encouraging usage in energy conversion devices derived from BC, obtained from *Bacillus subtilis* precursor. The as-prepared sample indicated improved capacitance than those of commercial carbons even at high current rates (Zhu et al. 2013). Shortly, high-power, flexible symmetrical supercapacitor was designed using nitrogen-doped carbon nanofibers resulting from ammonia-treated pyrolyzed BC source. The resultant device reported utmost specific power of 390.53 kW kg^{-1}

along with outstanding cyclic stability of 95.9% over 5000 GCD cycles (Chen et al. 2013b). Very recently, a novel approach was adopted to obtain large surface area-based, oxygen-doped porous carbon productively made via single-step carbonization cum activation procedure from bacterial cellulose, carboxymethyl cellulose, and citric acid composites. The so-obtained O-enriched carbon electrode recorded substantially improved specific capacitance of 350 F g^{-1} recorded @ 0.5 A g^{-1} current density with appreciable rate capability and electrochemical stability of 96% beyond 10,000 GCD cycles tests (Shu et al. 2020).

2.5.2 Fungi-Derived Carbons for Energy Storage Applications

Agaricus, a popular and naturally abundant mushroom variety, on being subjected to carbonization under inert atmosphere and subsequently KOH activation yielded mesoporous carbons. The resultant material displayed large surface area and good capacitance response of 196 F g^{-1} at potential sweep rate of 5 mV s^{-1}, recording an operating cell voltage of 1 V in aqueous electrolyte along with good electrochemical constancy beyond 1000 GCD cyclic tests (Zhu et al. 2011). *Agaricus* was further used to obtain N, O-doped, hierarchically porous activated carbon frameworks with ultra-high surface area of 2264 m^2 g^{-1}, the doping level regulated by varying the mole ratio of KOH and carbon source. The so-prepared electrode material recorded appreciable capacitance of 158 F g^{-1} in organic electrolyte, achieving a good capacitance retention efficiency of 93% even under 50 times rise in current density as well as outstanding cyclic stability of 92% (capacitance retaining efficiency) after undergoing continuous 10,000 GCD cyclic tests (Wang and Liu 2014). A template-free strategy was employed to design three-dimensional inter-linked porous carbon (as depicted in Fig. 2.12a using yeast (*S. cerevisiae*) as precursors using dispersion, carbonization at different temperatures, and subsequent KOH activation procedures. The optimized derived carbon material (carbonization temperature at 750 °C) displayed outstanding capacitive response even at high voltage scan rates (Fig. 2.12b) as well as high current densities (Fig. 2.12c), recording utmost capacitance of 330 F g^{-1} recorded at 1 A g^{-1} current density along with enhanced electrochemical stability beyond 1000 continuous GCD cycles (Sun et al. 2013).

In another approach, systematic fabrication of derived carbons were carried out using the crown-top and stem of two different mushrooms, viz., *Ganoderma lucidum* and *Calocybe indica*, from white and brown rot classes, respectively. They were separately subjected to microwave-assisted H_3PO_4 activation, carbonization treatment, and subsequently potassium hydroxide activation to yield activated nanocarbons that largely varied in surface area, pore size, and capacitive properties. Among the two, the former sample showed high BET surface area of 2432.4 m^2 g^{-1} with improved thermal properties and utmost specific capacitance of 271.94 F g^{-1} in addition to good electrochemical stability beyond 10,000 GCD cycles (Gannavarapu et al. 2019). Guo et al. used a very common white fungus called *Tremella*, composed of heteropolysaccharide varieties to obtain highly activated O-functionalized highly porous nanocarbons with exceptionally large surface area of 3760 m^2 g^{-1}. The

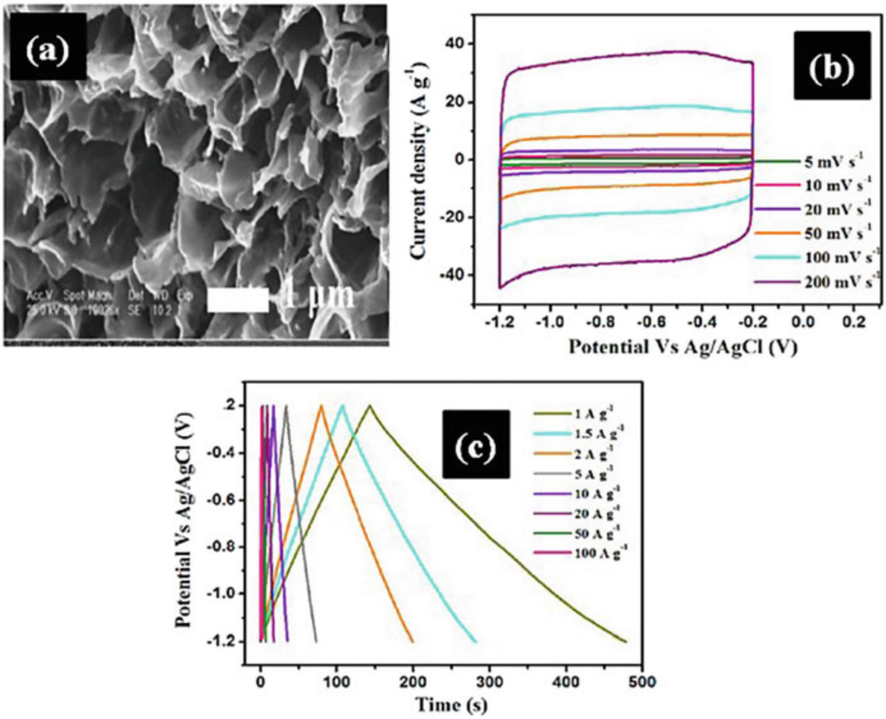

Fig. 2.12 (**a**) SEM image and (**b**) CV profiles of the optimized sample of inter-linked porous carbon using yeast (*S. cerevisiae*) carbonized at 750 °C at various voltage sweep rates and (**c**) Galvanostatic Charging/Discharging profiles of the same sample at varying current densities (Sun et al. 2013)

as-prepared material recorded good capacitance, advanced cycle performance in different aqueous electrolytes with wide cell voltage of 1 V in 6 M KOH, 1.6 V in 1 M Na_2SO_4, and high value of 3.0 V in pure ionic electrolyte EMIM BF_4, respectively. The symmetric cell setup using ionic liquid electrolyte recorded fine specific energy of 28 Wh kg^{-1} even at high specific power value of 19,700 W kg^{-1} (Guo et al. 2017). Aniline functionalized fungus was used as precursor for preparing N-doped carbon materials prepared by carbonization and then alkali-assisted (KOH) activation processes. The resultant carbon possessed high porosity and inter-linked arrangement that recorded high surface area of 2339 m^2 g^{-1} with fast ion diffusion rate. The material displayed appreciable specific capacitance of 218 F g^{-1} @ 0.1 A g^{-1} current density in addition to exceptional electrochemical reversibility and rate capacity even beyond 5000 GCD cycles (Wang et al. 2017d). Similarly, S- and N-co-doped carbon fiber networks are designed using the composites formed from bio-concentration of various toxic organic dye pollutants with fungal hyphae as an effective green strategy of converting toxic wastes to important resources. Among them, the N and S-co-doped carbon fiber obtained from methylene blue dye bio-concentration on hyphae displayed utmost specific capacitance of 235 F g^{-1} @ 1 A g^{-1} specific current, undergoing a net capacitance loss of only 27.2% on 20-fold rise in current rate (Lei et al. 2018). Again, bamboo fungus as starting component was put through two-step pyrolytic procedures to obtain hierarchical, nitrogen-doped porous carbons with honeycomb structure. The optimized sample showed high surface area (1708 m^2 g^{-1}) and recorded utmost capacitance value of 228 F g^{-1}, good and even capacitive signatures. The symmetrical cell setup using the same recorded high specific energy of 4.3 Wh kg^{-1} with almost no capacitance loss even after surviving continuous 10,000 GCD cyclic tests recorded at high current density of 10 A g^{-1} (Zou et al. 2019). A nitrogen-doped 3D porous activated carbon network was produced on $ZnCl_2$ activation followed by high-temperature carbonization of mycelium pellets with thread-type chain morphology in presence of ammonium chloride. The obtained mass displayed nearly symmetric rectangular CV curves even at high potential scan rates, recording utmost capacitance of 237.2 F g^{-1} at the voltage sweep rate of 10 mV s^{-1}, about 1.5 times superior to that of pure and undoped derived carbon analogue. Its unique morphology and surface functionalization synergistically improve the capacitive signature of the doped sample (Hao et al. 2018).

2.5.3 Microbe-Derived Carbon-Based Nanocomposites as Energy Storage Materials

The above discussion clearly indicates that the microbe-derived carbons have shown genuine encouraging results of better processability, charge transport properties, large surface and higher mass loading, porosity, and mechanical flexibility that have motivated their usage in fabrication of carbon-based nanocomposites for energy storage applications. However, to further improve their low theoretical capacitance values of these derived carbon materials, introduction of electroactive components is mandatory in order to achieve high power/energy density devices (Schopf and Es-Souni 2017). Such electroactive materials include semiconducting metallic compounds, conducting polymers, etc. with remarkable theoretical capacitances but limited with low conductivity, large and irreversible volume changes, sluggish charge transfer rates, and poor cycling performance leading to substandard electrochemical stability (Abdah et al. 2020). Thus, synergic cooperation of the components can eliminate their individual shortcomings, promoting higher conductivity and shortened charge transport pathways, as well as introduce improved reaction kinetics and morphological stability. Various nanocomposite materials have been synthesized using these carbons blended with either other carbon nanomaterials, like graphenes, CNTs, carbon nanofibers, and conducting polymers, or metal nanoparticles like Pd, Ag, etc. or metal compounds Fe_3O_4, Co_3O_4, Ni_3S_2, MnO_2, $CoFe_2O_4$, etc. which has been discussed in the subsequent sections.

In situ growth of polyaniline on bacterial cellulose followed by subsequent pyrolysis and KOH activated yielded N- and O-functionalized carbon powders.

The resultant material displayed appreciable volumetric capacitance of 28.3 F cm^{-3}, smooth charge transfer rates, and excellent cycle life of 100% over 2500 GCD cycles measured at specific current of 0.1 A g^{-1} using PVA/H_2SO_4 gel electrolyte (Lv et al. 2017).

Since metallic compounds offer very high pseudocapacitance, a facile designing strategy for obtaining binder-free metal oxides anchored on the carbon papers derived from BC gel was formulated by impregnating desired metal ions within the gel followed by drying and then subsequently carbonizing under suitable conditions to produce the resultant electrode material. Self-supporting three-dimensional bacterial cellulose-derived carbon-fiber network blended with N-doped carbon-coated Fe_3O_4 obtained via combination of hydrothermal and carbonization processes were used for supercapacitor applications. The electrode material displayed large areal as well as volume capacitances of 1.36 F cm^{-2} and 2300 F cm^{-3}, respectively, @ 3 mA cm^{-2} areal current density. In addition, the electrode also responded to appreciable cycle life undergoing only 11.5% capacitance loss beyond 4000 cycles within the working potential range of −1.2 to 0 V in aqueous KOH electrolyte (Lv et al. 2018). An asymmetric cell was assembled using three-dimensional networks of MnO_2 coated bacterial cellulose-derived carbon nanofiber and nitrogen-doped bacterial cellulose nanomaterial as positive and negative electrodes. The optimized gadget displayed a cell output potential of 2.0 V in presence of 1 M aqueous Na_2SO_4 electrolyte. Further, the cell also recorded appreciable specific energy of 32.91 Wh kg^{-1} along with maximum power output of 284.63 kW kg^{-1} and cycling efficiency of 95.4% after 2000 nonstop charging/ discharging cycles (Chen et al. 2013c). In another report, nitrogen-doped carbon web-like structure was processed via carbonization of polyaniline-coated bacterial cellulose composite that was subsequently blended with MnO_2 (carbon-MnO_2) and assembled to form activated carbon (AC) // carbon-MnO_2 asymmetric cell configuration that displayed high specific energy of 63 Wh kg^{-1} in 1 M Na_2SO_4 electrolyte, accomplishing a cell output potential of ~1.1 V along with 92% capacitance retaining efficacy even beyond 5000 cycles of GCD tests (Long et al. 2014). Ni_3S_2 nanoparticles were hydrothermally deposited onto carbon nanofibers (CNFs) obtained from carbonized BC, as depicted in the TEM image of Fig. 2.13a, illustrating large capacitance of 883 F g^{-1} @ 2 A g^{-1} current density as well as improved good cycle stability compared to its metal sulfide component in alkaline electrolyte. The asymmetric supercapacitor Ni_3S_2@CNFs//CNFs in aqueous KOH electrolyte recorded high operating voltage of 1.7 V in addition to high specific energy of 25.8 Wh kg^{-1} @ specific power of 425 W kg^{-1}, undergoing only 3% capacitance loss even after continuous 2500 GCD cycles. The Ragone plot for the Ni_3S_2@CNFs//CNFs cell, as reflected in Fig. 2.13b, indicates much superior performance compared to other asymmetric supercapacitors made with synthetic carbon materials. The system was also successful in lighting LED; the corresponding setup has been shown in the inset of Fig. 2.13b, glowed for 3 min on being charged in just 20 s, indicating its capability as high-performance energy storage system (Yu et al. 2014).

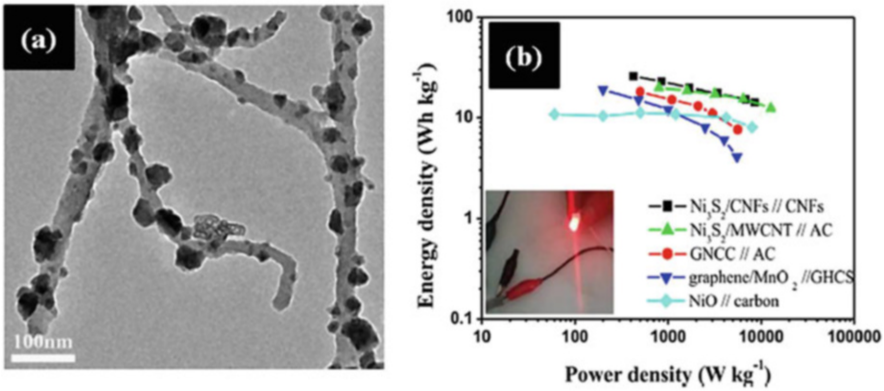

Fig. 2.13 (**a**) TEM image of Ni_3S_2/CNFs sample. (**b**) Comparison of electrochemical performances based on Ragone plots of Ni_3S_2/CNFs//CNFs, Ni_3S_2/MWCNT-Nitrogen-doped bacterial cellulose pellicles (MWCNTNC)//AC (activated-carbon), graphene-nickel cobaltite nanocomposite (GNCC) //AC, graphene/MnO_2 //graphitic hollow carbon spheres (GHCS), and NiO//carbon asymmetric cell configurations. The inset shows red LED glow for the Ni_3S_2/CNFs// CNFs asymmetric device (Yu et al. 2014)

Lately, it has been perceived that aerogels with large surface area and mesoporous cross-linked morphology work as brilliant electrode materials in supercapacitors. Explorations executed with molybdenum oxides loaded on derived carbon papers obtained from BC gel, as expected, illustrated exceptional redox capacitance along with better charge transport kinetics offered by the interconnected fibrillar carbon matrix of the composite (Miyajima et al. 2016). High pseudocapacitive nickel sulfide was grown in situ on bacterial cellulose-derived carbon sheet aerogels (CA) that recorded not only capacitive performance as high as 1606 F g^{-1} recorded at specific current of 1 A g^{-1} but also high capacitive response even at large currents (69% of initial capacitance restored even at current density of 10 A g^{-1}), as well as achieving 91.2% capacitance retaining ability over 10,000 continuous CV cyclic tests, recorded at large potential sweep rate of 100 mV s^{-1}. Furthermore, the asymmetric supercapacitor NiS@CA//CA delivered specific energy of ~21.5 Wh kg^{-1} @ specific power of 700 W kg^{-1} procuring a cell output potential of 1.4 V in aqueous KOH electrolyte, enduring cycling stability of ~87.1% even after 10,000 CV cycles scans (Zuo et al. 2017b).

2.6 Conclusion and Future Prospects

This book chapter highlights on the fruitful correlation between microbe-derived substances and their electrochemical behavior to be functional for smart energy storage devices. Herein, current advancements on the fabrication and designing of microbe-derived supercapacitor electrodes have been detailed. Several microbes and their by-products have contributed as essential as well sustainable constituents for developing high-performance energy devices. This is made feasible by means of their exclusive capabilities toward large scalability due to fast rate of reproduction, biomineralization, tunable genetic modification, and self-assembling characteristics. Their superior structural stability and interconnected-morphology offer suitable matrix for easy accessibility of intercalating electrolyte ions to numerous electroactive centers; promote superior electronic conductivity; as well as exhibit greater potentiality in electroactive mass loading capacity. In addition, bacteria and fungi can be easily subjected to carbonization to yield mono-or multi-heteroatom-doped carbon compounds with tunable doping quantities that considerably influence as well as alter the composition, electronic properties, and surface characteristics, thus considerably upgrading the supercapacitive signatures. Further, the rationally designed nanocomposites prepared with these microbe-derived heteroatom-doped porous carbons under the influence of synergism report superior electrochemical performance compared to their other synthetic nanocarbon (such as graphene, etc.) material composites that is very impressing especially considering green state-of-the-art research and technology.

Though numerous interesting achievements have come up with this bio-synthetic strategy, till date a number of challenges do exist. Limitations of inferior productivity, poor control on material quality, contaminations, and difficulty in separations still exist in biosynthesis of nanomaterials!

Hence, to further improve the electrochemical performances of these microbe-based energy storage systems, proper selection of microorganisms—having unique characteristics, morphologies, and composition that can come up with desired heteroatoms—is to be carried out. In addition, target-directed synthesis using appropriate bio-compounds with high speculated charge storage capacities, high-quality physicochemical features, and mechanical flexibility can lead to advanced products. Moreover, cost-effective, scalable, and efficient synthesis techniques are urged that would significantly improve the conversion efficiency of the microorganisms to high-quality porous carbon materials. Additional stress has to be imposed on parametric investigations related to the carbonized ash contents, their nature, porosity, etc., and accordingly their influence on electrochemical behavior must be projected in the near future. Further detailed insight on the mechanism and transformation of phases in the biomineralization process that result in the formation of in situ inorganic nanomaterial as well as thorough knowledge on the determining factors that guide their electrochemical behavior, molecular interactions, and chemistries are essential. Again, deep conception of the genetic engineering of viruses is essential that can lead to useful surface functional groups for better binding of electrode materials to the microbe-matrix. Importantly, innovations on the in situ characterization techniques and genetics-related computations are indispensable for finer interpretation of the dynamics and chemistry of self-assembly process, RNA or DNA chains modification strategies, and allied issues. Even though microbe-based materials and their derived products display high potentiality in energy-associated applications, serious issues related to their safety while handling and disposal, environment benignity of the degraded products, production costs in comparison to traditionally employed materials must be considered during commercialization.

Nonetheless, with the pace at which the research areas of microbial electrochemical science and technologies are progressing at present, it is obvious that microbe-based supercapacitor electrode materials will keep long-term promise and successfully address the energy problems of the society soon. Hence, the author hopes that this chapter will dish up as a scaffold for ongoing and energetic thinking in the reading minds that will certainly contribute in shaping and maturation of R&D of this interdisciplinary field of microbiology and electrochemistry for a better tomorrow!

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References

Abbas Q, Raza R, Shabbir I, Olabi AG (2019) Heteroatom doped high porosity carbon nanomaterials as electrodes for energy storage in electrochemical capacitors: a review. J Sci Adv Mater Devices 4(3):341–352. https://doi.org/10.1016/j.jsamd.2019.07.007

Abdah MAAM, Hawa N, Azman N, Kulandaivalu S, Sulaiman Y (2020) Review of the use of transition-metal-oxide and conducting polymer-based fibres for high-performance supercapacitors. Mater Des 186:108199. https://doi.org/10.1016/j.matdes.2019.108199

Achromobacter (n.d.). https://en.wikipedia.org/wiki/Achromobacter

Afif A, Rahman SMH, Azad AT, Zaini J, Islan MA, Azad AK (2019) Advanced materials and technologies for hybrid supercapacitors for energy storage—a review. J Energy Storage 25:100852. https://doi.org/10.1016/j.est.2019.100852

Agaricus (n.d.). https://en.wikipedia.org/wiki/Agaricus

Akinwolemiwa B, Chen GZ (2018) Fundamental consideration for electrochemical engineering of supercapattery. J Braz Chem Soc 29:960–972. https://doi.org/10.21577/0103-5053.20180010

Allred DB, Sarikaya M, Baneyx F, Schwartz DT (2005) Electrochemical nanofabrication using crystalline protein masks. Nano Lett 5:609–613. https://doi.org/10.1021/nl047967b

An C, Zhang Y, Guo H, Wang Y (2019) Metal oxide-based supercapacitors: progress and prospective. Nanoscale Adv 1:4644–4658. https://doi.org/10.1039/C9NA00543A

Atalay FE, Asma D, Kaya H, Ozbey E (2015) The fabrication of metal oxide nanostructures using Deinococcus radiodurans bacteria for supercapacitor. Mater Sci Semicond Process 38:314–318. https://doi.org/10.1016/j.mssp.2014.12.002

Atalay FE, Asma D, Kaya H, Bingol A, Yaya P (2016) Synthesis of NiO nanostructures using cladosporium cladosporioides fungi for energy storage applications. Nanomater Nanotechnol 28:6. https://doi.org/10.5772/63569

Atalay FE, Kaya H, Bingol A, Asma D (2017) La-based material for energy storage applications. Acta Phys Pol A 131(3):453. https://doi.org/10.12693/APhysPolA.131.453

Augustyn V, Simon P, Dunn B (2014) Pseudocapacitive oxide materials for high-rate electrochemical energy storage. Energy Environ Sci 7:1597–1614. https://doi.org/10.1039/C3EE44164D

Bacillus subtilis (n.d.). https://en.wikipedia.org/wiki/Bacillus_subtilis

Bai Y, Liu R, Li E, Li X, Liu Y, Yuan G (2018a) Graphene/carbon nanotube/ bacterial cellulose assisted supporting for polypyrrole towards flexible supercapacitor applications. J Alloys Compd 777:524–530. https://doi.org/10.1016/j.jallcom.2018.10.376

Bai Q, Xiong Q, Li C, Shen Y, Uyama H (2018b) Hierarchical porous carbons from a sodium alginate/bacterial cellulose composite for high-performance supercapacitor electrodes. Appl Surf Sci 455:795–807. https://doi.org/10.1016/j.apsusc.2018.05.006

Bhattacharya K, Raha S (2002) Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. Mycopathologia 155:135–141. https://doi.org/10.1023/A:1020475411125

Bi Z, Kong Q, Cao Y, Sun G, Su F, Wei X, Li X, Ahmad A, Xie L, Chen C (2019) Biomass-derived porous carbon materials with different dimensions for supercapacitor electrodes: a review. J Mater Chem A 7:16028. https://doi.org/10.1039/C9TA04436A

Biswal M, Banerjee A, Deo M, Ogale S (2013) From dead leaves to high energy density supercapacitors. Energy Environ Sci 6:1249–1259. https://doi.org/10.1039/C3EE22325F

Borenstein A, Hanna O, Attias R, Luski S, Brousse T, Aurbach D (2017) Carbon-based composite materials for supercapacitor electrodes: a review. J Mater Chem A 5:12653–12672. https://doi.org/10.1039/C7TA00863E

Brousse T, Belanger D, Long JW (2015) To be or not to be pseudocapacitive? J Electrochem Soc 162:A5185–A5189. https://doi.org/10.1149/MA2014-02/3/157

Bu Y, Cao M, Jiang Y, Gao L, Shi Z, Xiao X, Wang M, Yang G, Zhou Y, Shen Y (2018) Ultra-thin bacterial cellulose/poly(ethylenedioxythiophene) nanofibers paper electrodes for all solid-state flexible supercapacitors. Electrochim Acta 271:624–631. https://doi.org/10.1016/j.electacta.2018.03.155

Burke A (2000) Ultracapacitors: why, how, and where is the technology. J Power Sources 91:37–50. https://doi.org/10.1016/S0378-7753(00)00485-7

Cai J, Xu W, Liu Y, Zhu Z, Liu G, Ding W, Wang G, Wang H, Luo Y (2019) Robust construction of flexible bacterial cellulose@Ni(OH)2 paper: toward high capacitance and sensitive H2O2 detection. Eng Sci 5:21–29. https://doi.org/10.30919/es8d669

Campbell B, Ionescu R, Favors Z, Ozkan CS, Ozkan M (2015) Bio-derived, binderless, hierarchically porous carbon anodes for Li-ion batteries. Sci Rep 5:14575. https://doi.org/10.1038/srep14575

Campbell B, Ionescu R, Ozkan CS, Ozkan M (2016) Structural and compositional characterization of fungus-derived pyrolytic carbon architectures. Adv Mater Sci Eng 2016:9843875. https://doi.org/10.1155/2016/9843875

Chae JH, Zhou X, Chen GZ (2012) From electrochemical capacitors to supercapatteries. Green 2:41–54. https://doi.org/10.1515/green-2011-0007

Chang P-K, Scharfenstein LL, Wei Q, Bhatnagar D (2010) Development and refinement of a high-efficiency gene-targeting system for Aspergillus flavus. J Microbiol Methods 81:240. https://doi.org/10.1016/j.mimet.2010.03.010

Chang Y, Zhou L, Xiao Z, Liang J, Kong D, Li Z, Zhang X, Li X, Zhi L (2017) Embedding reduced graphene oxide in bacterial cellulose-derived carbon nanofibril networks for supercapacitors. ChemElectroChem 4(10):2448–2452. https://doi.org/10.1002/celc.20170062

Chaturvedi UC, Shrivastava R (2005) Interaction of viral proteins with metal ions: role in maintaining the structure and functions of viruses. FEMS Immunol Med Microbiol 43 (2):105–114. https://doi.org/10.1016/j.femsim.2004.11.004

Chen GZ (2017) Supercapacitor and supercapattery as emerging electrochemical energy stores. Int Mater Rev 62(4):173–202. https://doi.org/10.1080/09506608.2016.1240914

Chen C, Kennel EB, Stiller AH, Stansberry PG, Zondlo JW (2006) Carbon foam derived from various precursors. Carbon 44:1535–1543. https://doi.org/10.1016/j.carbon.2005.12.021

Chen X, Gerasopoulos K, Guo J, Brown A, Wang C, Ghodssi R, Culver JN (2010) Virus-enabled silicon anode for lithium-ion batteries. ACS Nano 4:5366–5372. https://doi.org/10.1021/nn100963j

Chen J, Li C, Shi GQ (2013a) Graphene materials for electrochemical capacitors. J Phys Chem Lett 4:1244–1253. https://doi.org/10.1021/jz400160k

Chen L-F, Huang Z-H, Liang H-W, Yao W-T, Yu Z-Y, Yu S-H (2013b) Flexible all-solid-state high-power supercapacitor fabricated with nitrogen-doped carbon nanofiber electrode material derived from bacterial cellulose. Energy Environ Sci 6(11):3331. https://doi.org/10.1039/c3ee42366b
Chen L-F, Huang Z-H, Liang H-W, Guan Q-F, Yu S-H (2013c) Bacterial-cellulose-derived carbon nanofiber@MnO2 and nitrogen-doped carbon nanofiber electrode materials: an asymmetric supercapacitor with high energy and power density. Adv Mater 25:4746–4752. https://doi.org/10.1002/adma.201204949
Chen L-F, Huang Z-H, Liang H-W, Gao H-L, Yu S-H (2014) Three-dimensional heteroatom-doped carbon nanofiber networks derived from bacterial cellulose for supercapacitors. Adv Funct Mater 24:5104–5111. https://doi.org/10.1002/adfm.201400590
Chen X, Paul R, Dai L (2017) Carbon-based supercapacitors for efficient energy storage. Natl Sci Rev 4(3):453–489. https://doi.org/10.1093/nsr/nwx009
Christen T, Carlen MW (2000) Theory of Ragone plots. J Power Sources 91(2):2010–2016. https://doi.org/10.1016/S0378-7753(00)00474-2
Chu S, Gerasopoulos K, Ghodssi R (2016) Tobacco mosaic virus-templated hierarchical Ni/NiO with high electrochemical charge storage performances. Electrochim Acta 220:184–192. https://doi.org/10.1016/j.electacta.2016.10.106
Cladosporium cladosporioides (n.d.). https://en.wikipedia.org/wiki/Cladosporium_cladosporioides
Cleaves HJ, Butch C, Burger PB, Goodwin J, Meringer M (2019) One among millions: the chemical space of nucleic acid-like molecules. J Chem Inf Model 59(10):4266. https://doi.org/10.1021/acs.jcim.9b00632
CNTSEM.JPG (n.d.). https://commons.wikimedia.org/w/index.php?curid=12837127 (By Material scientist at English Wikipedia, CC BY-SA 3.0)
Conway BE (1991) Transition from 'supercapacitor' to 'battery' behavior in electrochemical energy storage. J Electrochem Soc 138(6):1539–1548. https://doi.org/10.1149/1.2085829
Correa CR, Kruse A (2018) Bio-based functional carbon materials: production, characterization, and applications—a review. Materials (Basel) 11(9):1568. https://doi.org/10.3390/ma11091568
de Petris S (1967) Ultrastructure of the cell wall of Escherichia coli and chemical nature of its constituent layers. J Ultrastruct Res 19(1–2):45–83. https://doi.org/10.1016/S0022-5320(67)80059-5
Deinococcus_radiodurans (n.d.). https://en.wikipedia.org/wiki/Deinococcus_radiodurans
Deng S, Zhang Y, Xie D, Yang L, Wang G, Wang X, Zheng X, Zhu J, Yu Y, Pan G, Xia X, Tu J (2019a) Oxygen vacancy modulated Ti2Nb10O29-x embedded onto porous bacterial cellulose carbon for highly efficient lithium ion storage. Nano Energy 58:355–364. https://doi.org/10.1016/j.nanoen.2019.01.051
Deng X, Zhu S, Li J, He F, Liu E, He C, Shi C, Li Q, Ma L, Zhao N (2019b) Bio-inspired three-dimensional carbon network with enhanced mass-transfer ability for supercapacitors. Carbon 143:728–735. https://doi.org/10.1016/j.carbon.2018.11.055
Dickeya dadantii (n.d.). https://en.wikipedia.org/wiki/Dickeya_dadantii
Ding A, Wang B, Zheng J, Weng B, Li C (2018) Sensitive dopamine sensor based on three dimensional and macroporous carbon aerogel microelectrode carbon aerogels. Int J Electrochem Sci 13:4379–4389. https://doi.org/10.20964/2018.05.43
Divyashree A, Hegde G (2015) Activated carbon nanospheres derived from bio-waste materials for supercapacitor applications—a review. RSC Adv 5:88339–88352. https://doi.org/10.1039/C5RA19392C
Dong D, Zhang Y, Sutaria S, Konarov A, Chen P (2013) Binding mechanism and electrochemical properties of M13 phage-sulfur composite. PLoS One 8(11):e82332. https://doi.org/10.1371/journal.pone.0082332
Dong L, Xu C, Li Y, Huang Z, Kang F, Yang Q, Zhao X (2016) Flexible electrodes and supercapacitors for wearable energy storage: a review by category. J Mater Chem A 4:4659–4685. https://doi.org/10.1039/C5TA10582J

Douglas T, Young M (2006) Viruses: making friends with old foes. Science (New York, N.Y.) 312 (5775):873–875. https://doi.org/10.1126/science.1123223
Du JH, Pei SF, Ma LP, Cheng HM (2014) Carbon nanotube- and graphene-based transparent conductive films for optoelectronic devices. Adv Mater 26:1958–1991. https://doi.org/10.1002/adma.201304135
Dubey R, Guruviah V (2019) Review of carbon-based electrode materials for supercapacitor energy storage. Ionics 25:1419–1445. https://doi.org/10.1007/s11581-019-02874-0
Dupont MF, Donne SW (2016) Charge storage mechanisms in electrochemical capacitors: effects of electrode properties on performance. J Power Sources 326:613–623. https://doi.org/10.1016/j.jpowsour.2016.03.073
Dutta S, Kim J, Ide Y, Kim JH, Hossain MSA, Bando Y, Yamauchi Y, Wu KC-W (2017) 3D network of cellulose-based energy storage devices and related emerging applications. Mater Horiz 4:522–545. https://doi.org/10.1039/C6MH00500D
Enock TK, King'ondu CK, Pogrebnoi A, Jande YAC (2017) Status of biomass derived carbon materials for supercapacitor application. Int J Electrochem 2017:6453420. , 14 pages. https://doi.org/10.1155/2017/6453420
Enterobacter (n.d.). https://en.wikipedia.org/wiki/Enterobacter
Esa F, Tasirin SM, Rahman NA (2014) Overview of bacterial cellulose production and application. Agric Agric Sci Proc 2:113–119. https://doi.org/10.1016/j.aaspro.2014.11.017
Escherichia coli (n.d.). https://en.wikipedia.org/wiki/Escherichia_coli
Fan XZ, Pomerantseva E, Gnerlich M (2013) Tobacco mosaic virus: a biological building block for micro/nano/bio systems. J Vac Sci Technol A 31:050815. https://doi.org/10.1116/1.4816584
Fang Q, Zhou X, Deng W, Zheng Z, Liu Z (2016) Freestanding bacterial cellulose-graphene oxide composite membranes with high mechanical strength for selective ion permeation. Sci Rep 6:33185. https://doi.org/10.1038/srep33185
Fang X, Wang Y, Wang Z, Jiang Z, Dong M (2019) Microorganism assisted synthesized nanoparticles for catalytic applications. Energies 12:190. https://doi.org/10.3390/en12010190
Fischlechner M, Donath E (2007) Viruses as building blocks for materials and devices. Angew Chem Int Ed 46(18):3184–3193. https://doi.org/10.1002/anie.200603445
Gannavarapu KP, Azizighannad S, Molli M, Pandey M, Muthukumar S, Mitra S, Dandamudi RB (2019) Nanoporous hierarchical carbon structures derived from fungal basidiocarps for high performance supercapacitors. Energy Storage 1:e58
Gao K, Shao Z, Wu X, Wang X, Zhang Y, Wang W, Wang F (2013) Paper-based transparent flexible thin film supercapacitors. Nanoscale 5:5307–5311. https://doi.org/10.1039/C3NR00674C
Geobacter sulfurreducens (n.d.). https://en.wikipedia.org/wiki/Geobacter_sulfurreducens
Gerasopoulos K, Pomerantseva E, McCarthy M, Brown A, Wang C, Culver J, Ghodssi R (2012) Hierarchical three-dimensional microbattery electrodes combining bottom-up self-assembly and top-down micromachining. ACS Nano 6:6422–6432. https://doi.org/10.1021/nn301981p
Ghosh A, Guo J, Brown AD, Royston E, Wang C, Kofinas P, Culver JN (2012) Virus-Assembled flexible electrode-electrolyte interfaces for enhanced polymer-based battery applications. J Nanomater 2012:795892. https://doi.org/10.1155/2012/795892
Gidwani M, Bhagwani A, Rohra N (2014) Supercapacitors: the near future of batteries. Int J Eng Invent 4:22–27
Gnerlich M, Pomerantseva E, Gregorczyk K, Ketchum D, Rubloff G, Ghodssi R (2013) Solid flexible electrochemical supercapacitor using tobacco mosaic virus nanostructures and ALD ruthenium oxide. J Micromech Microeng 23:114014. https://doi.org/10.1088/0960-1317/23/11/114014
Gnerlich M, Ben-Yoav H, Culver JN, Ketchum DR, Ghodssi R (2015) Selective deposition of nanostructured ruthenium oxide using Tobacco mosaic virus for micro-supercapacitors in solid Nafion electrolyte. J Power Sources 293:649–656. https://doi.org/10.1016/j.jpowsour.2015.05.053

González A, Goikolea E, Barrena JA, Mysyk R (2016) Review on supercapacitors: technologies and materials. Renew Sust Energ Rev 58:1189–1206. https://doi.org/10.1016/j.rser.2015.12.249

Gopinath V, Priyadarshini S, Loke MF, Arunkumar J, Marsili E, MubarakAli D, Velusamy P, Vadivelu J (2017) Biogenic synthesis, characterization of antibacterial silver nanoparticles and its cell cytotoxicity. Arab J Chem 10:1107–1117. https://doi.org/10.1016/j.arabjc.2015.11.011

Guan L, Yu L, Chen GZ (2016) Capacitive and non-capacitive faradaic charge storage. Electrochim Acta 206:464–478. https://doi.org/10.1016/j.electacta.2016.01.213

Guan F, Chen S, Sheng N, Chen Y, Yao J, Pei Q, Wang H (2018) Mechanically robust reduced graphene oxide/bacterial cellulose film obtained via biosynthesis for flexible supercapacitor. Chem Eng J 360:829–837. https://doi.org/10.1016/j.cej.2018.11.202

Guo N, Li M, Sun X, Wang F, Yang R (2017) Tremella derived ultrahigh specific surface area activated carbon for high performance supercapacitor. Mater Chem Phys 201:399–407. https://doi.org/10.1016/j.matchemphys.2017.08.054

Gwon H, Kim HS, Lee KU, Seo DH, Park YC, Lee YS, Ahn BT, Kang K (2011) Flexible energy storage devices based on graphene paper. Energy Environ Sci 4:1277–1283. https://doi.org/10.1039/C0EE00640H

Hao J, Huang Y, He C, Xu W, Yuan L, Shu D, Song X, Meng T (2018) Bio-templated fabrication of three-dimensional network activated carbons derived from mycelium pellets for supercapacitor applications. Sci Rep 8:562. https://doi.org/10.1038/s41598-017-18895-6

He Y, Chen W, Gao C, Zhou J, Li X, Xie E (2013) An overview of carbon materials for flexible electrochemical capacitors. Nanoscale 5:8799. https://doi.org/10.1039/C3NR02157B

Heinemann JA, Walker S (2019) Environmentally applied nucleic acids and proteins for purposes of engineering changes to genes and other genetic material. Biosafety Health 1(3):113–123. https://doi.org/10.1016/j.bsheal.2019.09.003

Higgins D, Dworkin J (2012) Recent progress in Bacillus subtilis sporulation. FEMS Microbiol Rev 36:131–148. https://doi.org/10.1111/j.1574-6976.2011.00310.x

Hirayama D, Saron C, Botelho EC, , Costa M L, Ancelotti A, Carlos A, (2017). Polypropylene composites manufactured from recycled carbon fibers from aeronautic materials waste. Mater Res, 20: 519-525. https://doi.org/10.1590/1980-5373-mr-2016-1022

Hirneola_auricula-judae_(xndr).jpg (n.d.). https://en.wikipedia.org/wiki/Auricularia#/media/File:Hirneola_auricula-judae_(xndr).jpg

Hu W, Chen S, Yang Z, Liu L, Wang H (2011) Flexible electrically conductive nanocomposite membrane based on bacterial cellulose and polyaniline. J Phys Chem B 115(26):8453–8457. https://doi.org/10.1021/jp204422v

Hu Z, Li S, Cheng P, Yu W, Li R, Shao X, Lin W, Yuan D (2016) N, P-co-doped carbon nanowires prepared from bacterial cellulose for supercapacitor. J Mater Sci 51:2627–2633. https://doi.org/10.1007/s10853-015-9576-x

Hyun WJ, Park OO, Chin BD (2013) Foldable graphene electronic circuits based on paper substrates. Adv Mater 25:4729–4734. https://doi.org/10.1002/adma.201302063Tolle

Iro ZS, Subramani C, Dash SS (2016) A brief review on electrode materials for supercapacitor. Int J Electrochem Sci 11:10628–10643. https://doi.org/10.20964/2016.12.50

Islam N, Li S, Rena G, Zu Y, Warzywoda J, Wang S, Fan Z (2017) High-frequency electrochemical capacitors based on plasma pyrolyzed bacterial cellulose aerogel for current ripple filtering and pulse energy storage. Nano Energy 40:107–114. https://doi.org/10.1016/j.nanoen.2017.08.015

Islam N, Hoque MNF, Zu Y, Wang S, Fan Z (2018) Carbon nanofiber aerogel converted from bacterial cellulose for kilohertz AC-supercapacitors. MRS Adv 3(15–16):855–860. https://doi.org/10.1557/adv.2018.139

Isogai A, Hänninen T, Fujisawa S, Saito T (2018) Review: catalytic oxidation of cellulose with nitroxyl radicals under aqueous conditions. Prog Polym Sci 86:122–148. https://doi.org/10.1016/j.progpolymsci.2018.07.007

Jana A, Scheer E, Polarz S (2017) Synthesis of graphene-transition metal oxide hybrid nanoparticles and their application in various fields. Beilstein J Nanotechnol 8:688–714. https://doi.org/10.3762/bjnano.8.74

Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S (2017) Environmental Escherichia coli: ecology and public health implications—a review. J Appl Microbiol 123:570–581. https://doi.org/10.1111/jam.13468

Jayalakshmi M, Balasubramanian K (2008) Simple capacitors to supercapacitors—an overview. Int J Electrochem Sci 3:1196–1217

Jiajia W, Zhanwen D, Ping X, Zhijiang C (2020) Fabrication of flexible polyindole/carbon nanotube/bacterial cellulose nanofiber nonwoven electrode doped by D-tartaric acid with high electrochemical performance. Cellulose 27:6353–6366. https://doi.org/10.1007/s10570-020-03199-2

Jiang Y, Liu J (2019) Definitions of pseudocapacitive materials: a brief review. Energy Environ Mater 2:30–37. https://doi.org/10.1002/eem2.12028

Jiao SQ, Zhou AG, Wu MZ, Hu HB (2019) Kirigami patterning of MXene/bacterial cellulose composite paper for all-solid-state stretchable micro-supercapacitor arrays. Adv Sci 6:1900529. https://doi.org/10.1002/advs.201900529

Kaewnopparat S, Sansernluk K, Faroongsarng D (2008) Behavior of freezable bound water in the bacterial cellulose produced by Acetobacter xylinum: an approach using thermoporosimetry. AAPS Pharm Sci Tech 9(2):701–707. https://doi.org/10.1208/s12249-008-9104-2

Kim SY, Hong J, Kavian R, Lee SW, Hyder MN, Horn YS, Hammond PT (2013) Rapid fabrication of thick spray layer-by-layer carbon nanotube electrodes for high power and energy devices. Energy Environ Sci 6:888–897. https://doi.org/10.1039/C2EE23318E

Kim BC, Hong J-Y, Wallace GG, Park HS (2015) Recent progress in flexible electrochemical capacitors: electrode materials, device configuration, and functions. Adv Energy Mater 5:1500959. https://doi.org/10.1002/aenm.201500959

Klaus T, Joerger R, Olsson E, Granqvist C-G (1999) Silver-based crystalline nanoparticles, microbially fabricated. Proc Natl Acad Sci U S A 96(24):13611–13614. https://doi.org/10.1073/pnas.96.24.13611

Klemm D, Kramer F, Moritz S, Lindström T, Ankerfors M, Gray D, Dorris A (2011) Nanocelluloses: a new family of nature-based materials. Angew Chem Int Ed 50:5438–5466. https://doi.org/10.1002/anie.201001273

Kotz R, Carlen M (2000) Principles and applications of electrochemical capacitors. Electrochim Acta 45(15–16):2483–2498. https://doi.org/10.1016/S0013-4686(00)00354-6

Krishnan S, Manavathu EK, Chandrasekar PH (2009) Aspergillus flavus: an emerging non-fumigatus Aspergillus species of significance. Mycoses 52:206–222. https://doi.org/10.1111/j.1439-0507.2008.01642.x

Lahoz R, Ibeas JG (1968) The autolysis of Aspergihs flavus in an alkaline medium. Microbiology 53:101

Lakshmi SD, Avti PK, Hegde G (2018) Activated carbon nanoparticles from biowaste as new generation antimicrobial agents: a review. Nano Struct Nano Objects 16:306–321. https://doi.org/10.1016/j.nanoso.2018.08.001

Larcher D, Tarascon JM (2015) Towards greener and more sustainable batteries for electrical energy storage. Nat Chem l7:19–29. https://doi.org/10.1038/nchem.2085

Le Fevre LW, Cao J, Kinloch IA, Forsyth AJ, Dryfe RAW (2019) Systematic comparison of graphene materials for supercapacitor electrodes. Chem Open 8(4):418–428. https://doi.org/10.1002/open.201900004

Lee YJ, Yi H, Kim WJ, Kang K, Yun DS, Strano MS, Ceder G, Belcher AM (2009) Fabricating genetically engineered high-power lithium-ion batteries using multiple virus genes. Science 324 (5930):1051–1055. https://doi.org/10.1126/science.1171541

Lee K-Y, Qian H, Tay FH, Blaker JJ, Kazarian SG, Bismarck A (2013) Bacterial cellulose as source for activated nanosized carbon for electric double layer capacitors. J Mater Sci 48:367–376. https://doi.org/10.1007/s10853-012-6754-y

Lee K-Y, Aitomäki Y, Berglund LA, Oksman K, Bismarck A (2014) On the use of nanocellulose as reinforcement in polymer matrix composites. Compos Sci Technol 105:15–27. https://doi.org/10.1016/j.compscitech.2014.08.032
Lei W, Han L, Xuan C, Lin R, Liu H, Xin HL, Wang D (2016) Nitrogen-doped carbon nanofibers derived from polypyrrole coated bacterial cellulose as high-performance electrode materials for supercapacitors and Li-ion batteries. Electrochimica Acta 210:130–137. https://doi.org/10.1016/j.electacta.2016.05.158
Lei J, Guo Q, Yao W, Duan T, Chen P, Zhu W (2018) Bioconcentration of organic dyes via fungal hyphae and their derived carbon fibers for supercapacitors. J Mater Chem A 6:10710–10717. https://doi.org/10.1039/C8TA02655F
Li X, Wei B (2013) Supercapacitors based on nanostructured carbon. Nano Energy 2(2):159–173. https://doi.org/10.1016/j.nanoen.2012.09.008
Li S, Huang D, Yang J, Zhang B, Zhang X, Yang G, Wang M, Shen Y (2014a) Freestanding bacterial cellulose–polypyrrole nanofibres paper electrodes for advanced energy storage device. Nano Energy 9:309–317. https://doi.org/10.1016/j.nanoen.2014.08.004
Li S, Huang D, Zhang B, Xu X, Wang M, Yang G, Shen Y (2014b) Electrodes: flexible supercapacitors based on bacterial cellulose paper electrodes (Adv. Energy Mater. 10/2014). Adv Energy Mater 4. https://doi.org/10.1002/aenm.201470050
Li Q, Liu D, Jia Z, Csetenyi L, Gadd GM (2016) Fungal biomineralization of manganese as a novel source of electrochemical materials. Curr Biol 26(7):950–955. https://doi.org/10.1016/j.cub.2016.01.068
Li E, Liu R, Huang S, Mei J, Xu J, Yuan G (2017) Flexible N-doped active carbon/bacterial cellulose paper for supercapacitor electrode with high areal performance. Synth Met 226:104–112. https://doi.org/10.1016/j.synthmet.2017.02.008.F
Li C, Xie B, He Z, Chen J, Long Y (2019) 3D structure fungi-derived carbon stabilized stearic acid as a composite phase change material for thermal energy storage. Renew Energy 140:862–873. https://doi.org/10.1016/j.renene.2019.03.121
Liang H-W, Guan Q-F, Zhu Z, Song L-T, Yao H-B, Lei X, Yu S-H (2012) Highly conductive and stretchable conductors fabricated from bacterial cellulose. NPG Asia Mater 4:e19. https://doi.org/10.1038/am.2012.34
Lin S-P, Calvar IL, Catchmark JM, Liu J-R, Demirci A, Cheng K-C (2013) Biosynthesis, production and applications of bacterial cellulose. Cellulose 20:2191–2219. https://doi.org/10.1007/s10570-013-9994-3
Lingzhi (mushroom) (n.d.). https://en.wikipedia.org/wiki/Lingzhi_(mushroom) (*Ganoderma lingzhi*)
Liu Y, Goebl J, Yin Y (2013) Templated synthesis of nanostructured materials. Chem Soc Rev 42:2610. https://doi.org/10.1039/C2CS35369E
Liu Y, Zhou J, Zhu E, Tang J, Liu X, Tang W (2015a) Facile synthesis of bacterial cellulose fibres covalently intercalated with graphene oxide by one-step cross-linking for robust supercapacitors. J Mater Chem C 3:1011–1017. https://doi.org/10.1039/C4TC01822B
Liu Y, Zhou J, Tang J, Tang W (2015b) Three-dimensional chemically bonded polypyrrole/bacterial cellulose/graphene composites for high-performance supercapacitors. Chem Mater 27(20):7034–7041. https://doi.org/10.1021/acs.chemmater.5b03060
Liu J, Zheng Y, Hong Z, Cai K, Zhao F, Han H (2016a) Microbial synthesis of highly dispersed PdAu alloy for enhanced electrocatalysis. Sci Adv 2:e1600858. https://doi.org/10.1126/sciadv.1600858
Liu R, Ma L, Huang S, Mei J, Xu J, Yuan G (2016b) Large areal mass, flexible and freestanding polyaniline/bacterial cellulose/graphene film for high-performance supercapacitors. RSC Adv 6:107426–107432. https://doi.org/10.1039/C6RA21920A
Liu R, Ma L, Huang S, Mei J, Li E, Yuan G (2016c) Large areal mass, high scalable and flexible cobalt oxide/graphene/bacterial cellulose electrode for supercapacitors. J Phys Chem C 120(50):28480–28488. https://doi.org/10.1021/acs.jpcc.6b10475

Liu R, Ma L, Huang S, Mei J, Xu J, Yuan G (2017) A flexible polyaniline/graphene/bacterial cellulose supercapacitor electrode. New J Chem 41:857–864. https://doi.org/10.1039/C6NJ03107B

Liu KK, Jiang Q, Kacica C, Derami HG, Biswas P, Singamaneni S (2018a) Flexible solid-state supercapacitor based on tin oxide/reduced graphene oxide/bacterial nanocellulose. RSC Adv 8:31296–31302. https://doi.org/10.1039/c8ra05270k

Liu Y, Chen J, Cui B, Yin P, Zhang C (2018b) Design and preparation of biomass-derived carbon materials for supercapacitors: a review. C-J Carbon Res 4:53. https://doi.org/10.3390/c4040053

Lomonossoff GP, Wege C (2018) TMV particles: the journey from fundamental studies to bionanotechnology applications. Adv Virus Res 102:149–176. https://doi.org/10.1016/bs.aivir.2018.06.003

Long CL, Qi DP, Wei T, Yan J, Jiang LL, Fan ZJ (2014) Nitrogen-doped carbon networks for high energy density supercapacitors derived from polyaniline coated bacterial cellulose. Adv Funct Mater 24:3953–3961. https://doi.org/10.1002/adfm.201304269

Luo H, Xiong G, Yang Z, Raman SR, Si H, Wan Y (2014) A novel three-dimensional graphene/bacterial cellulose nanocomposite prepared by in situ biosynthesis. RSC Adv 4:14369–14372. https://doi.org/10.1039/C4RA00318G

Luo H, Ao H, Li G, Li W, Xiong G, Zhu Y, Wan Y (2017) Bacterial cellulose/graphene oxide nanocomposite as a novel drug delivery system. Curr Appl Phys 17:249–254. https://doi.org/10.1016/j.cap.2016.12.001

Luo H, Xie J, Xiong L, Zhu Y, Yang Z, Wan Y (2019) Fabrication of flexible, ultra-strong, and highly conductive bacterial cellulose-based paper by engineering dispersion of graphene nanosheets. Compos Part B 162:484–490. https://doi.org/10.1016/j.compositesb.2019.01.027

Lv X, Li G, Li D, Huang F, Liu W, Wei Q (2017) A new method to prepare no-binder, integral electrodes-separator, asymmetric all-solid-state flexible supercapacitor derived from bacterial cellulose. J Phys Chem Solids 110:202–210. https://doi.org/10.1016/j.jpcs.2017.06.017

Lv X, Li G, Zhou H, Li D, Zhang J, Pang Z, Lv P, Cai Y, Huang F, Wei Q (2018) Novel freestanding N-doped carbon coated Fe_3O_4 nanocomposites with 3D carbon fibers network derived from bacterial cellulose for supercapacitor application. J Electroanal Chem 810:18–26. https://doi.org/10.1016/j.jelechem.2017.12.082

Lyu L, Seong K, Ko D, Choi J, Lee C, Hwang T, Cho Y, Jin X, Zhang W, Pang H, Piao Y (2019) Recent development of biomass-derived carbons and composites as electrode materials for supercapacitors. Mater Chem Front 3:2543. https://doi.org/10.1039/C9QM00348G

M13 bacteriophage (n.d.). https://en.wikipedia.org/wiki/M13_bacteriophage

Ma L, Liu R, Niu H, Zhao M, Huang Y (2016a) Flexible and freestanding electrode based on polypyrrole/graphene/bacterial cellulose paper for supercapacitor. Compos Sci Technol 137:87–93. https://doi.org/10.1016/j.compscitech.2016.10.027

Ma L, Liu R, Niu H, Wang F, Liu L, Huang Y (2016b) Freestanding conductive film based on polypyrrole/bacterial cellulose/graphene paper for flexible supercapacitor: large areal mass exhibits excellent areal capacitance. Electrochim Acta 222:429–437. https://doi.org/10.1016/j.electacta.2016.10.195

Ma L, Liu R, Niu H, Xing L, Liu L, Huang Y (2016c) Flexible and freestanding supercapacitor electrodes based on nitrogen-doped carbon networks/graphene/bacterial cellulose with ultrahigh areal capacitance. ACS Appl Mater Interfaces 8(49):33608–33618. https://doi.org/10.1021/acsami.6b11034

Ma L, Bi Z, Xue Y, Zhang W, Huang Q, Zhang L, Huang Y (2020) Bacterial cellulose: an encouraging eco-friendly nano-candidate for energy storage and energy conversion. J Mater Chem A 8:5812–5842. https://doi.org/10.1039/C9TA12536A

Maiti UN, Lim J, Lee KE, Lee WJ, Kim SO (2014) Three-dimensional shape engineered, interfacial gelation of reduced graphene oxide for high rate, large capacity supercapacitors. Adv Mater 26:615–619. https://doi.org/10.1002/adma.201303503

Majumdar D (2016) Functionalized-graphene/polyaniline nanocomposites as proficient energy storage material: an overview. Innov Energy Res 5(2):145(1-9)

Majumdar D (2018) An overview on ruthenium oxide composites—challenging material for energy storage applications. Mater Sci Res India 15(1):30–40. https://doi.org/10.13005/msri/150104

Majumdar D (2019a) Polyaniline as proficient electrode material for supercapacitor applications: polyaniline nanocomposites: innovative materials for supercapacitor applications—PANI nanocomposites for supercapacitor applications. In: Ramdani N (ed) Polymer nanocomposites for advanced engineering and military applications. IGI Global, Hershey . ISBN13: 9781522578383|ISBN10: 1522578382, EISBN13: 9781522578390. https://doi.org/10.4018/978-1-5225-7838-3.ch007

Majumdar D (2019b) PANI nanocomposites for supercapacitor applications. In: Ramdani N (ed) Polymer nanocomposites for advanced engineering and military applications. IGI Global, Hershey. ISBN13: 9781522578383|ISBN10: 1522578382, EISBN13: 9781522578390. https://doi.org/10.4018/978-1-5225-7838-3.ch008

Majumdar D, Bhattacharya SK (2017) Sonochemically synthesized hydroxy-functionalized graphene–MnO2 nanocomposite for supercapacitor applications. J Appl Electrochem 47 (7):789–801. https://doi.org/10.1007/s10800-017-1080-3

Majumdar D, Maiyalagan T, Jiang Z (2019a) Recent progress in ruthenium oxide-based composites for supercapacitor applications. ChemElectroChem 6(17):4343–4372. https://doi.org/10.1002/celc.201900668

Majumdar D, Mandal M, Bhattacharya SK (2019b) V2O5 and its' carbon-based nanocomposites for supercapacitor applications. ChemElectroChem 6:1623. https://doi.org/10.1002/celc.201801761

Majumdar D, Mandal M, Bhattacharya SK (2020) Journey from supercapacitors to supercapatteries: recent advancements in electrochemical energy storage systems. Emerg Mater 3:347–367. https://doi.org/10.1007/s42247-020-00090-5

Malvankar NS, Mester T, Tuominen MT, Lovley DR (2012) Supercapacitors based on c-type cytochromes using conductive nanostructured networks of living bacteria. ChemPhysChem 13:463–468. https://doi.org/10.1002/cphc.201100865

Mei B-A, Munteshari O, Lau J, Dunn B, Pilon L (2018) Physical interpretations of Nyquist plots for EDLC electrodes and devices. J Phys Chem C 122(1):194–206. https://doi.org/10.1021/acs.jpcc.7b10582

Meng C, Gall OZ, Irazoqui PP (2013) A flexible super-capacitive solid-state power supply for miniature implantable medical devices. Biomed Microdevices 15(6):973–983. https://doi.org/10.1007/s10544-013-9789-1

Meng Q, Cai K, Chen Y, Chen L (2017) Research progress on conducting polymer based supercapacitor electrode materials. Nano Energy 36:268–285. https://doi.org/10.1016/j.nanoen.2017.04.040

Micrococcus (n.d.). https://en.wikipedia.org/wiki/Micrococcus

Miller RA, Presley AD, Francis MB (2007) Self-assembling light-harvesting systems from synthetically modified tobacco mosaic virus coat proteins. J Am Chem Soc 129(11):3104–3109. https://doi.org/10.1021/ja063887t

Miller EE, Hua Y, Tezel FH (2018) Materials for energy storage: review of electrode materials and methods of increasing capacitance for supercapacitors. J Energy Storage 20:30–40. https://doi.org/10.1016/j.est.2018.08.009

Miyajima N, Jinguji K, Matsumura T, Matsubara T, Sakane H, Akatsu T, Tanaike O (2016) A simple synthesis method to produce metal oxide loaded carbon paper using bacterial cellulose gel and characterization of its electrochemical behavior in an aqueous electrolyte. J Phys Chem Solids 91:122–127. https://doi.org/10.1016/j.jpcs.2016.01.007

Moradi M, Li Z, Qi J, Xing W, Xiang K, Chiang Y-M, Belcher AM (2015) Improving the capacity of sodium ion battery using a virus-templated nanostructured composite cathode. Nano Lett 15:2917–2921. https://doi.org/10.1021/nl504676v

Najib S, Erdem E (2019) Current progress achieved in novel materials for supercapacitor electrodes: mini review. Nanoscale Adv 1:2817. https://doi.org/10.1039/C9NA00345B

Nam KT, Kim D-W, Yoo PJ, Chiang C-Y, Meethong N, Hammond PT, Chiang Y-M, Belcher AM (2006) Virus-enabled synthesis and assembly of nanowires for lithium ion battery electrodes. Science 312:885–888. https://doi.org/10.1126/science.1122716

Naoi K, Simon P (2008) New materials and new configurations for advanced electrochemical capacitors. Electrochem Soc Interface 17:34–37

Neurospora crassa (n.d.). https://en.wikipedia.org/wiki/Neurospora_crassa

Ni D, Wang L, Sun Y, Guan Z, Yang S, Zhou K (2010) Amphiphilic hollow carbonaceous microspheres with permeable shells. Angew Chem Int Ed Engl 49(25):4223–4227. https://doi.org/10.1002/anie.201000697

Nurfarahin AH, Mohamed MS, Phang LY (2018) Culture medium development for microbial-derived surfactants production—an overview. Molecules (Basel, Switzerland) 23(5):1049. https://doi.org/10.3390/molecules23051049

Obreja VVN (2008) On the performance of supercapacitors with electrodes based on carbon nanotubes and carbon activated material—a review. Physica E 40(7):2596–2605. https://doi.org/10.1016/j.physe.2007.09.044.C

Oh D, Dang X, Yi H, Allen MA, Xu K, Lee YJ, Belcher AM (2012) Graphene sheets stabilized on genetically engineered M13 viral templates as conducting frameworks for hybrid energy-storage materials. Small 8(7):1006–1011. https://doi.org/10.1002/smll.201102036

Omidi-Khaniabadi Y, Jafari A, Nourmoradi H, Taheri F, Saeedi S (2015) Adsorption of 4-chlorophenol from aqueous solution using activated carbon synthesized from aloe vera green wastes. J Adv Environ Health Res 3(2):120–129

Peng S, Xu Q, Fan L, Wei C, Bao H, Xu W, Xu J (2016) Flexible polypyrrole/cobalt sulfide/ bacterial cellulose composite membranes for supercapacitor application. Synth Met 222:285–292. https://doi.org/10.1016/j.synthmet.2016.11.002

Peng S, Fan L, Wei C, Liu X, Zhang H, Xu W, Xua J (2017) Flexible polypyrrole/copper sulfide/ bacterial cellulose nanofibrous composite membranes as supercapacitor electrodes. Carbohydr Polym 157:344–352. https://doi.org/10.1016/j.carbpol.2016.10.004

Phallus indusiatus (n.d.). https://en.wikipedia.org/wiki/Phallus_indusiatus

Picheth GF, Pirich CL, Sierakowski MR, Woehl MA, Sakakibara CN, Fernandes de Souza C, Martin AA, da Silva R, de Freitas RA (2017) Bacterial cellulose in biomedical applications: a review. Int J Biol Macromol 104(A):97–106. https://doi.org/10.1016/j.ijbiomac.2017.05.171

Pires DP, Cleto S, Sillankorva S, Azeredo J, Lu TK (2016) Genetically engineered phages: a review of advances over the last decade. Microbiol Mol Biol Rev 80(3):523–543. https://doi.org/10.1128/MMBR.00069-15

Pomerantseva E, Gerasopoulos K, Chen X, Rubloff G, Ghodssi R (2012) Electrochemical performance of the nanostructured biotemplated V2O5 cathode for lithium-ion batteries. J Power Sources 206:282–287. https://doi.org/10.1016/j.jpowsour.2012.01.127

Poonam SK, Arora A, Tripathi SK (2019) Review of supercapacitors: materials and devices. J Energy Storage 21:801–825. https://doi.org/10.1016/j.est.2019.01.010

Pseudomonas aeruginosa 01.jpg (n.d.). https://commons.wikimedia.org/wiki/File:Pseudomonas_aeruginosa_01.jpg

Raza W, Ali F, Raza N, Luo Y, Kim K-H, Yang J, Kumar S, Mehmood A, Kwon EE (2018) Recent advancements in supercapacitor technology. Nano Energy 52:441–473. https://doi.org/10.1016/j.nanoen.2018.08.013

Ren Y, Wong SM, Lim LY (2010) Application of plant viruses as nano drug delivery systems. Pharm Res 27:2509–2513. https://doi.org/10.1007/s11095-010-0251-2

Reverberi AP, Kuznetsov NT, Meshalkin VP, Salerno M, Fabiano B (2016) Systematical analysis of chemical methods in metal nanoparticles synthesis. Theor Found Chem Eng 50:59–66. https://doi.org/10.1134/S0040579516010127

Rhizobia (n.d.). https://en.wikipedia.org/wiki/Rhizobia

Saccharomyces cerevisiae (n.d.). https://en.wikipedia.org/wiki/Saccharomyces_cerevisiae

Santoro C, Arbizzani C, Erable B, Ieropoulos I (2017) Microbial fuel cells: from fundamentals to applications. A review. J Power Sources 356:225–244. https://doi.org/10.1016/j.jpowsour.2017.03.109

Sarcina (bacterium) (n.d.). https://en.wikipedia.org/wiki/Sarcina_(genus)

Schopf D, Es-Souni M (2017) Thin film nanocarbon composites for supercapacitor applications. Carbon 115:449–459. https://doi.org/10.1016/j.carbon.2017.01.027

Selvakumar R, Seethalakshmi N, Thavamani P, Naidu R, Megharaj M (2014) Recent advances in the synthesis of inorganic nano/microstructures using microbial biotemplates and their applications. RSC Adv 4:52156–52169. https://doi.org/10.1039/c4ra07903e

Shang T, Xu Y, Li P, Han J, Wu Z, Tao Y, Yang Q-H (2020) A bio-derived sheet-like porous carbon with thin-layer pore walls for ultrahigh-power supercapacitors. Nano Energy 70:104531. https://doi.org/10.1016/j.nanoen.2020.104531

Shen SH, Zhou RF, Li YH, Liu B, Pan GX, Liu Q, Xiong QQ, Wang XL, Xia XH, Tu JP (2019) Bacterium, fungus, and virus microorganisms for energy storage and conversion. Small Methods 3:1900596. https://doi.org/10.1002/smtd.201900596

Shi S, Xu C, Yang C, Li J, Du H, Li B, Kang F (2013) Flexible supercapacitors. Particuology 11 (4):371–377. https://doi.org/10.1016/j.partic.2012.12.004

Shim H-W, Jin Y-H, Seo S-D, Lee S-H, Kim D-W (2011) Highly reversible lithium storage in bacillus subtilis-directed porous Co_3O_4 nanostructures. ACS Nano 5:443–449. https://doi.org/10.1021/nn1021605

Shim H-W, Lim A-H, Kim J-C, Jang E, Seo S-D, Lee G-H, Kim TD, Kim D-W (2013) Scalable one-pot bacteria-templating synthesis route toward hierarchical, porous-Co3O4 superstructures for supercapacitor electrodes. Sci Rep 3:2325. https://doi.org/10.1038/srep02325

Shu Y, Bai Q, Fu G, Xiong Q, Li C, Ding H, Shen Y, Uyama H (2020) Hierarchical porous carbons from polysaccharides carboxymethyl cellulose, bacterial cellulose, and citric acid for supercapacitor. Carbohydr Polym 227:115346. https://doi.org/10.1016/j.carbpol.2019.115346

Simon P, Gogotsi Y (2008) Materials for electrochemical capacitors. Nat Mater 7:845–854. https://doi.org/10.1038/nmat2297

Singh V, Chakarvarti SK (2016) Bio-templates and their uses in nanomaterials synthesis: a review. Am J Bioeng Biotechnol 2(1):1–14. https://doi.org/10.7726/ajbebt.2016.1001

Stephanopoulos N, Ortony JH, Stupp SI (2013) Self-assembly for the synthesis of functional biomaterials. Acta Mater 61(3):912–930. https://doi.org/10.1016/j.actamat.2012.10.046

Stoller MD, Ruoff RS (2010) Best practice methods for determining an electrode material's performance for ultracapacitors. Energy Environ Sci 3:1294–1301. https://doi.org/10.1039/C0EE00074D

Stumpf TR, Yang X, Zhang J, Cao X (2018) In situ and ex situ modifications of bacterial cellulose for applications in tissue engineering. Mater Sci Eng C 82:372–383. https://doi.org/10.1016/j.msec.2016.11.121

Subbiah KA, Balan V (2015) A comprehensive review of tropical milky white mushroom (Calocybe indica P&C). Mycobiology 43(3):184–194. https://doi.org/10.5941/MYCO.2015.43.3.184

Sumboja A, Liu J, Zheng WG, Zong Y, Zhang H, Liu Z (2018) Electrochemical energy storage devices for wearable technology: a rationale for materials selection and cell design. Chem Soc Rev 47:5919–5945. https://doi.org/10.1039/C8CS00237A

Sun H, Cao L, Lu L (2012) Bacteria promoted hierarchical carbon materials for high-performance supercapacitor. Energy Environ Sci 5:6206–6213. https://doi.org/10.1039/C2EE03407G

Sun H, He W, Zong C, Lu L (2013) Template-free synthesis of renewable macroporous carbon via yeast cells for high-performance supercapacitor electrode materials. ACS Appl Mater Interfaces 5:2261–2268. https://doi.org/10.1021/am400206r

Tang L, Han J, Jiang Z, Chen S, Wang H (2015) Flexible conductive polypyrrole nanocomposite membranes based on bacterial cellulose with amphiphobicity. Carbohydr Polym 117:230–235. https://doi.org/10.1016/j.carbpol.2014.09.049

Tarascon J (2009) Viruses electrify battery research. Nat Nanotechnol 4:341–342. https://doi.org/10.1038/nnano.2009.137

Tasaki S, Nakayama M, Shoji W (2017) Morphologies of Bacillus subtilis communities responding to environmental variation. Develop Growth Differ 59:369–378. https://doi.org/10.1111/dgd.12383

Tobacco mosaic virus (n.d.). https://en.wikipedia.org/wiki/Tobacco_mosaic_virus

Tolle FJ, Fabritius M, Mulhaupt R (2012) Emulsifier-free graphene dispersions with high graphene content for printed electronics and freestanding graphene films. Adv Funct Mater 22:1136–1144. https://doi.org/10.1002/adfm.201102888

Tremella fuciformis (n.d.). https://en.wikipedia.org/wiki/Tremella_fuciformis

Tshikantwa TS, Ullah MW, He F, Yang G (2018) Current trends and potential applications of microbial interactions for human welfare. Front Microbiol 9:1156. https://doi.org/10.3389/fmicb.2018.01156

Ukanwa KS, Patchigolla K, Sakrabani R, Anthony E, Mandavgane S (2019) A review of chemicals to produce activated carbon from agricultural waste biomass. Sustainability 11:6204. https://doi.org/10.3390/su11226204

Ummartyotin S, Juntaro J, Sain M, Manuspiya H (2012) Development of transparent bacterial cellulose nanocomposite film as substrate for flexible organic light emitting diode (OLED) display. Ind Crop Prod 35(1):92–97. https://doi.org/10.1016/j.indcrop.2011.06.025

Vilona D, Lorenzo RD, Carraro M, Licini G, Trainotti L, Bonchio M (2015) Viral nano-hybrids for innovative energy conversion and storage schemes. J Mater Chem B 3:6718–6730. https://doi.org/10.1039/C5TB00924C

Wang J, Kaskel S (2012) KOH activation of carbon-based materials for energy storage. J Mater Chem 22:23710–23725. https://doi.org/10.1039/C2JM34066F

Wang J, Liu Q (2014) Fungi-derived hierarchically porous carbons for high-performance supercapacitors. RSC Adv 5:4396–4403. https://doi.org/10.1039/C4RA13358G

Wang DW, Li F, Zhao JP, Ren WC, Chen ZG, Tan J, Wu ZS, Gentle I, Lu GQ, Cheng HM (2009) Fabrication of graphene/polyaniline composite paper via in situ anodic electropolymerization for high-performance flexible electrode. ACS Nano 3:1745–1752. https://doi.org/10.1021/nn900297m

Wang H, Zhu E, Yang J, Zhou P, Sun D, Tang W (2012) Bacterial cellulose nanofiber-supported polyaniline nanocomposites with flake-shaped morphology as supercapacitor electrodes. J Phys Chem C 116:13013–13019. https://doi.org/10.1021/jp301099r

Wang H, Bian L, Zhou P, Tang J, Tang W (2013) Core–sheath structured bacterial cellulose/polypyrrole nanocomposites with excellent conductivity as supercapacitors. J Mater Chem A 1:578–584. https://doi.org/10.1039/C2TA00040G

Wang Z, Tammela P, Zhang P, Strømme M, Nyholm L (2014a) High areal and volumetric capacity sustainable all-polymer paper-based supercapacitors. J Mater Chem A 2:16761–16769. https://doi.org/10.1039/C4TA03724C

Wang J, Senkovska I, Kaskel S, Liu Q (2014b) Chemically activated fungi-based porous carbons for hydrogen storage. Carbon 75:372–380. https://doi.org/10.1016/j.carbon.2014.04.016

Wang Y, Guo J, Wang T, Shao J, Wang D, Yang YW (2015a) Mesoporous transition metal oxides for supercapacitors. Nanomaterials 5:1667–1689. https://doi.org/10.3390/nano5041667

Wang X, Ai W, Li N, Yu T, Chen P (2015b) Hierarchically porous N-doped carbon nanosheets derived from grapefruit peels for high-performance supercapacitors. J Mater Chem A 3:12873. https://doi.org/10.1002/slct.201600133

Wang B, Lv X, Chen S, Li Z, Sun X, Feng C, Wang H, Xu Y (2016a) In vitro biodegradability of bacterial cellulose by cellulase in simulated body fluid and compatibility in vivo. Cellulose 23:3187–3198. https://doi.org/10.1007/s10570-016-0993-z

Wang F, Kim H-J, Park S, Kee C-D, Kim S-J, Oh I-K (2016b) Bendable and flexible supercapacitor based on polypyrrole-coated bacterial cellulose core-shell composite network. Compos Sci Technol 128:33–40. https://doi.org/10.1016/j.compscitech.2016.03.012

Wang H, Yang Y, Guo L (2017a) Renewable-biomolecule-based electrochemical energy-storage materials. Adv Energy Mater 7(1-6):1700663. https://doi.org/10.1002/aenm.201700663
Wang F, Wu X, Yuan X, Liu Z, Zhang Y, Fu L, Zhu Y, Zhou Q, Wu Y, Huang W (2017b) Latest advances in supercapacitors: from new electrode materials to novel device designs. Chem Soc Rev 46:6816–6854. https://doi.org/10.1039/C7CS00205J
Wang J, Dong S, Ya BD, Xiaodong W, Hui H, Yongyao D, Zhang XX (2017c) Pseudocapacitive materials for electrochemical capacitors: from rational synthesis to capacitance optimization. Natl Sci Rev 4(1):71–90. https://doi.org/10.1093/nsr/nww072
Wang K, Xu M, Wang X, Gu Z, Fan QH, Gibbons W, Croat J (2017d) Porous carbon derived from aniline-modified fungus for symmetrical supercapacitor electrodes. RSC Adv 7:8236–8240. https://doi.org/10.1039/C6RA27600H
Wei L, Karahana HE, Zhai S, Yuan Y, Qian Q, Goh K, Ng AK, Chen Y (2016) Microbe-derived carbon materials for electrical energy storage and conversion. J Energy Chem 25(2):191–198. https://doi.org/10.1016/j.jechem.2015.12.001
Wilkinson JF (1963) Carbon and energy storage in bacteria. J Gen Microbiol 32:171–176. https://doi.org/10.1099/00221287-32-2-171
Windt WD, Aelterman P, Verstraete W (2005) Bioreductive deposition of palladium (0) nanoparticles on Shewanella oneidensis with catalytic activity towards reductive dechlorination of polychlorinated biphenyls. Environ Microbiol 7:314. https://doi.org/10.1111/j.1462-2920.2005.00696.x
Winter M, Brodd RJ (2004) What are batteries, fuel cells, and supercapacitors? Chem Rev 104:4245–4270. https://doi.org/10.1021/cr020730k
Wu R, Cui L, Chen L, Wang C, Cao C, Sheng G, Yu H, Zhao F (2013) Effects of bio-Au nanoparticles on electrochemical activity of Shewanella oneidensis wild type and ΔomcA/mtrC mutant. Sci Rep 3:3307. https://doi.org/10.1038/srep03307
Wu H, Zhang Y, Yuan W, Zhao Y, Luo S, Yuan X, Zheng L, Cheng L (2018) Highly flexible, foldable and stretchable Ni-Co layered double hydroxide/polyaniline/bacterial cellulose electrodes for high performance all-solid-state supercapacitors. J Mater Chem A 6:16617–16626. https://doi.org/10.1039/C8TA05673K
Xiea J, Yang P, Wang Y, Qi T, Lei Y, Li CM (2018) Puzzles and confusions in supercapacitor and battery: theory and solutions. J Power Sources 401:213–223. https://doi.org/10.1016/j.jpowsour.2018.08.090
Xu J, Zhu L, Bai Z, Liang G, Liu L, Fang D, Xu W (2013) Conductive polypyrrole–bacterial cellulose nanocomposite membranes as flexible supercapacitor electrode. Org Electron 14:3331–3338. https://doi.org/10.1016/j.orgel.2013.09.042
Xu X, Zhou J, Nagaraju DH, Jiang L, Marinov VR, Lubineau G, Alshareef HN, Oh M (2015) Flexible, highly graphitized carbon aerogels based on bacterial cellulose/lignin: catalyst-free synthesis and its application in energy storage devices. Adv Funct Mater 25:3193–3202. https://doi.org/10.1002/adfm.201500538
Xu Q, Fan L, Yuan Y, Wei C, Bai Z, Xu J (2016) All-solid-state yarn supercapacitors based on hierarchically structured bacterial cellulose nanofiber-coated cotton yarns. Cellulose 23:3987–3997. https://doi.org/10.1007/s10570-016-1086-8
Xue Q, Sun J, Huang Y, Zhu M, Pei Z, Li H, Wang Y, Li N, Zhang H, Zhi C (2017) Recent progress on flexible and wearable supercapacitors. Small 13:1701827. https://doi.org/10.1002/smll.201701827
Xylaria (n.d.). https://en.wikipedia.org/wiki/Xylaria
Yang Y, Wang B, Zhu J, Zhou J, Xu Z, Fan L, Zhu J, Podila R, Rao AM, Lu B (2016) Bacteria absorption-based Mn2P2O7–carbon@reduced graphene oxides for high-performance lithium-ion battery anodes. ACS Nano 10:5516–5524. https://doi.org/10.1021/acsnano.6b02036
Yang H, Ye S, Zhou J, Liang T (2019) Biomass-derived porous carbon materials for supercapacitor. Front Chem 7:274. https://doi.org/10.3389/fchem.2019.00274

Yano H, Sugiyama J, Nakagaito AN, Nogi M, Matsuura T, Hikita M, Handa K (2005) Optically transparent composites reinforced with networks of bacterial nanofibers. Adv Mater 17:153–155. https://doi.org/10.1002/adma.200400597

Yao J, Ji P, Sheng N, Guan F, Zhang M, Wang B, Chen S, Wang H (2018) Hierarchical core-sheath polypyrrole@carbon nanotube/bacterial cellulose macrofibers with high electrochemical performance for all solid- state supercapacitors. Electrochim Acta 283:1578–1588. https://doi.org/10.1016/j.electacta.2018.07.086

Yassine M, Fabri D (2017) Performance of commercially available supercapacitors. Energies 10:1340. https://doi.org/10.3390/en10091340

Yu W, Lin W, Shao X, Hu Z, Li R, Yuan D (2014) High performance supercapacitor based on Ni3S2/carbon nanofibers and carbon nanofibers electrodes derived from bacterial cellulose. J Power Sources 272:137–143. https://doi.org/10.1016/j.jpowsour.2014.08.064

Yuan Y, Zhou J, Rafiq MI, Dai S, Tang J, Tang W (2018) Growth of Ni-Mn layered double hydroxide and polypyrrole on bacterial cellulose nanofibers for efficient supercapacitors. Electrochim Acta 295:82–91. https://doi.org/10.1016/j.electacta.2018.10.090

Zang F, Chu S, Gerasopoulos K, Culver JN, Ghodssi R (2017) Biofabrication of tobacco mosaic virus nano scaffolded supercapacitors via temporal capillary microfluidics. Nanotechnology 28:265301. (9pp). https://doi.org/10.1088/1361-6528/aa742f

Zhan C, Nagui M, Lukatskaya M, Kent PRC, Gogotsi Y, Jiang D (2018) Understanding the MXene pseudocapacitance. J Phys Chem Lett 9(6):1223–1228. https://doi.org/10.1021/acs.jpclett.8b00200

Zhang LL, Zhao XS (2009) Carbon-based materials as supercapacitor electrodes. Chem Soc Rev 38:2520. https://doi.org/10.1039/B813846J

Zhang Y, Liu X, Wang S, Li L, Dou S (2017) Bio-nanotechnology in high-performance supercapacitors. Adv Energy Mater 7:1700592. https://doi.org/10.1002/aenm.201700592

Zhang X, He M, He P, Li C, Liu H, Zhang X, Ma Y (2018) Ultrafine nano-network structured bacterial cellulose as reductant and bridging ligands to fabricate ultrathin K-birnessite type MnO2 nanosheets for supercapacitors. Appl Surf Sci 433:419–427. https://doi.org/10.1016/j.apsusc.2017.10.053

Zhang P, Wang Z, Liu L, Klausen LH, Wang Y, Mi JL, Dong M (2019) Modulation the electronic property of 2D monolayer MoS2 by amino acid. Appl Mater Today 14:151–158. https://doi.org/10.1016/j.apmt.2018.12.003

Zhao N, Wu F, Xing Y, Qu W, Chen N, Shang Y, Yan M, Li Y, Li L, Chen R (2019) Flexible hydrogel electrolyte with superior mechanical properties based on poly(vinyl alcohol) and bacterial cellulose for the solid-state zinc–air batteries. ACS Appl Mater Interfaces 11 (17):15537–15542. https://doi.org/10.1021/acsami.9b00758

Zhou P, Wang H, Yang J, Tang J, Sun D, Tang W (2012) Bio-supported palladium nanoparticles as a phosphine-free catalyst for the Suzuki reaction in water. RSC Adv 2:1759–1761. https://doi.org/10.1039/C2RA01015A

Zhu H, Wang X, Yang F, Yang X (2011) Promising carbons for supercapacitors derived from fungi. Adv Mater 23:2745–2748. https://doi.org/10.1002/adma.201100901

Zhu H, Yin J, Wang X, Wang H, Yang X (2013) Microorganism-derived heteroatom-doped carbon materials for oxygen reduction and supercapacitors. Adv Funct Mater 23:1305–1312. https://doi.org/10.1002/adfm.201201643

Zou Z, Lei Y, Li Y, Zhang Y, Xiao W (2019) Nitrogen-doped hierarchical meso/microporous carbon from bamboo fungus for symmetric supercapacitor applications. Molecules 24:3677. https://doi.org/10.3390/molecules24203677

Zuo W, Li R, Zhou C, Li Y, Xia J, Liu J (2017a) Battery-supercapacitor hybrid devices: recent progress and future prospects. Adv Sci 4(1–21):1600539. https://doi.org/10.1002/advs.201600539

Zuo L, Fan W, Zhang Y, Huang Y, Gao W, Liu T (2017b) Bacterial cellulose-based sheet-like carbon aerogels for the in situ growth of nickel sulfide as high performance electrode materials for asymmetric supercapacitors. Nanoscale 9:4445–4455. https://doi.org/10.1039/C7NR00130D

Application of Microbes in Climate-Resilient Crops

3

Clement Kiing Fook Wong

Abstract

Agriculture productivity has suffered major losses due to global climate change. The United Nations has outlined a total of 17 Sustainable Development Goals (SDGs) which include zero hunger and climate action. To achieve both of these goals, sustainable measures are needed to ensure continuous production of agriculture as crops are exposed to climate change induced stress factors. The application of beneficial microbes, ranging from plant growth promoting bacteria to arbuscular mycorrhizal fungi (AMF), is a low-cost, environmental-friendly, and sustainable method in producing climate-resilient crops. These microbes were reported to confer crop tolerance to abiotic stresses via various mechanisms without compromising crop growth and yield attributes. In line with the SDG goals, this method can be conveniently adopted in poor and developing countries as it is cost-effective and requires lesser technical expertise than other currently available methods. Therefore, this book chapter aims to highlight and review previous attempts of applying various strains of beneficial microbes in the improvement of crop tolerance against heat, cold, submergence, drought, and salinity stresses. Potential limitations of applying beneficial microbes are discussed in detail and suggestions for future research directions are outlined to improve the utilization of microbes as well as to facilitate the successful adoption of this method worldwide.

C. K. F. Wong (✉)
Department of Agricultural and Food Science, Faculty of Science, Universiti Tunku Abdul Rahman, Jalan Universiti, Kampar, Perak, Malaysia
e-mail: kfwong@utar.edu.my

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Keywords

Abiotic stress · Agriculture productivity · Beneficial microbes · Climate change · SDGs · Crop tolerance

3.1 Introduction

Food insecurity has emerged as a major concern as agriculture productivity is being hampered by climate change (Kumar and Verma 2018). With the human population expected to rise exponentially to 8.9 billion by the year 2050, extensive agriculture activities are being practiced to fulfill food supply shortage but such practices have exhausted and polluted most fertile lands through the excessive use of synthetic fertilizers, pesticides, and herbicides (Singh et al. 2011; Masciarelli et al. 2014). The agroecosystem is known to be the most vulnerable towards climate change. Various abiotic factors resulting from inconsistent precipitation patterns, fluctuating temperatures, and prolonged dry spells are the primary reasons that have incurred about 50% of economic loss in the agriculture sector (Chodak et al. 2015). In developing countries badly affected by climate change, insufficient food supply and hunger remain as challenging problems (Downing 1991; Janssens et al. 2020).

In order to address the negative impacts of climate change on agriculture, extensive researches were conducted to develop strategies to cope with abiotic stresses, including breeding for stress tolerant crops, shifting of cultivation calendars, and improving resource management practices such as irrigation and fertilizer management (Grover et al. 2011; Dharmarathna et al. 2014; Arbuckle et al. 2015; Gilliham et al. 2016). Most of these approaches can be cost-intensive and most importantly, soil infertility remains unresolved. Conversely, the application of beneficial microbes is a cost-effective means to boost crop growth, enhance soil fertility through nutrient recycling, and also to induce stress tolerant responses in crops through direct and indirect mechanisms (Saxena et al. 2005; Enebe and Babalola 2018). In this chapter, the utilization of microbes in producing climate-resilient crops and their potential pitfalls were reviewed extensively. Recommendations for future research to address these limitations were also included to empower the use of naturally occurring microbes to combat climate change in the agriculture sector. In line with the United Nations Sustainable Development Goals of climate action and zero hunger (2015–2030), developing climate-resilient crops using microbes can be a sustainable and low-cost alternative that could be adopted worldwide to improve agriculture productivity and to reduce hunger especially in poor and developing countries.

3.2 Heat Stress Tolerance

In the next half of the century, an increase in earth's temperature by 2–4 °C is predicted (IPCC 2007). The rising of temperature is not desirable in the agriculture sector as most cultivated crops are not bred for heat stress tolerance (Zhu et al. 2017). Heat stress severely affects crop growth and productivity by inducing changes in crop physiology and biochemical responses, which are largely associated with seed germination arrest, reduced pollination, impaired photosynthesis mechanism, inactivation of host defense system, interference of nutrient uptake, and generation of harmful reactive oxygen species (ROS) (Hatfield and Prueger 2015; Ali et al. 2019). Frequent heat waves have devastated most agricultural productions around the world. In 2003, China reported rice yield losses amounting to 5.18 million tons due to a prolonged heat wave (Tian et al. 2009). The heat wave, that surged through the European countries in 2003, has also caused major agriculture yield losses (Ciais et al. 2005). The agriculture sector is expected to suffer losses of up to 40% if climate change-induced heat stress remains unresolved (Lobell and Tebaldi 2014).

The earliest study conducted on the potential of AMF in improving heat tolerance of crops was in 1986. Cotton plants inoculated with *Glomus intraradices*, *G. ambisporum,* and *Gigaspora margarita* showed enhanced plant growth and root length at high temperatures ranging from 24 to 36 °C with 57–80% increase in root colonization rate coupled with enhanced nutrient acquisition (Smith and Roncadori 1986). Subsequent studies also revealed that AMF was able to exert its plant growth promotion ability at higher temperatures which further proved that AMF could induce heat tolerance in crops (Raju et al. 1990; Haugen and Smith 1992; Martin and Stuz 2004; Matsubara et al. 2004; Bunn et al. 2009). Although AMF-treated crops showed improved plant biomass and root growth, certain nutrient uptake such as phosphorus (P) was reduced in AMF-treated crops during heat stress (Martin and Stuz 2004; Matsubara et al. 2004). It was suggested that less P was transferred to host plant due to lower colonization levels which caused the plant to invest more carbon in roots leading to higher root mass (Martin and Stuz 2004). The antioxidant and reactive oxygen species (ROS) scavenging activity of Cyclamen, an ornamental flowering plant was also enhanced after inoculation with *G. fasciculatum* in which treated plants showed reduction in leaf browning when exposed to heat treatment (Maya and Matsubara 2013). Similar results were reported in Asparagus plants inoculated with *G. intraradices* whereby the host antioxidative activity and nutrient acquisition were greatly enhanced during heat stress (Yeasmin et al. 2019).

Maize plants inoculated with *G. etunicatum* showed similar biomass to uninoculated plants but enhanced photosynthesis rate, water use efficiency, water content, and soil water holding capacity were observed in treated plants when exposed to heat stress (Zhu et al. 2011). Zhu et al. (2017) described that AMF regulated their own aquaporins and plant aquaporins to transport water efficiently to the host plant during heat stress. The primary function of aquaporins is to regulate water absorption capacity and hydraulic conductivity of roots in order to facilitate water flow across membranes (Kruse et al. 2006). The regulation of aquaporins by AMF could have improved the water use efficiency of maize plants. In addition, increased stomatal conductance during AMF symbiosis is also beneficial to improve gas exchange capacity and increase photosynthetic activity in maize plants (Zhu et al. 2010a, 2011). Cabral et al. (2016) discovered that the inoculation of wheat with a mixture of *Rhizophagus*, *Funneliformis,* and *Claroideoglomus* sp. affected the carbon (C) source–sink relationship during heat treatment. Due to increased C content in the spikes, the increased number of grains and enhanced photosynthetic rate were reported in wheat (Cabral et al. 2016). In contrast, higher temperature increased C allocation from plants to fungus resulting in increased carbon consumption by AMF and consequently, causing lower plant biomass (Hawkes et al. 2008). Such contrasting findings could be due to genotype factor between AMF and inoculation host. Duc et al. (2018) discovered the compatibility between AMF and host plants affected the heat tolerance mechanisms in plants. For example, the AMF *Septoglomus constrictum* and *S. deserticola* regulated heat-induced oxidative stress by decreasing peroxidation of the membrane lipid peroxidation, concentration of hydrogen peroxide, and increasing ROS scavenging activity in tomato plants. However, tomato plants inoculated with *S. constrictum* resulted in better heat tolerance due to enhanced stomatal conductivity, leaf water status, and relative moisture content compared to *S. deserticola* and uninoculated plants. Each AMF genotype differed in the capacity of mycelia spreading, colonization behavior, and viability in soil and these factors contributed to different host plant growth responses towards AMF (Giovannetti et al. 2001; Lee et al. 2013).

Biopriming of wheat seeds coated with talc powder containing thermotolerant *Pseudomonas putida* strain AKMP7 significantly improved the biomass and grain yield under heat stress (Ali et al. 2011). Inoculated plants showed reduced membrane injury and reduced antioxidant activity indicating microbial priming effect. Accumulation of essential metabolites such as proline, chlorophyll, sugars, and amino acids in treated wheat plants was reported but the microbial-mediated tolerance mechanisms in relation to these biochemical changes were not explained in detail. Seed biopriming with *Azospirillum brasilense* also increased the heat tolerance of two wheat cultivars by reducing the host defense mechanisms (the ascorbate–glutathione pathway antioxidant enzymes and heat shock proteins) as compared to control treatment (El-Daim et al. 2014). The upregulation of heat shock transcription factors at the early inoculation stage and reduced expression at a later stage indicated the microbial priming effect which enhanced wheat tolerance towards heat stress (El-Daim et al. 2014).

The application of thermotolerant *Bacillus cereus* SA1 improved biomass and chlorophyll in soybeans when exposed to heat stress (Khan et al. 2020). The endogenous phytohormone abscisic acid (ABA) was reduced which was followed by the increased in gibberellic acid (GA), salicylic acid (SA) concentration, and antioxidant enzyme activity after microbial inoculation. In general, the accumulation of GA is antagonistic towards ABA synthesis. GA improves plant growth, whereas the plant stress hormone ABA is reduced which in turn increases stomatal conductance leading to enhanced moisture and nutrient uptake (Verma et al. 2016). SA was reported to modulate antioxidant enzyme activities and several studies indicated that exogenous application of SA reduced ROS generation and promoted better growth in plants under abiotic stresses (Egamberdieva et al. 2017). Soil drench application of an endophytic fungus *Paecilomyces formosus* showed an increase in rice biomass, while the endogenous phytohormones, ABA and jasmonic acid (JA), were reduced under heat treatment (Waqas et al. 2016). As reported earlier, lower ABA level could imply stomata opening resulting in increased moisture and nutrient absorption (Khan et al. 2014; Verma et al. 2016). The reason behind low JA levels in microbial-inoculated rice plants was not investigated further. However, it was speculated that the minimal increase or reduce endogenous JA could have contributed to the plant growth promoting effects (Waqas et al. 2016). A similar study also indicated that exogenous application of JA of not more than 5 μM improved heat tolerance of Arabidopsis (Clarke et al. 2009). In short, the modulation of host plant JA by beneficial microbes during heat stress could be further studied. Besides phytohormones, microbial extracellular polymeric substances (EPS) were also found to confer heat tolerance in crops. A thermotolerant *B. cereus* showed increased EPS production and improved biomass of tomato plants when subjected to heat stress (Mukhtar et al. 2020). The microbial EPS matrix was discovered to enhance soil aggregation which benefited plants by retaining soil moisture and nutrients under environmental stress but the exact role of EPS in improving heat tolerance in crops has not been studied (Costa et al. 2018). Moreover, this study also demonstrated the bacterium's ability to decrease ethylene (ET) production by increasing the ACC deaminase activity which hydrolyzed ACC, the ET biosynthesis precursor, into ammonia and alpha ketobutyrate (Mukhtar et al. 2020). Accumulation of host plant ET during biotic and abiotic stress is harmful as high levels of ET could reduce seed germination and root development (Barnawal et al. 2012).

3.3 Cold Stress Tolerance

Approximately 64% of the earth has an average minimum temperature of 0 °C and most of the commonly cultivated agricultural crops do not have the ability to acclimate to cold temperatures (Chinnusamy et al. 2007; Rihan et al. 2017). Cold stress could potentially affect crop growth, thereby causing adverse yield loss on a global scale (Pearce and Fuller 2001). Climate change is not only characterized by increasing average temperature but also extreme annual variation in climate temperatures such as heat waves and cold waves (Rigby and Porporato 2008). In 2002–2003, the cold wave in Punjab, India has caused damage to various fruit crops from 30% to complete 100% damage (Samra et al. 2003). Unusually low temperatures in 2008 were experienced in most seasonal countries including Iran and this cold wave has killed many woody species that has previously survived up to 40 years (Jalili et al. 2010). It is evident that developing cold stress resistant cultivars and improving cold tolerance in crops are of prime importance to ensure sustainable agriculture under such unpredictable cold waves.

Various studies demonstrated that crops inoculated with either AMF or beneficial microbes (bacteria or fungus) improved the crop photosynthesis rate and hence enhanced overall plant growth during cold stress (Paradis et al. 1995; Ait-Barka et al. 2006; Zhu et al. 2010a; Mishra et al. 2011; Ghorbanpour et al. 2018; Hajiboland et al. 2019; Ma et al. 2019; Bidabadi and Mehralian 2020). Better photosynthetic capacity of crops during cold stress could be due to the microbial ability to protect the photosynthetic apparatus and to increase the carbon sink in leaves (Ma et al. 2019). Higher carbon-sink strength in leaves during cold stress is indicative of improved cold tolerance in crops. Fernandez et al. (2012a) also reported that carbohydrate metabolism in bacteria-treated plants was significantly altered during cold stress in which soluble sugars accumulation in leaves was greater than in non-inoculated plants. Even so, further validation work is required to fully understand the relationship between carbon source–sink and photosynthesis of crops during cold stress in order to shed light to the microbial-induced cold tolerance mechanism. Moreover, microbial-treated cold stress tolerant crops were also characterized by their upregulated activity of antioxidant enzymes. This feature is essential to reduce ROS accumulation which could cause membrane damage and cell electrolyte leakage (Zhu et al. 2010b; Abdel-Latef and Chaoxing 2011; Theocharis et al. 2012; Subramanian et al. 2015, 2016; Chu et al. 2016; Xiao et al. 2017; Ghorbanpour et al. 2018; Hajiboland et al. 2019; Bidabadi and Mehralian 2020).

Microbial regulation of phytohormones in crops also played an important role in developing cold tolerance. Upregulation of JA was observed in rice and a forage crop, *Digitaria eriantha* when inoculated with AMF *G. mosseae* and *R. irregularis,* respectively, under cold stress condition. The biosynthesis of JA in crops could be due to induced systemic response (ISR) of plants as a result of microbial inoculation which primed the plants to activate their early defense response prior to the onset of cold stress (Romera et al. 2019). JA accumulation has also been implicated to enhance the expression of cold tolerance related genes in many plants (Sharma and Laxmi 2015; Yang et al. 2019). The production of ACC deaminase in hydrolyzing harmful ET accumulation in common beans during cold stress was also reported as high endogenous ET level inhibited growth (Tiryaki et al. 2019). The synthesis of SA was also observed in rice inoculated with *B. laterosporus* and *B. amyloliquefaciens* which were linked to the expression of cold stress tolerant genes (Kakar et al. 2016). However, SA was known to increase cold sensitivity in plants at high concentrations which suggested that fine-tuning of SA concentration is required to achieve cold tolerance in crops (Miura et al. 2010). In another study, the ABA level of wheat was reduced, while plant growth hormones such as cytokinin and auxin were enhanced after seed biopriming of wheat with two strains of cold-tolerant strains of *Bacillus* sp. (Zubair et al. 2019). Under normal condition, lower ABA levels were found to be essential in improving photosynthetic rate of Arabidopsis inoculated with *B. subtilis* perhaps because of increased stomatal conductance (Zhang et al. 2008). Nonetheless, additional studies are required to provide a clearer understanding on the ABA biosynthesis in microbial-treated plants under cold stress since ABA levels are often upregulated in non-inoculated plants to impart cold tolerance (Sah et al. 2016; Huang et al. 2017).

Accumulation of osmolytes such as proline and trehalose was also reported to promote cold tolerance in plants (Dierking et al. 2012; Fernandez et al. 2012b; Kakar et al. 2016; Ghorbanpour et al. 2018). These osmolytes were known to protect membrane integrity (prevent cell dehydration) which helps to retain plant's capability to uptake water and nutrient during stressful conditions (Thalmann and Santelia 2017). Water uptake was maintained in *G. intraradices*-inoculated common bean and rice plants since aquaporins, which are responsible in regulating water hydraulic potential of plant tissues, were expressed during cold stress (Aroca et al. 2006; Liu et al. 2014). Inoculation with beneficial microbes has also improved nutrient acquisition in plants under cold condition. AMF-inoculated plants were found to show cold tolerance by improving P and N uptake although uptake efficiency was reduced as temperature drops (Kytöviita and Ruotsalainen 2007; Ma et al. 2015). As AMF is less frequently found in cold region, its growth and colonization behavior could be affected (Newsham et al. 2009). The dark septate endophytes served as an alternative to AMF in which plants inoculated with strains such as *Phialocephala fortinii* enhanced N and P uptake during cold stress through mineral solubilization in soil which eventually led to improved plant biomass and cold tolerance (Ruotsalainen and Kytöviita 2004; Upson et al. 2009). Using the same microbial species, Murphy et al. (2014) further explained N availability in the soil affected the microbial ability to improve plant growth as low N was proposed to compromise the potential of the endophyte to produce tryptophan, which was the precursor to the synthesis of indole acetic acid (IAA)—a common phytohormone involved in modulating plant growth.

Cucumber seedlings colonized by AMF *G. mosseae* upregulated enzymes involved in secondary metabolite synthesis, specifically the pentose-phosphate and shikimate pathways which produced phenolics, flavonoids, and lignin (Chen et al. 2013). These metabolites were involved in plant defense against various stresses and were proposed to be involved in cold tolerance of the cucumber seedlings. Unfortunately, the specific roles of these pathways in cold tolerance were not discussed in detail. Other secondary metabolites, for instance, ergot alkaloids and unsaturated fatty acids were also found to accumulate in forage grasses naturally colonized by a fungal endophyte, *Epichloë* sp. compared to non-stressed plants but their exact roles in conferring cold tolerance are not known (Zhou et al. 2015; Chen et al. 2016).

3.4 Submergence Stress Tolerance

Flooding can be caused by extensive rainfall over prolonged periods of time or the overflowing of a water body over to agricultural land. Globally, about two-thirds of crop damage and loss are due to flooding from the year 2006 to 2016 (FAO 2017). For instance, extreme monsoon rains in Pakistan from 2010 to 2014 have caused major flood which resulted in a huge loss of approximately 11 billion tons of maize, rice, sugarcane, and cotton, amounting to more than US$16 billion (Rehman et al. 2015). In the USA, crop loss was estimated to be 60 million dollars due to overflowing of the Mississippi river in 2011 (Olson and Morton 2012). Flooding can be classified as waterlogging, in which the water covers the root area and also, as submergence in which the aboveground plant parts are totally covered in water (Sasidharan et al. 2017). As climate change worsens, the frequency of heavy precipitation is forecasted to rise in future across the globe (Wright et al. 2017). In order to mitigate further crop losses, a handful of studies have demonstrated the ability of beneficial microbes in enhancing waterlogging tolerance in crops as well as maintaining or improving the growth of plants.

The colonization of two AMFs, *G. mosseae* and *G. intraradices* of rice had a significant uptake of P and K in flooded areas in comparison to non-flooded plants (Hajiboland et al. 2009). Plant biomass was improved by up to 117% compared to uninoculated ones. Inoculated rice plants had higher root growth and thus, higher root surface for better P uptake. However, low colonization rate was observed in roots as AMFs are obligate aerobes. Similar findings by Tuo et al. (2015) reported that reduced colonization of another AMF fungi, *Funneliformis mosseae* was observed in peach seedlings cultivated under waterlogged condition. Even so, the chlorophyll a and b content as well as proline content was increased. It was suggested that the ability of AMF fungi to colonize root, albeit at lower rate compared to non-stressed plants, was due to the presence of low oxygen concentration present in the aerenchyma root cells (Tuo et al. 2015). Further microscopy work is required to verify the formation of aerenchyma cells in these peach seedlings in order to better associate with the colonization pattern of AMF. A previous study has indicated that aerenchyma cells were formed in soybean roots during waterlogging stress to recover from prolonged hypoxia condition (Thomas et al. 2005). In addition, the AMF could be tagged with green fluorescent protein (GFP) to validate if colonization occurs at the aerenchyma cells in root.

The inoculation of AMF *Diversispora spurca* also exhibited reduced colonization in citrus seedlings during waterlogging stress but the number of entry points and vesicle formations have led to the improved shoot and root biomass (Wu et al. 2013). Increased leaf catalase activity was reported in inoculated plants indicating lower oxidative damage in citrus seedlings. On the contrary, Sah et al. (2006) observed that common beans inoculated with AMF *Gigaspora margarita* and *G. rosea* showed increased root colonization, whereas colonization by AMF *G. intraradices* and *Entrophospora colombiana* remained the same under waterlogging conditions. A fine root AMF endophyte, *G. tenue* was found to withstand waterlogging stress compared to other AMF species (Orchard et al. 2016). In this study, the root colonization was increased for lotus but not for ryegrass perhaps due to the abundant aerenchyma cells found in the aquatic plants such as lotus which allowed the accumulation of oxygen for *G. tenue* to penetrate and thrive under hypoxic condition. Deepika and Kothamasi (2015) also explained that optimal soil moisture was an important factor that determined the survival and colonization rate of AMF as reduction of root biomass was observed in sorghum plants after inoculation with *R. irregularis* which consequently caused poor P uptake under waterlogged condition. It is also worthy to note that colonization of AMF has been reported to be genotype dependent which could mean that screening for suitable AMF species is crucial to exert the desired protective effects on crops against waterlogging (Orchard et al. 2016).

During waterlogged stress, plants produce the stress hormone ethylene (ET) and accumulation of this hormone eventually leads to ROS generation, cell membrane damage, and root growth inhibition (Glick 2014). To avert the harmful effects of ET, some beneficial bacteria produce the enzyme ACC deaminase that degrades the substrate ACC in plants (Ali and Kim 2018). Tomato plants treated with both *Enterobacter cloacae* or *P. putida* strain UW4 showed tolerance to flood stress due to expression of microbial ACC deaminase (Grichko and Glick 2001). Transgenic *Mesorhizobium* sp. containing the ACC deaminase gene from *P. putida* UW4 also improved the nodulation in chickpea plants under waterlogged condition than non-stress condition (Nascimento et al. 2012). Li et al. (2013) further investigated the effect of *P. putida* UW4 on the proteome profile of cucumber when subjected to submergence stress. In general, proteins involved in nitrogen metabolism, carbohydrate metabolism, antioxidant, and defense stress were greatly induced in microbial-inoculated cucumber roots which suggested that microbial-mediated submergence stress tolerance in plants is a dynamic regulation of various metabolic pathways (Li et al. 2013). The utilization of this bacterial strain should also consider the ecological context of the plant. Plants living in the wetlands or riparian regions have evolved the ability to elongate stems and leaves to avoid hypoxia condition through the generation of endogenous ET. The application of *P. putida* UW4 on wetland plants such as *Rumex palustris* has reduced ET production which in turn impeded stem elongation during flood stress (Ravanbakhsh et al. 2017).

Ongoing efforts in prospecting potential endophytes conferring crop tolerance to waterlogging stress were reported. A comparative study of naturally colonized *Hordeum brevisubulatum* by a foliar endophytic fungus, *Epichloë* sp. was found to have greater root biomass, tiller production, and chlorophyll content than endophyte-free plants under waterlogged condition (Song et al. 2015). The colonized plants induced the osmo-protective proline production and lower membrane damage which were indicated by lower MDA content and electrolyte leakage, as well as reduced oxidative damage (Song et al. 2015). In another study, the inoculation of foliar *Epichloë* sp. onto its natural host marsh bluegrass (*Poa leptocoma*) did not improve submergence tolerance compared to plants cultivated under normal condition (Adams et al. 2017). The endophyte-symbiotic plants produced lesser seed count, reduced germination rate, and seedling survival under flooded soils despite improved plant biomass was observed. In other words, more studies are warranted to unravel the colonization behavior of this endophyte in various crops to ascertain its potential as ameliorative agent against submergence stress. In another recent study, a novel styrene antioxidant NFA (*Z*-*N*-(4-hydroxystyryl) formamide) compound was isolated from a riparian endophytic fungus *Aspergillus fumigatus* and its application has improved submergence tolerance in Arabidopsis (Xue et al. 2020). This metabolite was found to regulate ROS accumulation, antioxidant enzymes, and reduced MDA content during submergence. Such novel study could pave the way for the search of other potential and novel metabolites present in endophytes that could improve crop tolerance against submergence stress.

3.5 Salinity and Drought Stress Tolerance

Salinity is a common problem in dry areas where high temperature and low rainfall lead to inadequate amount of rain to filter away the excessive salts from the salt-sensitive root zone (Shrivastava and Kumar 2015). Soil salinity remains as a menacing problem to the agriculture sector and the ongoing global climate change crisis could further accelerate the salinization process (FAO 2015). This phenomenon poses a serious threat in food security as agriculture land such as the delta areas of India, Myanmar, and Bangladesh, where rice is produced, are facing salinity problems (Abedin et al. 2014; Szabo et al. 2016). About one billion hectares of land across the world may encounter salinization with crop production loss of more than 20% (FAO 2015). The United States has lost about 3.7 billion dollars in crop yield annually because of salinity whereas about 2531% of yield loss was reported in Canada and Pakistan (Dove 2017; Ilyas 2017).

Reduced precipitation has also caused the frequent onset of drought globally which led to severe decline in crop yield (Lobell et al. 2011). On a global scale, about 21 and 40% of yield reductions were reported for wheat and maize due to drought from the year 1980 to 2015 (Daryanto et al. 2016). In Africa, unpredictable drought seasons reduced yield of cowpea from 34 to 68% (Farooq et al. 2017). The rising levels of carbon emissions including carbon dioxide and methane for the past 250 years are expected to cause an average spike of 0.2 °C in every decade (Fahad et al. 2017). In order to counter the negative impacts of drought and salinity on crop yield, applying beneficial microbes is a promising method to impart crop tolerance while enhancing crop yield under stressful conditions.

To date, there are numerous comprehensive reviews available that highlight the potential of beneficial microbes such as plant growth promoting microbes in protecting crops against excessive salt in soil (Porcel et al. 2012; Ruppel et al. 2013; Etesami and Beattie 2018; Numan et al. 2018; Egamberdieva et al. 2019; Evelin et al. 2019). Similar to salinity stress, the beneficial effects of applying microbes to confer drought tolerance in crops were reviewed extensively (Vurukonda et al. 2015; Fahad et al. 2017; Mathimaran et al. 2017; Kerry et al. 2018; Bahadur et al. 2019; de Vries et al. 2020). The crop tolerance mechanisms against salinity and drought stress as mediated by these microbes are summarized in Tables 3.1 and 3.2. Although the research progress is evident in understanding the ameliorative effect of beneficial microbes on crops during salinity and drought stress, most studies were confined to greenhouse and only a handful of short-term field experiments have been conducted. There are a number of research avenues that are worth looking at and perhaps, the research outcomes could lay the foundation of adopting this strategy for developing sustainable agriculture practices in saline and drought affected soils.

1. Understanding the molecular adaptations and physiological changes of crops during salinity and drought conditions due to microbial application is crucial as such knowledge can help in designing optimal application of suitable strains to achieve consistent management of crop loss under field condition.

Table 3.1 General salinity tolerance mechanisms in crops mediated by the application of plant growth promoting bacteria and AMF

Salinity tolerance mechanisms	
Plant growth promoting bacteria	Arbuscular mycorrhizal fungi (AMF)
Upregulation of ACC deaminase enzyme activity	Alteration of root architecture
Phytohormone regulation	Phytohormone regulation
Enhanced phosphate solubilization	Nutrient acquisition and ion homeostasis
Enhanced nitrogen fixation	Osmoregulation
Osmoregulation	Oxidative stress regulation
Production of exopolysaccharides (EPS)	Water status regulation
Oxidative stress regulation	Photosynthesis regulation

Table 3.2 General drought tolerance mechanisms in crops mediated by the application of plant growth promoting bacteria and AMF

Drought tolerance mechanisms	
Plant growth promoting bacteria	Arbuscular mycorrhizal fungi (AMF)
Upregulation of ACC deaminase enzyme activity	Alteration of root architecture
Phytohormone regulation	Phytohormone regulation
Osmoregulation	Osmoregulation
Production of exopolysaccharides (EPS)	Water status regulation
Oxidative stress regulation	Oxidative stress regulation
Improved nutrient acquisition	Improved nutrient acquisition
Improved photosynthesis capacity	Improved photosynthesis capacity
Alteration of cell wall architecture	

2. Other potential mineral elements such as sulfur uptake are an essential component in the ABA biosynthesis and oxidative stress pathways. AMF was reported to improve sulfur uptake (Allen and Shachar-Hill 2009) but the plant sulfur uptake as a result of AMF colonization during salinity and drought stress has yet to receive attention.
3. Cell wall strengthening is plant's basal defense against biotic and abiotic stresses (van der Does et al. 2017). However, the role of microbial-induced cell wall strengthening during salinity and drought stress is not well elucidated.
4. Saline irrigation water is often overlooked and prospecting salt-tolerant microbes (halophytes) from these areas is needed besides saline soils (Ruppel et al. 2013). In fact, the metagenomic approach could be utilized to pinpoint dominant strains and may provide clues to culturable strains to be used as salt ameliorative agents (Kim et al. 2019).
5. Investigations on the compatibility between beneficial microbes and crop under salinity and drought stress should be performed, particularly on the interaction of root exudates and microbial survival or colonization behavior (Etesami and Beattie 2018). This could potentially avoid the host genotype factor that could lead to inconsistent results.

6. The crosstalk between phytohormones involved in abiotic stress such as ABA, JA, SA, GA, and phytohormones in plants inoculated with beneficial microbes remains unclear. Understanding the regulation between these two groups of hormones could perhaps allow researchers to engineer the phytohormone regulation to produce drought and salinity tolerant crops (Kumar et al. 2016).
7. Lastly, field screening should be conducted for several crop cycles within 2 years and various geographical regions to evaluate if the selected strains could provide consistent protection and growth promotion to crops. As a field setting consists of diverse soil and environmental conditions, such study could provide a clearer picture on the sustainability of this control method.

3.6 Conclusion and Future Perspectives

Besides the aforementioned abiotic stresses, the accumulation of carbon dioxide (CO_2) and tropospheric ozone (O_3) is another major threat to be addressed in the face of climate change. Crops exposed to excessive CO_2 accumulation positively impact their growth and productivity, especially in C3 crops such as cereal crops, but significantly reduced their nutritional quality of the harvest (Deryang et al. 2016; Yadav et al. 2019). Interestingly, the existing soil microbial diversity was not adversely affected under CO_2 saturated conditions (Lesaulnier et al. 2008; Drigo et al. 2008). As beneficial microbes are known to improve growth and plant nutrient acquisition, it would be interesting if these strains could exert the same beneficial properties in crops under elevated CO_2 concentration. Such study could also help to overcome poor nutritional quality in agricultural produce as a result of increased CO_2 levels. The O_3 gas has also negatively affected photosynthesis and metabolism in plants in which the accumulation O_3 in leaves led to the generation of ROS, programmed cell death, diminished plant carbon reserves, reduced plant growth, and quality of crop produce (Tai et al. 2014; Malin et al. 2015; Pleijel et al. 2018). In addition, the diversity of soil microbes is also severely affected by the accumulation of O_3 (Agathokleous et al. 2020). Thus, the ill effects of O_3 on the application of beneficial microbes should not be overlooked.

The increase in ultraviolet radiation especially ultraviolet-B (UVB 280–315 nm) during prolonged heat and drought stress was found to pose deleterious effects of the plant growth, yield, chlorophyll content, and the photosystem II (Sharma et al. 2017). Extensive exposure of plants to UV-B inhibited photosynthesis rate and increased oxidative stress induced damage (Kakani et al. 2003; Liu et al. 2005). Perhaps, the use of beneficial microbes to overcome light stress in crops should be included in microbial-mediated drought or heat tolerance studies.

Most of the observed outcomes from previous abiotic stress tolerance studies were derived from laboratories and climate chamber trials where one stress is applied at a time (Schillaci et al. 2019). In cases of field trial, the application of microbes often failed to promote crop growth and tolerance as field environment is unpredictable and it often consists of a combination of abiotic stresses (Suzuki et al. 2014; Hardoim et al. 2015). To date, limited studies have investigated the interaction between microbes and crops when exposed to several abiotic stresses. However, mimicking such conditions similar to the field settings can be an alternative to unravel the role of microbes in climate change induced abiotic stress. Another important consideration is the changes of native soil and plant microbial community under abiotic stress condition. Understanding the shift in soil and plant microbiota under climate change could provide clues to researchers as to which community thrives under adverse conditions (Meena et al. 2017). This information is potentially beneficial in engineering soil or plant microbiome that is tailored to produce climate-resilient crops with enhanced productivity. In addition, the formulation of biofertilizers containing strains that are tolerant to extreme conditions (extremophiles) is highly desirable as they could be applied in variable field conditions (Vimal et al. 2017). Despite that, factors that affect the microbial viability during storage and after application should be taken into account while formulating an effective biofertilizer that could be utilized under diverse soil and environmental conditions.

References

Abdel-Latef AA, Chaoxing H (2011) Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiol Plant 33:1217–1225

Abedin MA, Habiba U, Shaw R (2014) Salinity scenario in Mekong, Ganges, and Indus River deltas water insecurity: a social dilemma. Emerald Group Publishing Limited, Bingley, pp 115–138

Adams AE, Kazenel MR, Rudgers JA (2017) Does a foliar endophyte improve plant fitness under flooding. Plant Ecol 218:711–723

Agathokleous E, Feng Z, Oksanen E et al (2020) Ozone affects plant, insect, and soil microbial communities: a threat to terrestrial ecosystems and biodiversity. Sci Adv 6:1176

Ait-Barka EA, Nowak J, Clement C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting Rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environ Microbiol 72:7246–7252

Ali S, Kim W-C (2018) Plant growth promotion under water: decrease of waterlogging-induced ACC and ethylene levels by ACC deaminase-producing bacteria. Front Microbiol 9:1096

Ali SZ, Sandhya V, Grover M et al (2011) Effect of inoculation with a thermotolerant plant growth promoting *Pseudomonas putida* strain AKMP7 on growth of wheat (*Triticum* spp.) under heat stress. J Plant Interact 6:239–246

Ali S, Rizwan M, Arif MS et al (2019) Approaches in enhancing thermotolerance in plants: an updated review. J Plant Growth Regul 39:456–480

Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. Plant Physiol 149:549–560

Arbuckle JG, Morton LW, Hobbs J (2015) Understanding farmer perspectives on climate change adaptation and mitigation: the roles of trust in sources of climate information, climate change beliefs and perceived risk. Environ Behav 47:205–234

Aroca R, Porcel R, Ruiz-Lozano JM (2006) How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? New Phytol 173:808–816

Bahadur A, Batool A, Nasir F et al (2019) Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. Int J Mol Sci 20:4199

Barnawal D, Bharti N, Maji D et al (2012) 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-containing rhizobacteria protect *Ocimum sanctum* plants during waterlogging stress via reduced ethylene generation. Plant Physiol Biochem 58:227–235

Bidabadi SS, Mehralian M (2020) Arbuscular mycorrhizal fungi inoculation to enhance chilling stress tolerance of watermelon. Gesunde Pflanzen 72:171–179

Bunn R, Lekberg Y, Zabinski C (2009) Arbuscular mycorrhizal fungi ameliorate temperature stress in thermophilic plants. Ecology 90:1378–1388

Cabral C, Ravnskov S, Tringovska I et al (2016) Arbuscular mycorrhizal fungi modify nutrient allocation and composition in wheat (*Triticum aestivum* L.) subjected to heat-stress. Plant Soil 408:385–399

Chen S, Jin W, Liu A et al (2013) Arbuscular mycorrhizal fungi (AMF) increase growth and secondary metabolism in cucumber subjected to low temperature stress. Sci Hortic 160:222–229

Chen N, He R, Chai Q et al (2016) Transcriptomic analyses giving insights into molecular regulation mechanisms involved in cold tolerance by Epichloä endophyte in seed germination of *Achnatherum inebrians*. Plant Growth Regul 80:367–375

Chinnusamy V, Zhu J, Zhu JK (2007) Cold stress regulation of gene expression in plants. Trends Plant Sci 12:444–451

Chodak M, Golebiewski M, Morawska-Ploskonka J et al (2015) Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. Ann Microbiol 65:1627–1637

Chu XT, Fu JJ, Sun YF et al (2016) Effect of arbuscular mycorrhizal fungi inoculation on cold stress-induced oxidative damage in leaves of *Elymus nutans* Griseb. S Afr J Bot 104:21–29

Ciais P, Reichstein M, Viovy N et al (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature 437:529–533

Clarke SM, Cristescu SM, Miersch O et al (2009) Jasmonates act with salicylic acid to confer basal thermotolerance in *Arabidopsis thaliana*. New Phytol 182:175–187

Costa OYA, Raaijmakers JM, Kuramae EE (2018) Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Front Microbiol 9:1636

Daryanto S, Wang L, Jacinthe PA (2016) Global synthesis of drought effects on maize and wheat production. PLoS One 11:e0156362

de Vries FT, Griffiths RI, Knight CG et al (2020) Harnessing rhizosphere microbiomes for drought-resilient crop production. Science 368:270–274

Deepika S, Kothamasi D (2015) Soil moisture—a regulator of arbuscular mycorrhizal fungal community assembly and symbiotic phosphorus uptake. Mycorrhiza 25:67–75

Deryang D, Elliot J, Christian F et al (2016) Regional disparities in the beneficial effects of rising CO_2 concentrations on crop water productivity. Nat Clim Change Lett 6:786–790

Dharmarathna WRSS, Herath S, Weerakoon SB (2014) Changing the planting date as a climate change adaptation strategy for rice production in Kurunegala district, Sri Lanka. Sustain Sci 9:103–111

Dierking RM, Young CA, Kallenbach RL (2012) Mediterranean and continental tall fescue: I. effects of endophyte status on leaf extension, proline, mono- and disaccharides, fructan, and freezing survivability. Crop Sci 52:451–459

Dove A (2017) Central California is losing $3.7 billion in crop yield every year. Report from Department of Civil and Environmental Engineering, Pittsburgh, PA, Carnegie Mellon University

Downing TE (1991) Vulnerability to hunger in Africa: a climate change perspective. Glob Environ Chang 1:365–380

Drigo B, Kowalchuk GA, van Veen JA et al (2008) Climate change goes underground: effects of elevated atmospheric CO2 on microbial community structure and activities in the rhizosphere. Biol Fertil Soils 44:667–679

Duc NH, Csintalan Z, Posta K (2018) Arbuscular mycorrhizal fungi mitigate negative effects of combined drought and heat stress on tomato plants. Plant Physiol Biochem 132:297–307

Egamberdieva D, Wirth SJ, Alqarawi AA et al (2017) Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Front Microbiol 8:2104

Egamberdieva D, Wirth S, Bellingrath-Kimura SD et al (2019) Salt-tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils. Front Microbiol 10:2791
El-Daim IAA, Bejai S, Meijer J (2014) Improved heat stress tolerance of wheat seedlings by bacterial seed treatment. Plant Soil 379:337–350
Enebe MC, Babalola OO (2018) The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. Appl Microbiol Biotechnol 102:7821–7835
Etesami H, Beattie GA (2018) Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. Front Microbiol 9:148
Evelin H, Devi TS, Gupta S et al (2019) Mitigation of salinity stress in plants by Arbuscular Mycorrhizal symbiosis: current understanding and new challenges. Front Plant Sci 10:470
Fahad S, Bajwa AA, Nazir U et al (2017) Production under drought and heat stress: plant responses and management options. Front Plant Sci 8:1147
FAO (2015) Status of the world's soil resources (SWSR)—main report. Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils, Rome
FAO (2017) The impact of disasters and crises on agriculture and food security. FAO, Rome, p 168
Farooq M, Gogoi N, Barthakur S et al (2017) Drought stress in grain legumes during reproduction and grain filling. J Agron Crop Sci 203:81–102
Fernandez O, Theocharis A, Bordiec S et al (2012a) *Burkholderia phytofirmans*-PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. Mol Plant-Microbe Interact 25:496–504
Fernandez O, Vandesteene L, Feil R et al (2012b) Trehalose metabolism is activated upon chilling in grapevine and might participate in *Burkholderia phytofirmans* induced chilling tolerance. Planta 236:355–369
Ghorbanpour A, Salimi A, Ghanbary MAT et al (2018) The effect of *Trichoderma harzianum* in mitigating low temperature stress in tomato (*Solanum lycopersicum* L.) plants. Sci Hortic 230:134–141
Gilliham M, Able JA, Roy JS (2016) Translating knowledge about abiotic stress tolerance to breeding programmes. Plant J 90:898–917
Giovannetti M, Fortuna P, Citernesi AS et al (2001) The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. New Phytol 151:717–724
Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39
Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol Biochem 39:11–17
Grover M, Ali SZ, Sandhya V et al (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stress. World J Microbiol Biotechnol 27:1231–1240
Hajiboland R, Aliasgharzad N, Barzeghar R (2009) Phosphorus mobilization and uptake in mycorrhizal rice (*Oryza sativa* L.) plants under flooded and non-flooded conditions. Acta Agric Slov 93:153–161
Hajiboland R, Joudmand A, Aliasgharzad N et al (2019) Arbuscular mycorrhizal fungi alleviate low temperature stress and increase freezing resistance as a substitute for acclimation treatment in barley. Crop Pasture Sci 70:218–233
Hardoim PR, van Overbeek LS, Berg G et al (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293–320
Hatfield JL, Prueger JH (2015) Temperature extremes: effect on plant growth and development. Weather Clim Extremes 10:4–10
Haugen LM, Smith SE (1992) The effect of high temperature and fallow period on infection of mung bean and cashew roots by the versicular-arbuscular mycorrhizal fungus *Glomus intraradices*. Plant Soil 145:71–80
Hawkes CV, Hartley IP, Ineson P et al (2008) Soil temperature affects carbon allocation within arbuscular mycorrhizal networks and carbon transport from plant to fungus. Glob Chang Biol 14:1181–1190

Huang X, Shi H, Hu Z et al (2017) ABA is involved in regulation of cold stress response in Bermudagrass. Front Plant Sci 8:1613

Ilyas F (2017) Sindh suffers 31pc crop loss annually due to waterlogging and salinity. https://www.dawn.com/news/1357033. Accessed 10 Oct 2020

IPCC Intergovernmental Panel on Climate Change (2007) Climate change 2007: impacts, adaptation and vulnerability. In: Perry ML, Canziani OF, Palutikof JP et al (eds) Contribution of working group II to fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, p 1000

Jalili A, Jamzad Z, Thompson K et al (2010) Climate change, unpredictable cold waves and possible brakes on plant migration. Glob Ecol Biogeogr 19:642–648

Janssens C, Havlik P, Krisztin T et al (2020) Global hunger and climate change adaptation through international trade. Nat Clim Chang 10:829–835

Kakani VG, Reddy KR, Zhao D et al (2003) Ultraviolet-B radiation effects on cotton (*Gossypium hirsutum* L.) morphology and anatomy. Ann Bot 91:817–826

Kakar KU, Ren X-I, Nawaz Z et al (2016) A consortium of rhizobacterial strains and biochemical growth elicitors improve cold and drought stress tolerance in rice (*Oryza sativa* L.). Plant Biol 18:471–483

Kerry RG, Patra S, Gouda S et al (2018) Microbes and their role in drought tolerance of agricultural food crops. In: Patra JK (ed) Microbial biotechnology. Springer Nature, Singapore, pp 253–273

Khan AL, Waqas M, Lee IJ (2014) Resilience of *Penicillium resedanum* LK6 and exogenous gibberellin in improving *Capsicum annuum* growth under abiotic stresses. J Plant Res 128:259–268

Khan MA, Asaf S, Khan AL et al (2020) Thermotolerant effect of plant growth-promoting *Bacillus cereus* SA1 on soybean during heat stress. BMC Microbiol 20:175

Kim K, Samaddar S, Chatterjee P et al (2019) Structural and functional responses of microbial community with respect to salinity levels in a coastal reclamation land. Appl Soil Ecol 137:96–105

Kruse E, Uehlein N, Kaldenhoff R (2006) The aquaporins. Genome Biol 7:206

Kumar A, Verma JP (2018) Does plant-microbe interaction confer stress tolerance in plants: a review? Microbiol Res 207:41–52

Kumar V, Sah KS, Khare T et al (2016) Engineering phytohormones for abiotic stress tolerance in crop plants. In: Ahammed G, Yu JQ (eds) Plant hormones under challenging environment factors. Springer, Dordrecht, pp 247–266

Kytöviita MM, Ruotsalainen AL (2007) Mycorrhizal benefit in two low arctic herbs increases with increasing temperature. Am J Bot 94:1309–1315

Lee EH, Eo JK, Ka KH (2013) Diversity of arbuscular mycorrhizal fungi and their roles in ecosystems. Mycobiology 41:121–125

Lesaulnier C, Papamichail D, McCorkle S (2008) Elevated atmospheric CO_2 affects soil microbial diversity associated with trembling aspen. Environ Microbiol 10:926–941

Li J, McConkey BJ, Cheng Z et al (2013) Identification of plant growth-promoting bacteria-responsive proteins in cucumber roots under hypoxic stress using a proteomic approach. J Proteome 84:119–131

Liu LX, Xu SM, Woo KC (2005) Solar UV-B radiation on growth, photosynthesis and the xanthophyll cycle in tropical acacias and eucalyptus. Environ Exp Bot 54:121–130

Liu Z, Ma L, He X et al (2014) Water strategy of mycorrhizal rice at low temperature through the regulation of PIP aquaporins with the involvement of trehalose. Appl Soil Ecol 84:185–191

Lobell DB, Tebaldi C (2014) Getting caught with our plants down: the risks of a global crop yield slowdown from climate trends in the next two decades. Environ Res Lett 9:074003

Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. Science 333:616–620

Ma J, Janouskova M, Li Y et al (2015) Impact of arbuscular mycorrhizal fungi (AMF) on cucumber growth and phosphorus uptake under cold stress. Funct Plant Biol 42:1158–1167

Ma J, Janouskova M, Ye L et al (2019) Role of arbuscular mycorrhiza in alleviating the effect of cold onthe photosynthesis of cucumber seedlings. Photosynthetica 57:86–95
Malin CB, Feng Z, Xin Y et al (2015) Ozone effects on wheat grain quality—a summary. Environ Pollut 197:203–213
Martin CA, Stuz JC (2004) Interactive effects of temperature and arbuscular mycorrhizal fungi on growth, P uptake and root respiration of *Capsicum annuum* L. Mycorrhiza 14:241–244
Masciarelli O, Llanes A, Luna V (2014) A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation. Microbiol Res 169:609–615
Mathimaran N, Sharma MP, Raju MB et al (2017) Arbuscular mycorrhizal symbiosis and drought tolerance in crop plants. Mycosphere 8:361–376
Matsubara Y, Hirano I, Sassa D et al (2004) Alleviation of high temperature stress in strawberry plants infected with arbuscular mycorrhizal fungi. Environ Control Biol 42:105–111
Maya MA, Matsubara Y (2013) Influence of arbuscular mycorrhiza on the growth and antioxidative activity in cyclamen under heat stress. Mycorrhiza 23:381–390
Meena KK, Sorty AM, Bitla UM et al (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:172
Mishra PK, Bisht SC, Ruwari P et al (2011) Alleviation of cold stress in inoculated wheat (*Triticum aestivum* L.) seedlings with psychrotolerant *Pseudomonads* from NW Himalayas. Arch Microbiol 193:497–513
Miura K, Lee J, Miura T et al (2010) SIZ1 controls cell growth and plant development in Arabidopsis through salicylic acid. Plant Cell Physiol 51:103–113
Mukhtar T, Rehman S, Smith D et al (2020) Mitigation of heat stress in *Solanum lycopersicum* L. by ACC-deaminase and exopolysaccharide producing *Bacillus cereus*: effects on biochemical profiling. Sustainability 12:2159
Murphy BR, Doohan FM, Hodkinson TR (2014) Yield increase induced by the fungal root endophyte *Piriformospora indica* in barley grown at low temperature is nutrient limited. Symbiosis 62:29–39
Nascimento F, Brigido C, Alho L et al (2012) Enhanced chickpea growth-promotion ability of a *Mesorhizobium* strain expressing an exogenous ACC deaminase gene. Plant Soil 353:221–230
Newsham KK, Upson R, Read DJ (2009) Mycorrhizas and dark septate root endophytes in polar regions. Fungal Ecol 2:10–20
Numan M, Bashir S, Khan Y (2018) Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants. A review. Microbiol Res 209:21–32
Olson KR, Morton LW (2012) The impacts of 2011 induced levee breaches on agricultural lands of Mississippi River Valley. J Soil Water Conserv 67:5A–10A
Orchard S, Standish RJ, Nicol D et al (2016) The response of fine root endophyte (*Glomus tenue*) to waterlogging is dependent on host plant species and soil type. Plant Soil 403:305–315
Paradis R, Dalpe Y, Charest C (1995) The combined effect of arbuscular mycorrhizas and short-term cold exposure on wheat. New Phytol 129:637–642
Pearce RS, Fuller MP (2001) Freezing of barley studied by infrared video thermography. Plant Physiol 125:227–240
Pleijel H, Broberg MC, Uddling J et al (2018) Current surface ozone concentrations significantly decrease wheat growth yield and quality. Sci Total Environ 613-614:687–692
Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agron Sustain Dev 32:181–200
Raju PS, Clark RB, Ellis JR et al (1990) Effects of species of VA-mycorrhizal fungi on growth and mineral uptake of sorghum of different temperatures. Plant Soil 121:165–170
Ravanbakhsh M, Sasidharan R, Voesenek LA et al (2017) ACC deaminase-producing rhizosphere bacteria modulate plant responses to flooding. J Ecol 105:979–986
Rehman A, Jingdong L, Du Y et al (2015) Flood disaster in Pakistan and its impact on agriculture growth. Glob Adv Res J Agric Sci 4:827–830
Rigby JR, Porporato A (2008) Spring frost risk in a changing climate. Geophys Res Lett 35:L12703

Rihan HZ, Al-Issawi M, Fuller MP (2017) Advances in physiological and molecular aspects of plant cold tolerance. J Plant Interact 12:143–157

Romera FJ, García MJ, Lucena C et al (2019) Induced systemic resistance (ISR) and Fe deficiency responses in dicot plants. Front Plant Sci 10:287

Ruotsalainen AL, Kytöviita MM (2004) Mycorrhiza does not alter low temperature impact on *Gnaphalium norvegicum*. Oecologia 140:226–233

Ruppel S, Franken P, Witzel K (2013) Properties of the halophyte microbiome and their implications for plant salt tolerance. Funct Plant Biol 40:940–951

Sah S, Reed S, Jayachandran K (2006) The effect of repeated short-term flooding on mycorrhizal survival in snap bean roots. HortScience 41:598–602

Sah SK, Reddy KR, Li J (2016) Abscisic acid and abiotic stress tolerance in crop plants. Front Plant Sci 7:571

Samra JS, Singh G, Ramakrishna YS (2003) Cold wave of 2002-03—impact on agriculture. Heritage Print Services Pvt Ltd, Hyderabad, pp 18–19

Sasidharan R, Bailey-Serres J, Ashikari M et al (2017) Community recommendations on terminology and procedures used in flooding and low oxygen stress research. New Phytol 214:1403–1407

Saxena AK, Lata Shende R, Pandey AK (2005) Culturing of plant growth promoting rhizobacteria. In: Gopi KP, Varma A (eds) Basic research applications of mycorrhizae. I K International Pvt Ltd, New Delhi, pp 453–474

Schillaci M, Gupta S, Walker R et al (2019) The role of plant growth-promoting bacteria in the growth of cereals under abiotic stress. In: Ohyama T (ed) Root biology—growth, physiology and functions. IntechOpen, London, pp 1–22

Sharma M, Laxmi A (2015) Jasmonates: emerging players in controlling temperature stress. Front Plant Sci 6:1129

Sharma S, Chatterjee S, Kataria S et al (2017) A review on responses of plants to UV-B radiation related stress. In: Singh VP, Singh S, Prasad SM et al (eds) UV-B radiation: from environmental stressor to regulator of plant growth. John Wiley & Sons, Chichester, pp 75–97

Shrivastava P, Kumar R (2015) Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123–131

Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353

Smith GS, Roncadori RW (1986) Responses of three versicular-arbuscular mycorrhizal fungi at four soil temperatures and their effects on cotton growth. New Phytol 104:89–95

Song M, Li X, Saikkonen K et al (2015) An asexual *Epichloë* endophyte enhances waterlogging tolerance of *Hordeum brevisubulatum*. Fungal Ecol 13:44–52

Subramanian P, Mageswari A, Kim K et al (2015) Psychrotolerant endophytic *Pseudomonas* sp. strains OB155 and OS261 induced chilling resistance in tomato plants (*Solanum lycopersicum* Mill.) by activation of their antioxidant capacity. Mol Plant-Microbe Interact 28:1073–1081

Subramanian P, Kim K, Krishnamoorthy R et al (2016) Cold stress tolerance in psychrotolerant soil bacteria and their conferred chilling resistance in tomato (*Solanum lycopersicum* Mill.) under low temperatures. PLoS One 11:e0161592

Suzuki N, Rivero RM, Shulaev V et al (2014) Abiotic and biotic stress combinations. New Phytol 203:32–43

Szabo S, Hossain MS, Adger WN et al (2016) Soil salinity, household wealth and food insecurity in tropical deltas: evidence from south-west coast of Bangladesh. Sustain Sci 11:411–421

Tai APK, Val Martin M, Heald CL (2014) Threat to future global food security from climate change and ozone air pollution. Nat Clim Chang 4:817–821

Thalmann M, Santelia D (2017) Starch as a determinant of plant fitness under abiotic stress. New Phytol 214:943–951

Theocharis A, Bordiec S, Fernandez O et al (2012) *Burkholderia phytofirmans* PsJN primes *Vitis vinifera* L. and confers a better tolerance to low nonfreezing temperatures. Mol Plant-Microbe Interact 25:241–249

Thomas AL, Guerreiro SMC, Sodek L (2005) Aerenchyma formation and recovery from hypoxia of the flooded root system of nodulated soybean. Ann Bot 96:1191–1198

Tian X, Luo H, Zhou H et al (2009) Research on heat stress of rice in China: progress and prospect. Chin Agric Sci Bull 25:166–168

Tiryaki D, Aydin I, Atici Ő (2019) Psychrotolerant bacteria isolated € from the leaf apoplast of cold-adapted wild plants improve the cold resistance of bean (*Phaseolus vulgaris* L.) under low temperature. Cryobiology 86:111–119

Tuo X-Q, Li S, Wu Q-S (2015) Alleviation of waterlogged stress in peach seedlings inoculated with *Funneliformis mosseae*: changes in chlorophyll and proline metabolism. Sci Hortic 197:130–134

Upson R, Read DJ, Newsham KK (2009) Nitrogen form influences the response of Deschampsia antarctica to dark septate root endophytes. Mycorrhiza 20:1–11

van der Does D, Boutrot F, Engelsdorf T et al (2017) The Arabidopsis leucine-rich repeat receptor kinase MIK2/LRR-KISS connects cell wall integrity sensing, root growth and response to abiotic and biotic stresses. PLoS Genet 13:e1006832

Verma V, Ravindran P, Kumar PP (2016) Plant hormone-mediated regulation of stress responses. BMC Plant Biol 16:86

Vimal SR, Singh JS, Arora NK et al (2017) Soil-plant-microbe interactions in stressed agriculture management: a review. Pedosphere 27:177–192

Vurukonda SSKP, Vardharajula S, Shrivastava M et al (2015) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184:13–24

Waqas M, Khan AL, Shahzad R et al (2016) Mutualistic fungal endophytes produce phytohormones and organic acids that promote japonica rice plant growth under prolonged heat stress. J Zhejiang Univ Sci B (Biomed & Biotechnol) 16:1011–1018

Wright AJ, Kroon H, Visser EJ et al (2017) Plants are less negatively affected by flooding when growing in species-rich plant communities. New Phytol 213:645–656

Wu Q-S, Zou Y-N, Huang Y-M (2013) The arbuscular mycorrhizal fungus *Diversispora spurca* ameliorates effects of waterlogging on growth, root system architecture and antioxidant enzyme activities of citrus seedlings. Fungal Ecol 6:37–43

Xiao ML, Qing LX, Qing QL et al (2017) Physiological responses of the two blueberry cultivars to inoculation with an arbuscular mycorrhizal fungus under low-temperature stress. J Plant Nutr 40:2562–2570

Xue Y, Gao Y, Liu C et al (2020) A styrene antioxidant NFA from riparian endophytic fungi enhances flooding tolerance in Arabidopsis. J Plant Interact 15:111–116

Yadav A, Bhatia A, Yadav S et al (2019) The effects of elevated CO2 and elevated O3 exposure on plant growth, yield and quality of grains of two wheat cultivars grown in north India. Heliyon 5: e02317

Yang J, Duan G, Li C (2019) The crosstalks between jasmonic acid and other plant hormone signaling highlight the involvement of jasmonic acid as a core component in plant response to biotic and abiotic stresses. Front Plant Sci 10:1349

Yeasmin R, Bonser SP, Motoki S et al (2019) Arbuscular mycorrhiza influences growth and nutrient uptake of Asparagus (*Asparagus officinalis* L.) under heat stress. HortScience 54:846–850

Zhang H, Xie X, Kim M-S et al (2008) Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels in planta. Plant J 56:264–273

Zhou L, Li C, Zhang X et al (2015) Effects of cold shocked *Epichloë* infected *Festuca sinensis* on ergot alkaloid accumulation. Fungal Ecol 14:99–104

Zhu X-C, Song F-B, Xu H-W (2010a) Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil 331:129–137

Zhu X-C, Song F-B, Liu TD et al (2010b) Arbuscular mycorrhizae reducing water loss in maize plants under low temperature stress. Plant Signal Behav 5:591–593

Zhu X-C, Song F-B, Liu S-Q et al (2011) Effects of arbuscular mycorrhizal fungus on photosynthesis and water status of maize under high temperature stress. Plant Soil 346:189–199

Zhu X, Song F, Liu F (2017) Arbuscular mycorrhizal fungi and tolerance of temperature stress in plants. In: Wu QS (ed) Arbuscular mycorrhizas and stress tolerance of plants. Springer Nature, Singapore, pp 163–194

Zubair M, Hanif A, Farzand A et al (2019) Genetic screening and expression analysis of psychrophilic *Bacillus* spp. reveal their potential to alleviate cold stress and modulate phytohormones in wheat. Microorganisms 7:337

Application of Microbes in Biotechnology, Industry, and Medical Field

4

Moises Bustamante-Torres, David Romero-Fierro, Jocelyne Estrella-Nuñez, Evelin Cuadros-Buenaventura, and Emilio Bucio

Abstract

Microbes encompass a wide range of a group of bacteria, archaea, protists, fungi, and viruses. The term microbes is commonly related to side effects. However, the advances in the biology branch have promoted the application of microbes in almost unlimited fields. Microorganisms can be classified into prokaryotes (bacteria and archaea) and eukaryotes (Protist and Fungi). Prokaryotes can survive in extreme conditions. They are employed as biofactories. However, eukaryotes have been used in the agroindustry and for some medical purposes. Besides, viruses are a type of microbes that are commonly applied in the medical industry. This chapter describes the application of microbes in several fields with great importance. Besides, new techniques with better sensibility and reduced costs are

M. Bustamante-Torres (✉)
Biomedical Engineering Department, School of Biological and Engineering, Yachay Tech University, Urcuqui, Ecuador

Department of Radiation Chemistry and Radiochemistry, Institute of Nuclear Sciences, National Autonomous University of Mexico, Mexico, Mexico
e-mail: moises.bustamante@yachaytech.edu.ec

D. Romero-Fierro · J. Estrella-Nuñez
Chemistry Department, School of Chemical and Engineering, Yachay Tech University, Urcuqui, Ecuador

E. Cuadros-Buenaventura
Biomedical Engineering Department, School of Biological and Engineering, Yachay Tech University, Urcuqui, Ecuador

E. Bucio (✉)
Department of Radiation Chemistry and Radiochemistry, Institute of Nuclear Sciences, National Autonomous University of Mexico, Mexico, Mexico
e-mail: ebucio@nucleares.unam.mx

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required to study and the understanding of the microbes. The versatility of these microbes has enhanced the study and application of them in biotechnology, industry, and medical field.

Keywords

Microbes · Application · New techniques · Biotechnology · Industry

4.1 Overview of Microorganisms

Microorganisms constitute most of the earth's biodiversity and are an integral part of the biosphere process (Amsellem et al. 2017). At first glance, the use of microorganisms is considered a wrong idea. The idea of microorganisms is related to diseases or producers of bad consequences. Throughout history, microorganisms have played fundamental roles in the evolution and the constant change of the world. Today, their applications are almost limitless, which makes them essential for the development of a huge range of industries and even the environment.

The emergence of eukaryotes in a world dominated by prokaryotes is one of the defining moments of modern microbial evolution (Bendich and Drlica 2000). Microbes are divided into two categories: prokaryotes, whose DNA interacts closely with the cytoplasm; eukaryotes, whose DNA is separated from the cytoplasm by a nuclear membrane (Murat et al. 2010). Eukaryotic chromosomes have characteristics that usually lack these characteristics in prokaryotic chromosomes, such as the presence of nuclear membrane and cytoplasmic space.

Microbes are tiny living things that despite their ubiquity usually cannot be seen with the unaided eye. This biodiverse group of organisms embraces bacteria, archaea, protist, fungi, and virus. Although microbes have commonly a negative connotation due to its infective and pathogenic nature, they also constitute an important component in the equilibrium of life in the entire environment (Tortora et al. 2019). In addition, their distinctive characteristics, which include high reproductive rate and biosynthesis capacity, make them attractive organisms for biotechnology application (Demain 2000a; Kouzuma and Watanabe 2014).

As evidence suggests, the application of microbes in biotechnology is recorded for the first time in 5000 BC, during the beginnings of large-scale winemaking activity (Borneman et al. 2013). However, microbial biotechnology began to be formally considered in the 1980s, when the first patent was granted for a genetically modified *Pseudomonas putida*. This engineered bacteria was intended for the organic digestion of compounds present in oil spills (Vitorino and Bessa 2017). This fact, together with the rapid advancement of various areas of science such as microbiology, molecular and synthetic biology, has greatly promoted the use of microbes in the different subtypes of biotechnology such as medical, agricultural, industrial, marine, food, and environmental (De Lorenzo et al. 2018; Gupta et al. 2016).

4.1.1 Prokaryotic Microorganisms

Prokaryotic microorganisms are divided into two domains: Bacteria and Archaea. These types of organisms are the smallest and simplest form of life (Tortora et al. 2019). Consequently, in comparison with eukaryotic microorganisms, prokaryotes have a short cell cycle (Harvey et al. 2000). Despite this, they constitute a large portion of the genetic diversity of life and possess an important metabolic diversity and in some cases exclusive to prokaryotes (i.e., routes in addition to those present in eukaryotes for CO_2 assimilation, anaerobic photosynthesis, fixation of N_2, and adaptation to extreme environmental conditions) (Ward 2002; Amann and Rossello 2001; Grogan 1990). These characteristics make prokaryotic organisms a very attractive target for biotechnological manipulation. This section will describe the principal characteristics that make these type of microbes relevant in the area of biotechnology.

Bacteria

Bacteria are single-celled organisms and can be found almost anywhere on earth. They range in size from 0.2 to 2μm in diameter and 2 to 8μm in length. Morphologically, it is characterized by the lack of defined nuclear and membrane-bound organelles (Tortora et al. 2019; Rogers 2011). Furthermore, its genetic material consists of a circular chromosome made up of double-stranded DNA (free of histones) located in the nucleoid of the cell, and plasmids (extrachromosomal circular double-stranded DNA). According to their shape, bacteria can be classified as coccus (spherical shape), bacillus (rod-shaped), and spiral (Tortora et al. 2019).

However, the main criteria for classifying bacteria are based on the biochemical composition and structure of their cell walls. This classification divides the bacterial domain into Gram-positive and Gram-negative (Beveridge 2001). Gram-positive bacteria are characterized by a single cell plasma membrane and a thick cell wall composed of peptidoglycan. In contrast, gram-negative bacteria have thinner cell walls than gram-positive bacteria. In addition to the cytoplasmic membrane, the outer membrane of Gram-negative bacteria also contains carbohydrate and protein receptor sites, allowing phage to attach (Moat et al. 2002; Snyder et al. 2013). For the manipulation of microorganisms, it is important to identify and classify them properly, since several processes and characteristics, such as cell division, transformation, resistance to antibiotics and adaptability, can vary between Gram-positive and Gram-negative bacteria (Moat et al. 2002).

Other characteristics relevant to the biotechnology of bacteria include that they are haploid organisms. In other words, bacteria only have one allele of each gene. Therefore, genetic manipulation and mutation identification require simple processes. In addition, bacteria are microorganisms that reproduce asexually by binary fission and have a very short generation time. Consequently, it is possible to obtain large quantities of identical organisms in relatively short periods of time (Snyder et al. 2013).

In biotechnology, bacteria are frequently used. In the food industry they are required as metabolic agents in the production of fermented foods (Behera et al.

2019). They are also used as biofactories for nucleic acids, enzymes, and other proteins, important elements in the food and pharmaceutical industries (Nigam 2013; Ferrer-miralles and Villaverde 2013). Bacteria have relatively simple genetic characteristics; therefore, they can be genetically manipulated for different purposes, such as the improvement or introduction of metabolic processes (Singh et al. 2011).

Archaea

Archaea are widely distributed in different types of habitats. However, a large part of these microorganisms are considered extremophiles since they inhabit environments with extreme temperature, pH, and/or salinity (Snyder et al. 2013). The diameter of archaea ranges from 0.1 to 15μm, and the length does not exceed 200μm (Alquéres et al. 2007). They are divided mainly into two phyla: Euryarchaeota, which comprises the methanogens, halophiles, and hyperthermophiles, and Crenarchaeota containing only sulfur-dependent thermophile (Snyder et al. 2013).

Being a prokaryotic organism, Archaea share common characteristics with Bacteria: they are unicellular organisms, do not have a nucleus defined by a membrane, lack organelles such as mitochondria, chloroplast, Golgi apparatus, and endoplasmic reticulum, and their genetic material consists of a single circular chromosome and plasmids (Snyder et al. 2013). However, these two types of microorganisms also have important characteristic points of divergence from each other. For example, the lipids of the Archaea membrane are composed of isoprenoid chains instead of fatty acids as is the case of Bacteria. Furthermore, the most common peptidoglycan in the cell wall of Bacteria: murein, is not present in Archaea, instead S-layer protein or pseudo-murein can be found (Bräsen et al. 2014). Also, although the metabolic genes of Bacteria and Archaea have evolutionary aspects in common, the transcriptional and translational machinery of Archaea more closely resembles that of Eukarya (Alquéres et al. 2007; Barry and Bell 2006).

Archaean microorganisms occupy an important place in the biotechnology industry, because due to their extremophilic nature, archaeans are known to produce enzymes and metabolites with high biotechnological potential (Straub et al. 2018). Furthermore, the unique metabolic characteristics of Archaea, such as methane production and other unusual pathways involved in carbohydrate metabolism, present novel resources (e.g., enzymes, metabolic pathways) for their biotechnological application (Bräsen et al. 2014).

4.1.2 Eukaryotic Microorganisms

Eukaryotic microbes are mainly divided into two groups: Protist and Fungi. As is characteristic of all eukaryotic cells, eukaryotic microbes have a nucleus surrounded by a nuclear membrane, in which chromosomes are found. In addition, they have organelles such as mitochondria or chloroplast, Golgi apparatus, and endoplasmic reticulum (Tortora et al. 2019; Gross et al. 1995). The cell division of these organisms usually occurs by mitosis, so the two resulting cells are equal to each other (Tortora et al. 2019). Unlike the more highly evolved eukaryotic cells, eukaryotic microorganisms are cells of smaller size and less complexity that cannot form real tissues (Gross et al. 1995). However, these types of microbes have characteristics that may be of great interest in microbial and environmental biotechnology.

Protist

Protists are a complex group of organisms, mostly unicellular, that inhabit most terrestrial, marine and aquatic ecosystems. In addition, they can live as parasites of other Protists, Fungi, plants, and animals. In the terrestrial environment, protists are the main predators of bacteria and fungi and are an important indicator of soil condition. Morphologically, they are very diverse, but metabolically, Protists are less diverse than Bacteria. According to their ways of nutrition, they can be divided into osmo-heterotrophs, phago-heterotrophs, and phototrophs, which have the ability to fix carbon. Protists include organisms such as slime molds, protozoa, and algae (Dunlap 2001; Sergio et al. 2018).

The functional diversity of Protists in the soil microbiome makes this group of eukaryotes a rich source of tools that can be used in agricultural biotechnology. For example, they can be introduced into the plant microbiome as pathogen control agents as well as a nutrient provider and growth stimulant. In addition, they can be used to improve the fertility and productivity of crops (Jousset 2017). Also, some algae, such as dinoflagellates, constitute the most important source of natural products, which may be of great interest in medical biotechnology (Piel 2010).

Fungi

Phylogenetically, Fungi are divided into four groups: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota (Dunlap 2001). Yeasts, which belong to the Ascomycota group, are unicellular organisms. However, most Fungi tend to be multicellular and filamentous. The individual filaments formed by the fungi are called hyphae, and the network formed by these are called mycelium. In some organisms, the mycelium can be divided by cell walls known as septum (Gross et al. 1995).

Fungi are heterotrophic organisms that can be found in different types of habitats. They can be saprophytes, that is, they inhabit in decomposing organic matter; plant and animal parasites; and symbiotic organisms such as mycorrhizae (Gross et al. 1995). Its reproduction is asexual (except in the Basidiomycota phylum) and occurs through the formation of spores in the hyphae. The organisms obtained from reproduction are genetically identical to their parents, and the germination of the spore is necessary for the formation of the organism (Tortora et al. 2019).

Fungi are highly used in the field of biotechnology. Organisms such as *S. cerevisiae*, *P. pastoris*, and *H. polymorpha* are highly used as a host for the production of recombinant proteins, many of which are used for pharmaceutical purposes. Furthermore, several of its secondary metabolites are of great industrial interest. For example, penicillin (antibiotic), cyclosporin A (immunosuppressive agent), lovastatin and pravastatin (hypocholesterolemic agent), carotenoid astaxanthin and b-carotene (pigment), and ergot alkaloids (mycotoxin) (Adrio and Demain 2003).

Virus

The main characteristic of viruses within the world of microbes is that they are not cellular entities. Therefore, although it remains in dispute, viruses are not considered living organisms (Herrero-Uribe 2011). Compared with other microorganisms, viruses are much smaller, ranging from 20 to 1000 nm. According to virus morphology, they can be divided into helical (rod-shaped), polyhedral (icosahedron-shaped), enveloped viruses (i.e., helical or polyhedral covered by envelope), and complex viruses (e.g., bacteriophages) (Tortora et al. 2019).

Structurally, viruses consist of nucleic acids and a capsid whose protein composition depends on the genetic information contained in the virus. In some cases, the capsid may be covered by an envelope composed of lipids, proteins, and carbohydrates. The genetic material of a virus can only exist in one form. These can be single-stranded DNA, double-stranded DNA, single-stranded RNA, or double-stranded RNA. Depending on the microorganisms, these can be linear or circular. For their replication, viruses need to host a living cell since they do not have the machinery for their replication on their own. This process is generally done in five steps: attachment, penetration, biosynthesis, maturation, and release (Tortora et al. 2019).

Viruses are easy to manipulate and in the medical industry, they have been widely used as an alternative to antibiotics for resistant bacteria, as vaccine vectors, as a delivery vehicle for gene therapy, and for the screening of libraries of antibodies and other proteins (phage display) (Haq et al. 2012). In addition, in agriculture, viruses have been used as biocontrol agents and through their genetic manipulation, the generation and growth time of seedlings has been accelerated (Maeda et al. 2020).

4.2 Principles

Microorganisms are important natural resources for the development of microbial biotechnology. The sequencing of microbial genomes, the identification of established functions, and the study of microbial metabolism are indispensable foundations for the establishment of genome sequence databases (Glazer and Nikaido 2007). The analysis and manipulation of the sequenced genomes have given rise to multiple identifications, production, optimization, and application processes.

4.2.1 Screening for Microbial Products

A screening method involves the extraction, isolation, and identification of a compound or components in a sample studied (Biniarz et al. 2017). The main goal of any microbial screening technology for biologically active compounds is to discover a new chemical entity or molecule with unreported biological activity (Monciardini et al. 2014). The discovery of these new biologically active compounds is biased toward the isolation and selection of the most common and easy-to-cultivable strains (Monciardini et al. 2014). Practically, screeninig can be considered as a isolation process or isolation of microorganisms. In the colony, inspections are conducted once for specific properties. The screening steps are critical and need to be carefully selected, because each step has its advantages and disadvantages and can give qualitative and quantitative results (Biniarz et al. 2017).

Important points in the detection of microorganisms and their respective metabolites are listed below.

– Develop and verify tests and evaluate their repeatability separately. At this point, preliminary screening can estimate the hit rate and quickly screen a large number of samples to obtain data for complete screening.
– High-performance screening, processing a large number of samples in a more simplified process by using an automated system.
– Development of a secondary screening to eliminate false positives.

Screening Methods

Appropriate isolation procedures play a vital role in preliminary screens to identify secondary metabolites (Yuan-Kun 2013). Traditional techniques sometimes lead to less than optimal results or present certain limitations. Novel screening methods with high sensitivity are needed to discover new enzymes for their application with diverse purposes. The screening methods need to be low-cost and high-throughput to be considered efficient.

Various techniques for microbial screening have been reported in different studies. Denaturing gradient gel electrophoresis (DGGE) is used as a rapid screening method for the production of biological hydrogen (Kumar et al. 2018), fluorescence technology high-throughput screening (HTS) is used for kinase screening (Morgan et al. 2004), and surface plasmon resonance (SPR biosensor is used for primary and secondary screening of acetylcholine) binding protein ligands (Retra et al. 2010), Nouws antibiotic test (NAT) screening in slaughtered animals (Pikkemaat et al. 2008), Limulus Amebocyte Lysate (LAL) screening in gram-negative bacteria (Seiter and Jay 1980), Microbial Luminescence System (MLS) test (Bottari et al. 2015) are used to assess biological contamination.

Taking into account the progress of synthetic biology, miniaturization, and automation technology, high-performance screening technology has been developed through HTS. HTS technique presents remarkable properties such as screening a significant amount of isolates, a high sensitivity, and determining the minimum inhibitory concentration of the active fraction against a series of indicator microorganisms (Walter et al. 2010; Leavell et al. 2020). Through this technique, the manual intervention is reduced, and at the same time the error rate. For this reason, the HTS technique has emerged as useful technology adopted by biotechnological fields (Sarnaik et al. 2020). There have been some reports of changes in the past, including high-throughput screening systems based on phenolic reaction transcription (Jeong et al. 2012), flow cytometry and light-sensitive sequencing (Sciarria et al. 2019), and extracellular electron transfer (ETT) (Tahernia et al. 2020).

4.2.2 Microbial Bioprocess

Microbial process has been useful in industrial applications through the bioprocess technology. The production strains and the culture conditions are key points in the bioprocesses of microbial agents. In microbial cultures, the mechanisms of the growth process are influenced by the activity of subcellular elements and complexes of enzymes (Panikov 1995). The main factors that influence production are the host of expression, temperature changes, acidity, the composition and tonicity of the media, among others, which must be carefully chosen for better adaptation and efficiency (Rohe et al. 2012). There are different reagents in the culture medium, which can act as restrictive precursors or promoters for the product. Precursors can lead the fermentation process to the formation of specific products (directed biosynthesis) without having to change the rate (Demain 2000b). The use of biological processes involves gentle reactions, which are more specific, more efficient, and produce biomass (renewable products).

The growth of microbial cultures is directly related to growth kinetics, which are affected by chemical and physical parameters. The growth of microorganisms is not linear. As shown in Fig. 4.1, there are different growth stages, which are represented by atypical growth curves.

Growth curves describe the density of cell populations in the media over time, which includes distinct phases, which are described below (Yuan-Kun 2013; Ram et al. 2019).

(a) *Lag phase:* During the cell adaptation phase, the cell size and weight increase.
(b) *Exponential phase:* During this period, the cells begin to grow and multiply, and the cells grow and divide rapidly at an approximately constant rate. After exponential growth, there is a decelerating growth phase.

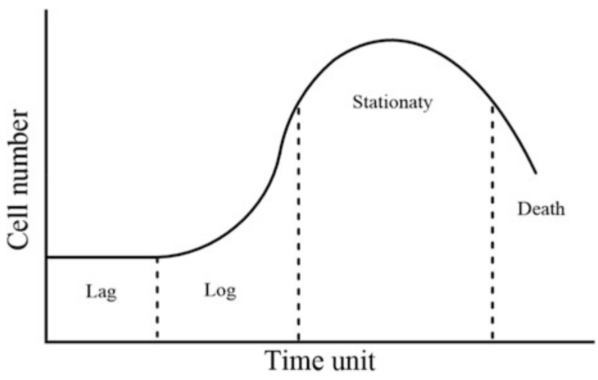

Fig. 4.1 Microbial growth curve

(c) *Stationary phase:* The growth rate is equal to death rate. It could happen when a nutrient is exhausted, or by presence of inhibitors or changes in the medium conditions.
(d) *Death phase:* The conditions of the medium become less favorable and the number of cells begins to decline.

Optimization

Synthetic biological technology has been raised great progress through the modification of factors and processes that are involved in the biotechnological industry. Metabolic engineering and genome editing have been used to modify genomic strains and optimize the structure of producers (Wen et al. 2019). Other promising strategies are based on heterologous expression, coculture systems, improvement of culture conditions, and the designing of bioreactor (Ray and Behera 2017). The strains improvement could reduce the cost of the processes, increase productivity, and obtain specialized characteristics and conditions.

Sustainable Technologies

Microbial technology is considered as a promising element because it can contribute substantively to environmental goals. They exhibit a wide spectrum of evolutionary, functional, and metabolic diversity (Timmis et al. 2017). There are important interactions in nature, such as the interaction between microorganisms and minerals. These interactions contribute to the development of sustainable microbial coal biotechnology, acting as a warehouse for a variety of new biomolecules and acid mine drainage (Mishra et al. 2015). One of the most promising areas of interest is related to bioelectricity generation which brings economic and environmental benefits (Mateo et al. 2014; Xiaobo 2020).

4.2.3 Enzymology

Enzymes are known as a potential biocatalyst, which is demanded by industrial applications for its beneficial properties like high specificity, fast action, low toxicity, product purity, and biodegradability. However, most of them need to be redesigned to improve their catalytic performance (Brahmachari 2016). The use of enzymes has been allowed to work at high temperatures, extreme pH, with organic solvents. Enzymes interfere in most biological processes and therefore in their industrial applications, mainly in fermentation processes. For this reason, microbial biotechnology has had an exponential growth with the study and manufacture of enzymes of various types such as lipases, carbohydrases, proteases, recombinant chymosin, among others (Demain 2000b). The discovery of new microbial enzymes with new characteristics and functions imply possibilities for new applications, mainly in organic synthesis, clinical analysis, pharmaceutical products, and fermentation processes, in addition to having a feasible use in environmental remediation processes (Ogawa 1999; Bhatt 2019) and recombinant DNA technology (Eun 1996).

4.2.4 Gene Manipulation

Enzymes obtained from natural sources are generally not suitable for industrial use. They are usually genetically modified to improve their high yield characteristics. In biotechnology, when the culture medium is manipulated, it involves the testing of hundreds of additives, which are considered to be the limiting precursors of the final product (Demain 2000b).

Through DNA sequences, phylogenetic relationships are obtained between new organisms. This method has a great limitation related to the great diversity of existing species and is only used for nearby species. Based on this precedent, the use of rRNA revolutionized the techniques of recognition and identification of new species. The use of rRNA is characterized by performing an identical function in each organism and by the slight evolutionary change in its sequence, making it an ideal referential marker (Glazer and Nikaido 2007). The exploration of the complete genome sequence from a free-living organism is carried out through the whole-genome shotgun sequencing method, which is shown in Fig. 4.2. This method is traditionally used to identify genome sequences of a given organism, or it is also used to capture a representative sequence of various organisms in a simultaneous way (Venter et al. 2004).

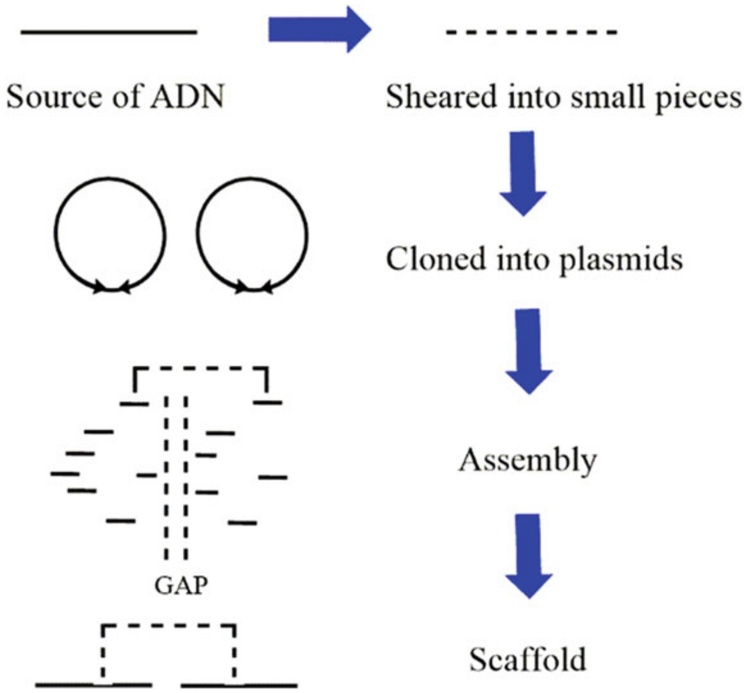

Fig. 4.2 Shotgun sequencing method

Hundreds of sequenced genomes have been collected, thanks to computer systems that have allowed better analysis of biological data.

There are certain bioinformatic parameters, which are important around the sequencing of genomes from various natural sources.

There are specific bioinformatic parameters, which are essential around the sequencing of genomes from various natural sources. Besides, there is a comparative analysis for gene identification and specific function based on genomics. Another relevant point is the visualization and cell simulation to analyze and model the organisms studied and their behaviors. Finally, the application in different areas (Bansal 2005).

Recombinant DNA Technology

In the biotechnology industry, the continuous progress of recombinant DNA technology is noteworthy. This relatively novel technology involves the production of essential and non-essential molecules. This technique is used to discover new secondary products through the introduction of genes (Demain 2000b; Murooka 1993). Recombinant DNA technology involves the modification of genetic material to obtain improved characteristics of a certain organism or its derivatives. This methodology involves inserting DNA fragments of the desired gene sequence or by blocking the expression of endogenous genes (Khan et al. 2016; Lodish et al. 2000).

4.3 Applications

Microbes have been wrongly known just because of their dangerous properties. Microbes describe a wide range of active applications. Unlike natural microorganisms, synthetic microorganisms have been genetically manipulated to perform specific activities and enhance their properties, which is beneficial due to low cost, safety, and wide range (Singh et al. 2014).

4.3.1 Industry

Food-Fermented Foods

In the food industry, microbial biotechnology is focused on improving the safety, quality, and consistency of bioprocessed products, as well as the yield, efficiency, and control of the processes adopted in this industry. Nowadays, biotechnology takes advantage of the biodiversity of described microorganisms, not only to use in important processes such as food fermentation, but also to obtain metabolites and enzymes that are required in the food industry as food ingredients, additives, and aids (Bhowmik and Patil 2018). This section describes how biotechnology, through the use of different types of microbes, has influenced the food industry in three different aspects: improvement of food quality, improvement of the efficiency and productivity of process, and the production of food additives.

Improvement of Food Quality

Fermentation is an anaerobic process which consists of a series of chemical reactions induced by different types of microorganisms or enzymes, which aims to convert sugars into alcohols or organic acids (Balaman 2019). Initially, this technique was used exclusively for the preservation of fruits and vegetables. However, nowadays fermentation is an important technique in the production of food products. Fermented products are part of the daily diet of human beings. Furthermore, the fermentation process is also used to increase the nutritional values of foods, enriching the substrates of proteins, minerals, essential amino acids, and fatty acids. It also contributes to the reduction of bacterial contamination and to eliminate anti-nutritive factors (Tamang et al. 2016; Petrova and Petrova 2020).

The improvement of the nutritional quality of foods through the fermentation process is evident in the production of yogurt. The beneficial compounds in yogurt, which include bioactive peptides, exopolysaccharides, and CLA, are obtained during the fermentation process, in which lactic acid bacteria are involved (Fernandez et al. 2017). These kind of bacteria produce an acidic environment during the fermentation of yogurt. This characteristic favors the bioavailability of the minerals present in yogurt, which are 50% more concentrated than in the raw material (milk) (El-abbadi et al. 2014).

The elimination of anti-nutritional factors from food is another way to improve the nutritional quality of the products. Oligosaccharides that cannot be metabolized by humans (e.g., raffinose, stachyose and verbascose), and whose α-D-Galactosidic bonds are not broken through the cooking process, constitute a very common anti-nutrient in foods such as soybean. Fermentation of soybeans with *Rhizopus oligosporus* molds, which are a great source of α-Galactosidases, allows the degradation of these oligosaccharides. The product obtained from this process is known as Tempeh (Bhowmik and Patil 2018). Other products, nutritionally favored in the fermentation process are described in Table 4.1.

Improvement Efficiency and Productivity of Process

The enzymatic variety that can be obtained from microbes is one of the most valuable resources that microbial biotechnology possesses. Since several enzymes, whose origin is mainly from bacteria and yeasts, are highly used to improve the efficiency and productivity of a number of processes carried out in the food industry.

A very common example is the use of xylanase and cellulase to selectively polish rice. This enzymatic treatment reduces the percentage of rice breakdown, improves nutrient retention, and increases the uptake rate of water, reducing cooking time (Das et al. 2008; Arora et al. 2007). The cellulase enzymes are mainly obtained from *Aspergillus* and *Trichoderma* (fungi) and *Bacillus* and *Paenibacillus* (bacteria). While Xylanase can be obtained from bacteria such as *Bacillus* sp. and *Pseudomonas* sp., as well as fungi such as *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. (Raveendran et al. 2018). From *Aspergillus fumigatus*, in particular, other nonlipolytic enzymes such as hemicellulase, chitanase, pectinase, and protease can also be obtained. These enzymes are used as a pre-treatment for oilseeds since they can degrade their cell wall, facilitating and increasing the performance of the oil

Table 4.1 Microorganisms involved in the improvement of nutritional and functional quality of fermented products

Fermented product	Raw product	Involved microorganism	Advantages	Source
Kimchi	Chinese cabbage 74–90%, radish 2.8–13.5%, garlic 1.4–2.0%, ginger 0.5–1.0%, onion 1.5–2.0%, green onion 1.0–3.5%, red pepper 1.8–3.0%	Starter culture: *Ln. mesenteroides*, *Ln. citreum*, and *Lb. plantarum*	Preservation of vegetables and improvement of nutritional quality	Patra et al. (2016)
Doenjang	Doenjang-meju and brine	*Bacillus, Enterococcus, Lactobacillus, Clostridium, Staphylococcus, Corynebacterium, and Oceanobacillus*	Improvement of nutritional quality	Kwak et al. (2012), Jung et al. (2016)
Kombucha	Tea	Bacteria phylum: *Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Deinococcus-Thermus, Firmicutes, Proteobacteria, and Verrucomicrobia* Yeast phylum: *Ascomycota*	Improvement of nutritional quality	Mitchell and Finn (2020)
Sausages	Pork meat	Starter culture: *Lactobacillus plantarum and Pediococcus damnosus*	Improvement of nutritional and functional quality	Kim et al. (2014)
Pasta	Wheat semolina	*Lactobacillus plantarum*	Improvement of nutritional quality	Capozzi et al. (2011)
Pasta	Durum semolina	*Lactobacillus alimentarius, Lactobacillus brevis, Lactobacillus sanfranciscensis, and Lactobacillus hilgardii*	Reduction of gluten content in the pasta	Di Cagno et al. (2005)
Gari	Cassava tubers	*Leuconostoc, Lactobacillus,* and *Streptococcus*	Elimination of anti-nutritional factors	Omolara (2014)

extraction process (Sarkar et al. 2004). Consequently, a reduction in costs and pollutants is achieved.

Moreover, enzymes of microbial origin are also an essential part in the elaboration of various products. The α-amylase produced by *Bacillus amyloliquefaciens*, *Bacillus stearothermophilus,* or *Bacillus licheniformis* and the glucoamylase produced by *Aspergillus niger* and *Aspergillus awamori*, both enzymes are used in the production of glucose and fructose syrups from starch (Raveendran et al. 2018).

Another way to optimize and improve processes in the food industry, based on microorganisms is fermentation. The fermentation of cocoa and coffee is not only carried out to enhance the flavor of their products, but also because it is an important process for the separation of tissues. The microorganisms related to this fermentation process are mainly yeasts such as *Kloeckera apiculata*, *Hanseniaspora uvarum*, *Pichia kluyveri,* and *Kluyveromyces marxianus*; and LAB, Enterobacteriaceae, and *Bacillus* (Bhowmik and Patil 2018*;* Schwan and Wheals 2010).

Food Additives

In the food industry, one of the greatest challenges historically has been the preservation of food, which so far has been solved through the use of additives. Food additives are also used to improve the taste, color, texture, and aroma of foods. In addition, they are used as antioxidants, emulsifiers, and thickeners (Bhowmik and Patil 2018).

Due to growing evidence of the adverse effects of chemical additives in foods, the popularity of additives of microbial origin has been increasing. Some of these additives, such as bioflavors, can biotechnologically be obtained de novo from the metabolic capacity of microbes, since many of the bioflavors are secondary metabolites or enzymes that are produced naturally by microorganisms. However, the bioflavors used in the food industry can also be obtained by technological biotransformation through fermentation by microorganisms (Schrader 2005). In addition, recombinant technology allows the design of competent microorganisms in the synthesis of different types of additives. In Table 4.2 some types of additives generated from different types of microorganisms used in the food industry are presented.

Agroindustry

The main objective of agricultural biotechnology is to provide the necessary tools to improve the yield of different agronomic practices, while reduce the negative environmental impact that the agroindustry actually produces (Gupta et al. 2016). Based on this objective, two approaches are identified in which biotechnology is currently working and microbial diversity plays an important role. These are: combat pests in crops, increase crop yields and quality of agricultural products.

Pest in Crops

Insects, weeds, and pathogenic microorganisms are the main cause of the loss of crops worldwide (Manosathiyadevan et al. 2017). Chemical pesticides are usually used to avoid pests that tend to attack crops. However, in addition to increasing the

Table 4.2 Different types of additives produced by microbes used in food industries

Additive type	Function	Compound	Features	Microorganism	Source
Bacteriocins	Preserve foods by inhibition or kill undesired microorganisms	Nisin	Inhibit: Gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus*)	*Lactococcus lactis*	Bhowmik and Patil (2018), Silva et al. (2018)
		Pediocin	Inhibit: LAB, clostridia, *Listeria*, *Staphylococci*	*Pediococcus pentosaceus*	
		Lacticin	Inhibit: LAB, clostridia, *Listeria monocytogenes*	*Lactococcus lactis*	
		Subtilin	Inhibit: Gram-positive bacteria	*Bacillus subtilis*	
		Microgard	Inhibit: Gram-negative bacteria, some yeast and mold	*Propionibacterium shermanii*	
		Sakacin	Inhibit: Gram-negative bacteria, some yeast and mold	*Lactobacillus bake*	
		Enterocin	Inhibit: *Listeria monocytogenes and Staphylococcus aureus*	*Enterococcus faecalis*	
		Aureocin	Inhibit: *Listeria monocytogenes*	*Staphylococcus aureus*	
Bioflavors	Diverse group of molecules with distinctive structure and functional groups that contribute to flavor and aroma of food	Pyrazines	Nutty and roasted flavor	Fungi: *Aspergillus* sp. *and Kluyveromyces lactis*	Bhowmik and Patil (2018), Bhari and Singh (2019)
				Bacteria: *Bacillus* sp., *Penicillium* sp., *Pseudomonas* sp., *Streptomyces* sp., *Streptococcus* and *Corynebacterium glutamicum*	
		Vanillin	Vanilla flavor	Fungi: *Phanerochaete chrysosporium, Pycnoporus cinnabarinus*	
		Methyl anthranilate	Fruity type flavor	Fungi: *Pycnoporus cinnabarinus and Trametes* sp.	

(continued)

Table 4.2 (continued)

Additive type	Function	Compound	Features	Microorganism	Source
		Limonin	Citric flavor	Bacteria: *Arthrobacter globiformis*	
		Lactone	Fruity type flavor	Fungi: *Cladosporium* sp.	
				Ceratocystis sp., *Saccharomyces* sp. and *Candida* sp.	
				Bacteria: *Sarcina* sp.	
		Terpene	Fruity type flavor	Fungi: *Ceratocystis* sp., *Kluyveromyces* sp., *Phellinus* sp. and *Lentinus* sp.	
		Diacetyl	Butter-like flavor	Bacteria: *Leuconostoc* sp., *Streptococcus* sp. and *Lactobacillus lactis*	
		Citronellol	Floral flavor	Fungi: *Ceratocystis variospora, Ceratocystis moniliformis* and *Trametes odorata*	
		Methyl ketones	Butter-like flavor	Bacteria: *Pseudomonas oleovorans*	
		Benzaldehyde	Fruity type flavor	Fungi: *Ischnoderma benzoinum*	
				Bacteria: *Pseudomonas putida*	
Pigments	Compounds that can serve as food colorants	Lutein	Yellow color	Protist: *Chlorella* and others *Microalgae*	Sen et al. (2019)
		Zeaxanthin	Yellow color	Bacteria: *Staphylococcus aureus, Flavobacterium* spp., *Paracoccus zeaxanthinifaciens,* and *Sphingobacterium multivorum*	

		Ankaflavin	Yellow color	Fungi: *Monascus* sp.	
		β-Carotene	Yellow-orange color	Protist: *Dunaliella salina*	
				Fungi: *Blakeslea trispora, Fusarium sporotrichioides, Mucor, circinelloides, Neurospora crassa, Phycomyces* and *Blakesleeanus*	
		Phycocyanin	Blue and green color	Protist: *Arthrospira* sp. and *Cyanobacteria*	
				Bacteria: *Pseudomonas* spp.	
		Phycoerythrin	Red color	Protist: *Porphyridium cruentum* and *Cyanobacteria*	
		Heptyl prodigiosin	Red color	Bacteria: *α-Proteobacteria*	
		Prodigiosin	Red color	Bacteria: *Serratia marcescens* and *Pseudoalteromonas rubra*	
		Lycopene	Red color	Fungi: *Fusarium sporotrichioides,* and *Blakeslea trispora*	
		Melanin	Black color	Fungi: *Saccharomyces* and *Neoformans*	
		Canthaxanthin	Orange and pink color	Fungi: *Monascus* spp.	
		Violacein	Purple color	Bacteria: *Janthinobacterium lividum, Pseudoalteromonas tunicata,* and *Chromobacterium violaceum*	
Enzymes		Proteases	Are essential in the production of dough and soy sauce and help in the degradation of plant proteins	Bacteria: *Bacillus* spp.	Raveendran et al. (2018)
				Fungi: *Aspergillus* spp. and *Saccharomyces* spp.	

(continued)

Table 4.2 (continued)

Additive type	Function	Compound	Features	Microorganism	Source
		Pectinases	Participate in: Clarification of fruit juices and wine	Fungi: *Aspergillus* spp.	
		Amylases	Participate in: Saccharification of starch for alcohol production and starch hydrolysis into dextrin, maltose, and glucose	Bacteria: *Bacillus* sp.	
				Fungi: *Aspergillus* spp.	
		ß-Glucanase	Participate in: Reduction of viscosity for mash filtration in brewing	Fungi: *Trichoderma*	
		Lactase	Participate in: Lactose intolerance reduction in people	Fungi: *Kluyveromyces lactis* and *Kluyveromyces fragilis*	
		Peroxidase	Participate in: Development of flavor, color, and nutritional quality of food	Fungi: *Phanerochaete chrysosporium*	
		Cellulase	Participate in: Cellulose degradation and ethanol production	Fungi: *Aspergillus* and *Trichoderma*	
				Bacteria: *Bacillus* and *Paenibacillus*	

price of agricultural production, it has been exposed that pesticides can have negative effects on human and environmental health (Dahab et al. 2017). Therefore, several alternatives have been proposed through microbial biotechnology.

In the case of the control of microorganisms, one of the alternatives consists in the use of microbial biological control agents (MBCA). MBCA consists of a group of microorganisms selected specifically for against pathogens, but also contain antimicrobial metabolites produced by these selected microbes. MBCAs protect cultures from pathogens in four ways. The first is that MBCAs release molecules that stimulate the immune system of the plant (MAMP), inducing resistance (priming). Microorganism-associated molecular patterns (MAMPs) can vary a lot, but some receptors for glucan, chitin, and xylan produced by Phytophthora megacephalus and Trichoderma have been identified. The second form of action of MBCAs is to create an environment of competition for resources with pathogens. For this mechanism to be efficient, MBCAs must contain highly competitive microbes under environmental conditions. The third and fourth mechanisms of action are direct interactions between MBCAs and pathogens, i.e. hyperparasitism and antibiosis through antimicrobial metabolites (Köhl et al. 2019).

For the control of insects, the spores of the Fungi microorganisms as bio-insecticides are a very promising option, since they are responsible for causing diseases to more than 200 different insects (Mostafiz 2012). For its action as an insecticide, the spores of selected Fungi are applied in the fields affected by insects. The spores adhere to the pathogen's cuticle, germinate, and penetrate the insect and invade the interior of the insect spreading its hyphae, which causes mechanical failure and the insect dies (Altinok et al. 2019). The use of the spore combination of *Beauveria bassiana* and *Trichoderma lignorum* as a bioinsecticide for the control of *Atta cephalotes* was recently reported (Felipe et al. 2019).

Nowadays, recombinant technology has allowed the development of new techniques for pest control. One of the most representative examples are Bt cultures. They are named in this way because they are genetically modified cultures that contain one or more genes that encode for one or more *Bacillus thuringiensis* (Bt) proteins. *Bacillus thuringiensis* is one of the most effective microorganisms as pesticide, since its spores contain crystal proteins (Cry and Cyt), which are highly insecticidal. These proteins are those produced by Bt cultures, therefore they are not usually invaded by pathogens (Koch et al. 2015).

Crop Yield and Product Quality

Fertilizers are a mixture of substances assimilable by plants that is widely used in the agricultural industry in order to increase soil nutrients and thus obtain crops with better performance and quality of their products. However, the excessive use of these can bring negative consequences to the environment such as air and water pollution, increased greenhouse gas emissions, and degraded soils (Chandini et al. 2019). Therefore, more environmentally friendly biotechnological alternatives such as bio-fertilizers have been proposed.

The metabolic capacity of some microorganisms has positioned them as powerful fertilizing agents. For example, microorganisms such as *Penicillium bilaii*,

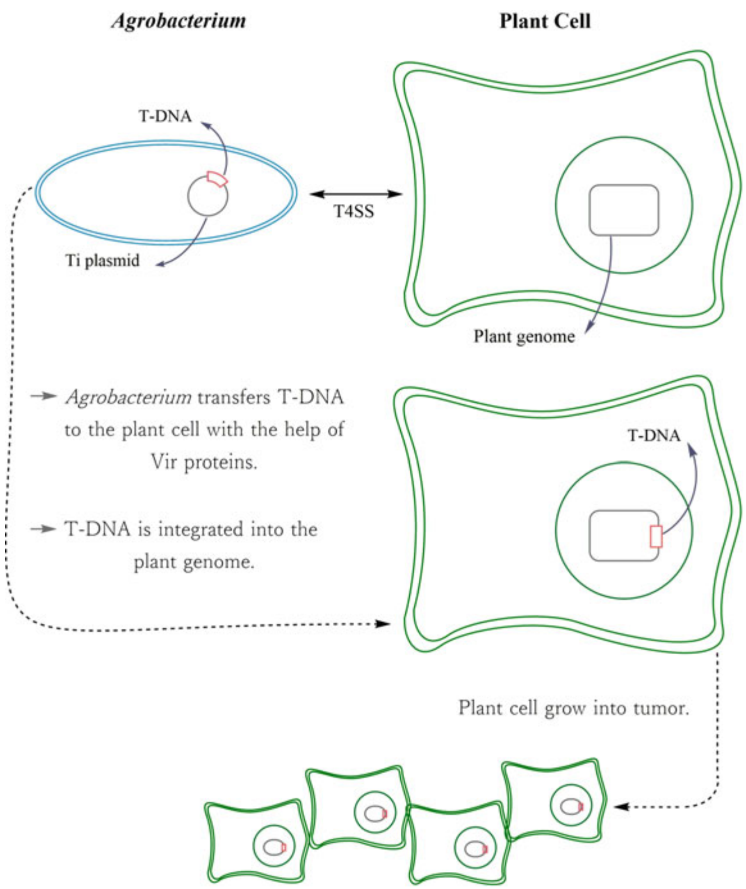

Fig. 4.3 Mechanism of Agrobacterium for gene transfer to plants

Arthrobacter chlorophenolicus, *Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus* are considered as fertilizing agents because they have the ability to solubilize the phosphate fixed in the soil so that it can be assimilated by plants. Other microorganisms of interest are N fixers such as *Rhizobium leguminosarum*, *Staphylococcus* sp., *Bacillus subtilis*, and *Gluconacetobacter diazotrophicus* used as inoculums (Schütz et al. 2018). In addition, some microorganisms (*Actinobacteria*) solubilize phosphate, fix nitrogen, and produce plant growth hormone, which stimulates the spread of roots horizontally, allowing the passage of more nutrients (Anilkumar et al. 2017).

On the other hand, as in the first approach, modern biotechnology makes genetic recombination available, which allows the introduction of genes of interest into the plant. For this, the pathogenicity mechanism of *Agrobacterium* as shown in Fig. 4.3 has been quite useful in the transformation process. They contain T-plasmid as an infectious agent that is used as the vector for exogenous genes (Krebs et al. 2014).

Today, this genus of bacteria, especially *Agrobacterium tumefaciens*, is the basis of one of the most used techniques in the transformation of plants (Baloglu et al. 2018). Some Agrobacterium-based transformations report an increase in the yield of blueberry crops (Song and Gao 2017), and early kiwifruit in vitro flowering (Moss et al. 2018). Although to a lesser extent, viruses have also been used in the transformation of plants. The use of Apple latent spherical vector virus was recently reported to accelerate breeding of Grapevine, reducing its generation time (Maeda et al. 2020).

Construction

In the construction area, microorganisms have been associated with the deterioration of the different materials used in this industry. However, microbial biotechnology has changed this connotation about microorganisms, through taking advantage of their innate metabolic characteristics in the development of construction processes and construction materials elaboration (Dapurkar and Telang 2017).

One of the earliest indications of construction biotechnology was the biotechnological production from microorganisms of polysaccharides and other metabolic products. In this context, microbial polysaccharides are used as admixtures for concrete, dry-mix mortars, injection grouts, and wall plasters (Stabnikov et al. 2015). The resulted characteristics of the admixtures vary depending on the polymer and the material in which it is added. For example, high molecular weight additives such as dextran (produced by *Leuconostoc mesenteroides* or *Streptococcus mutans*) and welan gum (produced by *bacteria Alcaligenes* sp.) when added to concrete reduce the porosity of concrete and can increase its viscosity and water retention capacity. This results in the improvement of the mechanical properties of the concrete, specifically the strength (Stabnikov et al. 2015; Ivanov 2017). In addition to modifying the viscosity and water retention, some admixtures such as Diutan gum (produced by *Sphingomonas* sp.) can generate pseudoplasticity to the material. Other metabolic products derived from microorganisms such as protein hydrolysates, lipids, sophorolipid, lignosulfate, and antioxidants are also used as additives in several of the materials already mentioned. They can have an effect on the shear resistance, mechanical properties, water retention, and fluidity of the material. In addition, these admixtures are used as detoxifiers, anti-corrosive agents, and deodorants (Dapurkar and Telang 2017).

Bio-cementation or also known as microbially induced carbonate precipitation (MICP) is another of the applications of microbial biotechnology in the construction industry. Bio-cementation is a process that occurs due to the precipitation of carbonate produced by the alkalinization of the medium caused by the metabolic activity of the microorganisms involved (Dapurkar and Telang 2017). Several microorganisms such as *Shewanella* (Ghosh et al. 2005), *Sporosarcina pasteurii* (Achal et al. 2011a), and *Bacillus* (Achal et al. 2011b) sp. have been used for bio-cementation processes to improve the strength and permeability of concrete and cement mortar that positively affect the durability of the material. In addition, this process allows the repair of cracks in concrete, either manually (Bang et al. 2001) or even by self-healing (Jonkers et al. 2010).

Microbes in the construction industry are also used to seek for more ecofriendly production alternatives, since concrete production is one of the largest contributors to greenhouse gas emissions. A recent study proposes a new method of bio-cementation using the metabolism of bacteria, which reduces the emission of CO_2 (Myhr et al. 2019).

Chemical Industry

Microbial biotechnology offers to be a good ally of the chemical industry, since the processes used in the production of industrial chemicals are linked to several disadvantages. These include: high production costs, use of non-renewable resources as raw material, high risk level, and poor waste management. Microbial biotechnology, for its part, offers highly competent alternatives for the production of chemicals, which have shown to reduce or eliminate most of these disadvantages. The potential of microbial biotechnology is based on four points. The first is the feedstock flexibility of microorganisms, since they can assimilate and process a wide range of materials. The second is based on the metabolic diversity of microorganisms. This allows all the processes necessary for the elaboration of a chemical product to be carried out within a single cell. Third, microorganisms are simple for their genetic manipulation, which allows any bioprocess to be efficiently designed. Fourth, the culture conditions of the microorganisms are moderate and do not require the use of toxic or flammable products (Burk and Van Dien 2015).

1,3-Propanediol was the first commercially produced compound by a genetically modified microorganism (Barton et al. 2015). Naturally some microorganisms of species such as *Citrobacter*, *Clostridium*, *Enterobacter*, *Klebsiella,* and *Lactobacillus* produce 1,3-propanediol from glycerol. Several genes of these and other species that produce 1,3-propanediol from glycerol were collected and analyzed. Then, through metabolic engineering, a recombinant strain that produces glycerol from D-glucose, the cheapest feedstock, was obtained (Nakamura and Whited 2003). In the chemical industry, other compounds such as 1,4-butanediol (Barton et al. 2015), succinic acid (Zeikus 1999), cis, cis-muconic acid (Yoshikawa et al. 1990), aromatic alcohols (Ghosh et al. 2008), and fine chemicals (Hara 2014) (physiologically active) have been produced from biotechnological techniques. In Fig. 4.4, differences between conventional production and bio-production of a chemical such as 1,4-butanediol are shown.

Cleaning

Bioremediation

Bioremediation is the process in which microorganisms are used for pollutant removal, is a highly promising method, and is cost-effective and efficient technology (Kumar et al. 2011; Azubuike et al. 2016).

Bacteria, microbes, archaea, and fungi are those microorganisms used for bioremediation, in this way they are called bioremediators. The design, control, and optimization of the bioremediation process is a complex system composed of multiple internal and external factors (Zouboulis and Moussas 2011).

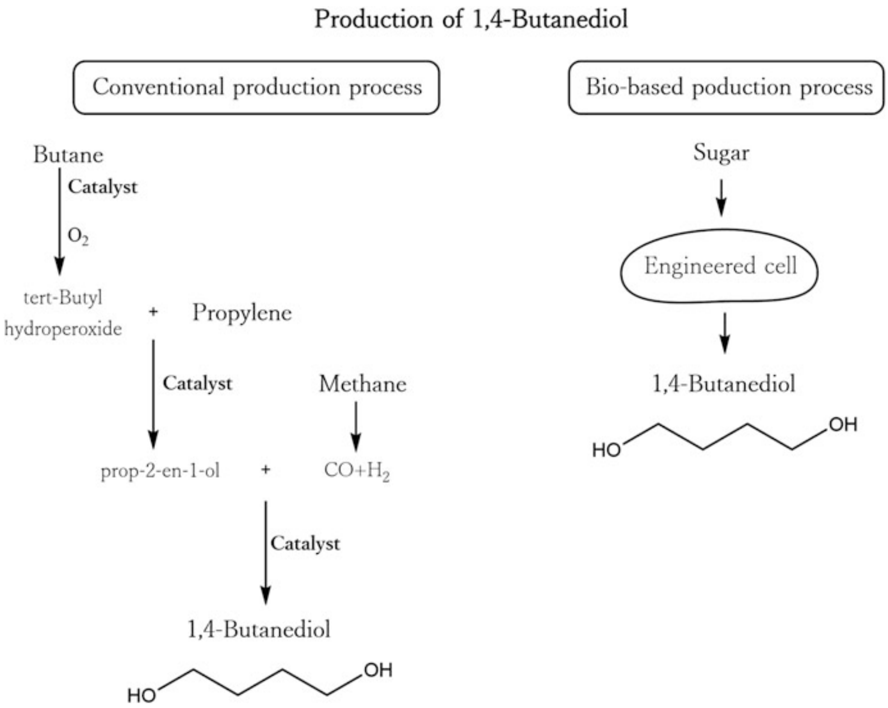

Fig. 4.4 Comparison between the conventional and bio-based production method of 1,4-butanediol

Some microorganisms are used to produce extracellular enzymes due to their properties, which can reduce the amount of pollutants in the environment. These microorganisms are called "microbial cleaners." Microbial cleaners are specially designed from several microorganisms for bioremediation (Spök 2009). Some microbes used for bioremediation including *Bacillus, Mycobacterium, Penicillium, Pseudomonas, Rhizoctomia,* among others (Gupta et al. 2017). However, they work synergistically to enhance their properties of biodegradation.

Chemical-Based Cleaning Products

The employing of chemical-based cleaning products is common in the whole world. The cleaning is a habit and lifestyle adopted for the population throughout the years. However, certain compounds used as cleaners can be harmful. EPA categorizes many of these chemicals as "volatile organic compounds" which can be harmful in different ways. These chemicals include phosphorus, nitrogen, and ammonia (Alton 2020).

The principal cleaners or disinfectants used are based on chlorine (Friedman et al. 2013), formaldehyde, phenolics (Bruins and Dyer 1995), hydrogen peroxide (Akuji and Chambers 2017), peracetic acid (Walters et al. 2019). However, they produce side effects to the day-to-day living, health, and the environment. Therefore, the use of new ways for cleaning arises in order to reduce the negative consequences by the normal cleaners.

As mentioned above, enzymes produced by microorganisms are potentially biodegradable and can be used in detergent products. Bacillus subtilis strains have been engineered to express modified genes and recombinant lipases (Hasan et al. 2010). Saccharomyces cerevisiae and Candida are fungal species used as cleaners (Gupta et al. 2017). Achromobacter, Actinobacillus, Alcaligenes, Arthrobacter, Rhodopseudomonas, Rhodobacter, and Lactobacillus are also used as detergents (Wassenaar 2008).

4.3.2 Environment

Microorganisms play an important role in their environment and contribute to the metabolism of all kinds of compounds, with important consequences for the functioning and maintenance of ecosystems. Thus, microorganisms can adapt to environments contaminated by toxic agents and transform them into harmless agents using the energy used in the process for their benefit (Kumar and Pal 2017). The main advantage of bioremediation mechanisms is that they can be applied in different settings, restoring the environment and preventing potential future contamination (Abatenh et al. 2017). These scenarios include wastewater treatment, solid treatment, metal recovery, and even the production of environmentally friendly fuels.

Wastewater Treatment

Humans have developed treatment systems that use microorganisms found in nature to neutralize household and industrial waste. The microorganisms in the wastewater treatment system remove the organic matter (dissolved in the form of particles), thereby converting it into new cell growth and by-products, that is, they are the main decomposition products (Adebayo and Obiekezie 2018). The microorganisms selected for wastewater treatment must not only use the capacity of organic matter, but also settle after the degradation process is completed, because bacteria and certain protozoa can aggregate to form flocs. It is easy to settle and a clear supernatant is obtained. The weight of filamentous bacteria and fungi is very small, and their surface area is large, so the sedimentation is poor, causing the problem of bulking (foam) (Wagner et al. 2002).

In a wastewater treatment plant, three stages of biodegradation take place during the degradation process:

- **Transfer:** Process by which organic matter comes into contact with microorganisms. It can be by absorption, the dissolved organic matter is transported inside the cell to be used as a source of nutrients, or by adsorption: the microbes adsorbed to the colloidal particles secrete enzymes that break them into particles that can be transported inside the cells (Rani et al. 2019).
- **Conversion:** The microbes are metabolizing their nutrients.

- **Stabilization:** When the microbes complete their capacity, their activity decreases and they sediment or flocculate easily (Rani et al. 2019).

Microbes in a biological waste treatment system are sensitive to many parameters. A very high organic load means that the microorganisms in the system are not enough to consume all the existing nutrients, or extreme temperatures can slow down the metabolism of bacteria in such a way that the decrease in organic matter does not meet the discharge requirements of the effluent (Coelho and Rezende 2015).

Therefore, for an effective treatment the addition of microorganisms is necessary to increase or restore the degradation process in biological treatments. The use of biotechnological products will not only increase the microbial population but will also allow the use of microorganisms that are better adapted to varying conditions of temperature, pH, salinity, etc. (Daims et al. 2006)

Solid Hazardous Treatment

Organic substances belonging to household or commercial solid waste can be biodegraded under controlled conditions until they reach a sufficiently stable state that they can be stored and used without adverse side effects (Shalaby 2011). Controlled conditions give the process a higher speed, reduce its uncertainty, and obtain a uniform final product (Mondal et al. 2019). The solid waste decomposition process can basically be carried out in two ways:

- Under aerobic conditions (in the presence of oxygen), organic matter is directly degraded into carbon dioxide, the most difficult to degrade organic matter is stabilized, and organic fertilizer products (compost) with stable quality are obtained.
- Under anaerobic conditions (in the absence of O_2), organic matter is partially degraded into CH_4 and CO_2 (biogas), and partially stabilized organic matter (Kobayashi and Rittmann 1982).

The two technologies can be implemented independently or in combination. There are experiences in which, in a first stage, anaerobic digestion is applied to obtain biogas and a composting process (maturation) is followed to completely stabilize the organic matter and obtain a high-quality compost (Rastogi et al. 2020).

Regardless of whether the process is aerobic or anaerobic, a biological treatment system consists of the following stages:

- **Pre-treatment:** Operations prior to the biological process, to adapt the waste and allow an adequate development of the process. Depending on the type of waste and the technology applied, the pre-treatment can be more or less intense. The pre-treatment normally includes the removal of unsuitable, crushing, mixing with additives (structuring material, co-substrates, etc.), homogenization, humidity adjustment, etc.
- **Biological treatment/s.**

- **Post-treatment:** Its objective is to refine the characteristics of the product obtained. Some of the possible operations are the classification according to size, the elimination of impurities, the humidity adjustment, the mixtures with inorganic fertilizers, etc.

Composting

Composting is the aerobic biological decomposition under controlled conditions to obtain a product with a high quality and sufficiently stable for storage and use without secondary effects (compost).

As the definition indicates, it is an aerobic biological process. For this reason, it is necessary to maintain optimal conditions so that the microorganisms responsible for the decomposition process can develop (Rastogi et al. 2020). The presence of oxygen is, in this case, the essential condition for the process to take place. Another important point to highlight from the definition is that the objective of the process is to obtain a stable quality product, compost. They indicated that all efforts have to focus on obtaining a quality compost, which can be useful in agriculture as a soil amendment and source of nutrients (Pan et al. 2012; Partanen et al. 2010).

The composting process can be used for a variety of wastes: biosolids (sludge) from sewage and industrial purifiers, livestock waste, plant re-waste from parks and gardens, waste from agri-food industries, and organic fraction of solid waste. As can be seen, composting can be applied to waste with very diverse characteristics (different C/N ratios, moisture content, nutrients, etc.). In many cases, however, it is necessary to mix residues with complementary properties for the process to develop properly (Pan et al. 2012).

For a compost to be considered of quality, it must have the following characteristics: (1) acceptable appearance and color, (2) correct sanitation, (3) low level of impurities and contaminants, (4) high level of agronomically useful components (N, P, K, etc.), and (5) constant composition (Rastogi et al. 2020).

Anaerobic Digestion

Anaerobic digestion is defined as anaerobic microbiological process (total oxygen absence) where organic matter is progressively degraded, by a heterogeneous bacterial population, to methane and carbon dioxide (Wang et al. 2018).

This type of decomposition is nothing more than a fermentation catalyzed by specific bacteria, which occurs sporadically in nature. It is the source of gas from swamps, natural gas from underground deposits, and even gas produced in the stomachs of ruminants.

In general, anaerobic digestion can be applied to any waste. However, the higher its organic matter content, the greater the biogas production and the more appropriate this treatment will be (Kumar and Sharma 2019).

The main advantages of anaerobic digestion include: (1) partially stabilizes and mineralizes organic matter, (2) has a positive energy balance, (3) homogenizes the composition of the waste. Likewise, it should be noted that this process is more sensitive than composting, so it is necessary to better understand the process and control more parameters, and it has a higher cost of implementation (Reineke 2005).

Metal Recovery

Industrial activities generate large-scale contamination with heavy metals (Cu, Zn, Pb, Cd, Cr, Ni, Hg, Co, Ag, Au) and radionuclides (U, Th) in the environment (Krebs et al. 1997). In the particular case of soils, they tend to affect fertility and/or their subsequent use, while in the case of aquifers and surface waters, they can seriously compromise the use of this resource as a source of water for human consumption (Krebs et al. 1997). The remediation of these contaminated environments through the use of chemical methods involves excessively high cost processes due to the specificity required. In addition, this type of solution is not suitable for in-situ repair processes because some metals cannot be processed due to competition from other metals (Ojuederie and Babalola 2017).

The application of effective remedial methods depends on the understanding of site hydrological and geological factors, the solubility and form of heavy metals, the attenuation and fixation process, and the degree to which metals can be dispersed horizontally and vertically when they migrate in the horizontal direction along the ground (Bal et al. 2019). On the other hand, the use of biological methods to repair the contaminated environment has high operational flexibility in both in-situ and ex-situ systems and can easily remove target metals.

The toxicity of heavy metals is very significant. The effects occur directly on organisms by preventing biological activity; that is to say, the inactivation of enzymes is caused by forming the bond between the metal and the -SH (sulfhydryl) group of the protein, thereby causing inactivation in different organisms. Reversal of destruction. In order for heavy metals to be toxic to organisms, they must be able to be captured, that is, the metals must be bioavailable (Ojuederie and Babalola 2017).

All interactions between microorganisms and metals or other elements (such as carbon, nitrogen, sulfur, and phosphorus) are fundamental components of the biogeochemical cycle (Rawlings 2002). Metal–microbiota interactions are studied in depth in the context of environmental biotechnology, in order to implement removal, recovery or detoxification methods for heavy metals and radionuclides (Gadd and Metals 2010).

Depending on the oxidation state of the metal and the species it forms, microorganisms can perform two possible transformations. The transformation will correspond to the mobilization of metals, that is, from the initial insoluble state corresponding to the solid phase (for example, metals related to soil, sulfide, or metal oxide) to the final soluble state (Gadd and Metals 2010). This process is called microbial leaching. The other corresponds to the immobilization of metals, that is, the transition from the initial soluble state in the aqueous phase to the final insoluble state in the solid phase. Conversely, there are multiple mechanisms in nature through which metal fixation can occur (Krebs et al. 1997; Rawlings 2002).

Microbial Biofuels

Microorganisms convert biomass into chemical compounds that can be used in the production of biofuels. This activity has been exploited for many years in the production of methanol, ethanol, and butanol, and more recently interest in the production of hydrogen, biodiesel, among other alternatives, has increased (Kumar and Kumar 2017). The main cost in the production of biofuels, in economic and environmental terms, is the raw material (biomass). The selection of the raw material is fundamental for the conversion to biofuel; the hydrolysis of biomass is required to produce a fermentable substrate. This step can involve physical, chemical, and enzymatic treatments (Sindhu et al. 2019; Bokinsky and Groff 2013).

In a natural way, a large number of metabolic processes occur in microorganisms that generate different compounds, both gaseous and liquid, where energy is stored that can be used as fuel (Speight 2011).

Biomethanol

Methanol has been obtained as an intermediate for degradation of methanotrophic bacteria, which use methane as an energy source to produce carbon dioxide. Methane is a type of biogas, which is produced by the action of microbes called methanogens. It is a very large and diverse group with three basic characteristics: (1) They form a large amount of methane as the main product of energy metabolism; (2) They are strictly anaerobic bacteria, (3) They belong to the field of archaea (Demirbaş 2008).

Methanogenic bacteria gain energy for growth by converting a limited amount of substrate into methane gas (Nakagawa et al. 2011). The synthesis of methane is the main energy source for the growth of methanogens. For this reason, methane production can be regarded as a form of anaerobic respiration, in which the methyl CO_2 from the carbon atom compound or the methyl carbon from the acetate is the electron acceptor. It should be mentioned that methanogens are very sensitive to oxygen (Kumar and Kumar 2017).

The large-scale methanol production process by microbial cells has several technical limitations. This is mainly because the metabolic processes of microorganisms usually produce a variety of products, by-products, and intermediates, which hinder the control and regulation of the global process that generates specific end products. This can be controlled by the use of specific enzymes that direct the reaction to the path required to obtain the desired product (Demirbaş 2008).

Bioethanol

Ethyl alcohol is a chemical product obtained from the fermentation of sugars found in plant products such as: cereals, beets, sugarcane, sorghum, or biomass. These sugars are combined in the form of sucrose, starch, hemicellulose, and cellulose (Ingale et al. 2014).

In the fermentation process of the sugar contained in the organic matter of plants, a hydrated alcohol is obtained. The water content is about 5%. After dehydration, it can be used as fuel and is called bioethanol. It is mixed with gasoline to produce a high-energy biofuel whose characteristics are very similar to gasoline, but significantly reduce the pollution emissions of traditional internal combustion engines (Prasad et al. 2019).

Ethanolic fermentation is by far the most exploited microbial process, and although there are several possible microorganisms responsible, it is undoubtedly the yeast Saccharomyces cerevisiae that is of the greatest industrial importance. However, it has been seen that the bacterium *Zymomonas mobilis* is the other microorganism that produces ethanol through homoethanolic fermentation. Among the by-products obtained from fermentation are: CO_2, low concentrations of methanol, glycerol, and water (Prasad et al. 2019).

Butanol

Butyl alcohol is one of the four-carbon primary alcohols having the molecular formula C_4H_9OH. It is a colorless liquid that produces irritating vapors that have an effect on the mucous membranes and at high concentrations it produces a narcotic effect (Singh and Nigam 2014).

Due to its properties it can directly replace gasoline, or it can serve as a fuel additive. Industrial production is based on a fermentation process carried out by *Clostridium acetobutylicum*, a microorganism that ferments carbohydrates and produces mainly butanol and acetone. However, different clostridia are capable of producing butanol, acetone, and isopropanol (Bokinsky and Groff 2013).

A characteristic of the process is that it is a biphasic fermentation. The first phase is acidogenic and it is an exponential phase, where acetate, butyrate, hydrogen, and CO_2 are formed as main products. The second phase is solvent-borne, the acids are re-assimilated and used in the production of acetone, butanol, and ethanol (Berezina et al. 2012).

Biodiesel

The oil used to make biodiesel is composed of triglycerides, and three fatty acids are esterified with one molecule of glycerol. Then, triglycerides and methanol react in a reaction called transesterification or alcoholysis. Transesterification reaction produces fatty acid methyl esters, called diesel and glycerol (Singh and Nigam 2014).

Unlike the production of biodiesel based on corn, soybean, or palm oil, obtaining with microorganisms has some advantages: (1) the production of oil per area is much higher than 4000 gallons/per year while that of plants is 50–60 gallons/acre per year, (2) microorganisms require much less water than terrestrial plants, (3) can be grown without using topsoil and do not compete for resources from conventional agriculture, (4) biomass production microalgae can be combined with waste CO_2 biofixation (1 kg of dry biomass requires about 1.8 kg of CO_2), (5) fertilizers, mainly nitrogen and phosphorus, can be supplemented by wastewater, (6) the cultivation of microorganisms does not need pesticides, (7) the residual biomass after extraction of the oils can be used as food, fertilizer, as a source for alcoholic fermentation or for methane production, and (8) the composition of the biomass. It can be modulated by varying the growing conditions (Sindhu et al. 2019). However, there are some limitations that must be considered: (1) selecting microalgae or microorganisms with high biomass production and high lipid production, (2) keeping the algae in laboratory conditions or in production systems, (3) carrying out large-scale production of the microorganism, and (4) the energy required to pump water, transfer the CO_2, mix the culture suspension, harvest and drain the biomass of the microorganisms (Speight 2011).

Some microalgae and other microorganisms can generate oils or biodiesel in a renewable way, they can be derived from fatty acids, diacyl or triacylglycerides. Some microalgae like *Botryococcus* can naturally accumulate long-chain terpenoids that can also be used in the production of biodiesel (Singh and Nigam 2014).

Medical Biotechnology

Microbiology plays a significant role in general medical devices, pharmacology and medicine. The main purpose during the use of microbes is to minimize risks. For long years, microbes have been studied as causes of disease. The lack of appropriate analytical methods showed a late comprehension about the importance of them.

Many microorganisms, such as viruses, have been studied through vaccines as antibiotics for drug-resistant bacteria. In the USA, methicillin-resistant Staphylococcus aureus (MRSA) and Clostridium difficile are the most common drug-resistant strains that cause disease. Many gram-negative bacilli with multiple drug resistance also fit the description of super bacteria (antibiotic-resistant microorganisms), and they have become the main last line of defense against these gram-negative super bacteria.

Microbiota is defined as the number of microorganisms into the human body and other multicellular organisms. The microbiota is associated with important functional roles, such as vitamin synthesis, digestion, and colonization resistance to intestinal pathogens (Autenrieth 2017).

The participation of microorganisms in the production of medical products or services involves the biological control of diseases and vaccine production (Vitorino and Bessa 2017). Vaccines are classified as attenuated, inactivated, DNA, or recombinant vaccines as shown in Table 4.3. In an attenuated vaccine, the pathogen (virus or bacteria) is alive and can induce an immune response similar to a real infection

Table 4.3 Types of vaccines

Vaccine	Properties	Application
Attenuated vaccine (A.V.)	High immunogenicity	Influenza, polio, rubella, tuberculosis, dengue, yellow fever
	Humoral immunity	
	One dose	
Inactivated vaccine	Safer than A.V.	Influenza, hepatitis A, cholera, pertussis
	Humoral immunity	
	Multiple doses	
DNA vaccines	Immunogenicity	Influenza, hepatitis B, HIV, HPV
	Use bacteria as carries of DNA plasmid	
Recombinant vaccines	Antibody production	Not yet available clinically
	Immunization	
	Intrinsically safe	

(Plotkin et al. 2008). In contrast, inactivated vaccines consist of the entire or fractioned part of a pathogen completely inactivated. Inactivated vaccine is safer than attenuated because the pathogen is dead. The DNA vaccine then consists of an expression plasmid that contains genes encoding one or more immunogenic antigens of interest (Robinson 1997). Finally, recombinant (gene) vaccines are prepared from viruses engineered with genes encoding antigens, inducing antibody production and immunity (Vitorino and Bessa 2017).

In addition, microorganisms have become an important factor in disease diagnosis. Microbiological assays describe controlled conditions for the growth of microorganisms with an appropriate antibiotic sensitivity pattern, which will work at a specific antibiotic concentration. Escherichia coli, Lactobacillus casei, and other microorganisms have been used to chemically detect the anti-tumor activity of microorganisms, because compounds with anti-tumor activity also often inhibit the growth of test microorganisms (Thayer et al. 1971; Abbott 1976). Although microbiological assays are cheaper, they are less sensitive.

Bacteriocins are a heterogeneous group of biologically active bacterial peptides or proteins synthesized by ribosomes, which show antibacterial activity against other bacteria (Karpiński and Szkaradkiewicz 2016). Bacteriocins are antibiotics produced by certain strains of microorganisms that are active against other strains of the same or related species (Gundogan 2014).

4.4 Conclusions

Advances in technology have promoted the development of new technologies related to microbial research. News and convenient methods with high sensitivity, such as DGGE, NAT, LALA MLS, SPR, and HTS, tend to study the importance of microorganisms. However, HTS (high-throughput screening) describes an effective technique that not only can isolate a large number of strains, but also has high sensitivity, limited manual interaction, and reduced error rates. It is important to consider that microbial production and research depend on growth conditions (hysteresis, exponential, static and death stages).

Microorganisms are essential for maintaining ecosystems. For a long time, microorganisms produced through biotechnology have been an important part of the world's largest industries, such as food, agriculture, construction, and chemical industries. The versatility of microorganisms allows it to be used in simple processes (such as food fermentation), but also in more complex processes (such as the production of recombinant proteins for therapeutic purposes). In addition, the application of genetically modified enzymes produced through microbial technology will work together to improve its performance. Microbial biotechnology has affected the reduction of production costs, the optimization of processes, and the improvement of product quality. At the same time, it has created a potential alternative method for reducing pollutant emissions in the industry. Environmental bioremediation is an area where microorganisms can also be applied. Because of all the advantages associated with these fields, microbes and environmental biotechnology have aroused great interest among researchers. However, there are still many options and areas to explore.

References

Abatenh E, Gizaw B, Tsegaye Z, Wassie M (2017) The role of microorganisms in bioremediation—a review. Open J Environ Biol 2(1):38–46. https://doi.org/10.17352/ojeb.000007

Abbott BJ (1976) Bioassay of plant extracts for anticancer activity. Cancer Treat Rep 60 (8):1007–1010

Achal V, Mukherjee A, Reddy MS (2011a) Effect of calcifying bacteria on permeation properties of concrete structures. J Ind Microbiol Biotechnol 38(9):1229–1234. https://doi.org/10.1007/s10295-010-0901-8

Achal V, Mukherjee A, Reddy MS (2011b) Microbial concrete: way to enhance the durability of building structures. J Mater Civil Eng 23(6):730–734. https://doi.org/10.1061/(ASCE)MT.1943-5533.0000159

Adebayo FO, Obiekezie SO (2018) Microorganisms in waste management. Res J Sci Technol 10 (1):28. https://doi.org/10.5958/2349-2988.2018.00005.0

Adrio JL, Demain AL (2003) Fungal biotechnology. Int Microbiol 6(3):191–199. https://doi.org/10.1007/s10123-003-0133-0

Akuji MA, Chambers DJ (2017) Hydrogen peroxide: more harm than good? Ventilation through an extraglottic tracheal tube: a technique for deep extubation and airway control. Anaesthesia 18 (6):958–959. https://doi.org/10.1093/bja/aex150

Alquéres S, Almeida R, Clementino M, Vieira R, Almeida W, Cardoso A, Martins O (2007) Exploring the biotechnologial applications in the archaeal domain. Braz J Microbiol 38 (3):398–405

Altinok HH, Altinok MA, Koca AS (2019) Modes of action of entomopathogenic Fungi. Curr Trends Nat Sci 8:117–124

Alton, L. How exactly do cleaning supplies affect the environment? https://blueandgreentomorrow.com/environment/how- exactly-cleaning-supplies-affect-environment/. Accessed 17 July 2020

Amann R, Rossello R (2001) The species concept for prokaryotes. FEMS Microbiol Rev 25:39–67

Amsellem L, Brouat C, Duron O, Porter SS, Vilcinskas A, Facon B (2017) Importance of microorganisms to macroorganisms invasions: is the essential invisible to the eye? Adv Ecol Res 57:99–146. https://doi.org/10.1016/bs.aecr.2016.10.005

Anilkumar RR, Edison LK, Pradeep NS (2017) Exploitation of fungi and actinobacteria for sustainable agriculture. In: Microbial biotechnology. Springer, Singapore, pp 135–162. https://doi.org/10.1007/978-981-10-6847-8_6

Arora G, Sehgal VK, Arora M (2007) Optimization of process parameters for milling of enzymatically pretreated basmati rice. J Food Eng 82:153–159. https://doi.org/10.1016/j.jfoodeng.2007.01.023

Autenrieth IB (2017) The microbiome in health and disease: a new role of microbes in molecular medicine. J Mol Med 95(1):1–3. https://doi.org/10.1007/s00109-016-1499-8

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques—classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32(11):180. https://doi.org/10.1007/s11274-016-2137-x

Bal B, Ghosh S, Das AP (2019) Microbial recovery and recycling of manganese waste and their future application: a review. Geomicrobiol J 36(1):85–96. https://doi.org/10.1080/01490451.2018.1497731

Balaman SY (2019) Biomass-based production systems. In: Decision-making for biomass-based production chains. Academic Press, London, pp 25–54. https://doi.org/10.1016/B978-0-12-814278-3.00002-9

Baloglu MC, Kavas M, Gürel S, Gürel E (2018) The use of microorganisms for gene transfer and crop improvement. In: New and future developments in microbial biotechnology and bioengineering. Elsevier, London, pp 1–17. https://doi.org/10.1016/B978-0-444-63987-5.00001-3

Bang SS, Galinat JK, Ramakrishnan V (2001) Calcite precipitation induced by polyurethane-immobilized bacillus pasteurii. Enzyme Microb Technol 28:404–409

Bansal AK (2005) Bioinformatics in microbial biotechnology—a mini review. Microb Cell Fact 4:19. https://doi.org/10.1186/1475-2859-4-19

Barry ER, Bell SD (2006) DNA replication in the archaea. Microbiol Mol Biol Rev 70(4):876–887. https://doi.org/10.1128/MMBR.00029-06

Barton NR, Burgard AP, Burk MJ, Crater JS, Osterhout RE, Pharkya P, Steer BA, Sun J, Trawick JD, Van Dien SJ, Hoon T, Harry Y (2015) An integrated biotechnology platform for developing sustainable chemical processes. J Ind Microbiol Biotechnol 42(3):349–360. https://doi.org/10.1007/s10295-014-1541-1

Behera SS, Ray RC, Das U (2019) Microorganisms in fermentation. In: Essentials in fermentation technology. Springer, Berlin. https://doi.org/10.1007/978-3-030-16230-6

Bendich AJ, Drlica K (2000) Prokaryotic and eukaryotic chromosomes: what's the difference? In: BioEssays. Wiley, Hoboken, pp 481–486. https://doi.org/10.1002/(sici)1521-1878(200005)22:5<481::aid-bies10>3.0.co;2-t

Berezina OV, Zakharova NV, Yarotsky CV, Zverlov VV (2012) Microbial producers of butanol. Appl Biochem Microbiol 48(7):625–638. https://doi.org/10.1134/S0003683812070022

Beveridge TJ (2001) Use of the gram stain in microbiology. Biotech Histochem 76(3):111–118

Bhari R, Singh RS (2019) Microbial production of natural flavours. In: Technology of handling, packaging, processing, preservation of fruits and vegetables: theory and practicals. New India Publishing Agency, New Delhi, pp 767–813

Bhatt P (2019) Smart bioremediation technologies: microbial enzymes, 1st edn. Academic Press, London, p 408

Bhowmik SN, Patil RT (2018) Application of microbial biotechnology in food processing. Elsevier B.V, Amsterdam. https://doi.org/10.1016/B978-0-444-63987-5.00005-0

Biniarz P, Lukaszewics M, Janek T (2017) Screening concepts, characterization and structural analysis of microbial-derived bioactive lipopeptides: a review. Crit Rev Biotechnol 37(3):393–410. https://doi.org/10.3109/07388551.2016.1163324

Bokinsky, G.; Groff, D.; Keasling, J. Chapter 11—Synthetic biology of microbial biofuel production: from enzymes to pathways to organisms Synthetic biology; Zhao, H.; Academic Press: Boston, 2013; pp. 207–223. doi: https://doi.org/10.1016/B978-0-12-394430-6.00011-X

Borneman AR, Schmidt SA, Pretorius IS (2013) At the cutting-edge of grape and wine biotechnology. Trends Genet 29(4):263–271. https://doi.org/10.1016/j.tig.2012.10.014

Bottari B, Santarelli M, Neviani E (2015) Determination of microbial load for different beverages and foodstuff by assessment of intracellular ATP. Trends Food Sci Technol 44:36–48. https://doi.org/10.1016/j.tifs.2015.02.012

Brahmachari G (2016) Biotechnology of microbial enzymes. Production, biocatalysis and industrial applications, 1st edn. Elsevier, London, p 632

Bräsen C, Esser D, Rauch B, Siebers B (2014) Carbohydrate metabolism in archaea: current insights into unusual enzymes and pathways and their regulation. Microbiol Mol Biol Rev 78(1):89–175. https://doi.org/10.1128/MMBR.00041-13

Bruins G, Dyer JA (1995) Environmental considerations of disinfectants used in agriculture. Rev Sci Tech 14(1):81–94

Burk MJ, Van Dien S (2015) Biotechnology for chemical production: challenges and opportunities. Trends Biotechnol 34(3):187–190. https://doi.org/10.1016/j.tibtech.2015.10.007

Capozzi V, Menga V, Diges AM, De Vita P, Van Sinderen D, Cattivelli L, Fares C, Spano G (2011) Biotechnological production of vitamin B2-enriched bread and pasta. J Agric Food Chem 59(14):8013–8020

Chandini, Kumar R, Kumar R, Prakash O (2019) The impact of chemical fertilizers on our environment and ecosystem (Chapter 5). In: Research trends in environmental sciences. AkiNik Publications, New Delhi, pp 69–86

Coelho LM, Rezende HC (2015) Bioremediation of polluted waters using microorganisms (Ch 1). In: Shiomi N (ed) Advances in bioremediation of wastewater and polluted soil, vol 1. IntechOpen, Rijeka. https://doi.org/10.5772/60770

Dahab AA, Jallow M, Albaho M (2017) Environmental and human health impacts of pesticide use in agriculture (Chapter 4). In: Pesticides, pp 2–31. Retrieved from https://www.researchgate.net/publication/322065517_Environmental_and_Human_Health_Impacts_of_Pesticide_Use_in_Agriculture

Daims H, Taylor MW, Wagner M (2006) Wastewater treatment: a model system for microbial ecology. Trends Biotechnol 24(11):483–489. https://doi.org/10.1016/j.tibtech.2006.09.002

Dapurkar D, Telang M (2017) A patent landscape on application of microorganisms in construction industry. World J Microbiol Biotechnol 33(7):138. https://doi.org/10.1007/s11274-017-2302-x

Das M, Gupta S, Kapoor V, Banerjee R, Bal S (2008) Enzymatic polishing of rice—a new processing technology. LWT Food Sci Technol 41:2079–2084. https://doi.org/10.1016/j.lwt.2008.02.007

De Lorenzo V, Prather KLJ, Chen G, Day EO, Von Kameke C, Oyarzún DA, Hosta-rigau L, Alsafar H, Cao C (2018) The power of synthetic biology for bioproduction, remediation and pollution control: the UN's sustainable development goals will inevitably require the application of molecular biology and biotechnology on a global scale. EMBO Rep 19(4):e45658. https://doi.org/10.15252/embr.201745658

Demain AL (2000a) Microb Biotechnol 18(January):89–93

Demain AL (2000b) Microbial biotechnology. Trends Biotechnol 18:26–31. https://doi.org/10.1016/S0167-7799(99)01400-6

Demirbaş A (2008) Biomethanol production from organic waste materials. Energy Sources Part A Recov Util Environ Eff 30:565–572. https://doi.org/10.1080/15567030600817167

Di Cagno R, De Angelis M, Alfonsi G, De Vincenzi M, Silano M, Vincentini O, Gobbetti M (2005) Pasta made from durum wheat semolina fermented with selected lactobacilli as a tool for a potential decrease of the gluten intolerance. Agric Food Chem 53(11):4393–4402. https://doi.org/10.1021/jf048341

Dunlap PV (2001) Microbial diversity. In: Levin SA (ed) Encyclopedia of biodiversity, vol 4. Academic Press, San Diego, pp 191–205. https://doi.org/10.1016/B978-0-12-384719-5.00435-4

El-abbadi NH, Dao MC, Meydani SN (2014) Yogurt: role in healthy and active aging. Am J Clin Nutr 99(5 Suppl):1263S–1270S. https://doi.org/10.3945/ajcn.113.073957.Yogurt

Eun H-M (1996) Enzymology primer for recombinant DNA technology, 1st edn. Academic Press, London, p 728

Felipe F, Daza F, Roman GR, Rodriguez MV, Andres I, Vargas G, Heano HC, Cereda MP, Alberto R, Mulet C (2019) Spores of Beauveria Bassiana and Trichoderma Lignorum as a bioinsecticide for the control of Atta Cephalotes. Biol Res 52:51. https://doi.org/10.1186/s40659-019-0259-y

Fernandez MA, Panahi S, Daniel N, Tremblay A, Marette A (2017) Yogurt and cardiometabolic diseases: A critical review of potential mechanisms. Adv Nutr 8(6):812–829. https://doi.org/10.3945/an.116.013946

Ferrer-miralles N, Villaverde A (2013) Bacterial cell factories for recombinant protein production; expanding the catalogue. Microb Cell Fact 12:113

Friedman ND, Walton AL, Boyd S, Tremonti C, Low J, Styles K et al (2013) The effectiveness of a single-stage versus traditional three-staged protocol of hospital disinfection at eradicating vancomycin-resistant Enterococci from frequently touched surfaces. Am J Infect Control 41(3):227–231

Gadd G, Metals M (2010) Minerals and microbes: geomicrobiology and bioremediation. Microbiology 156(3):609–643. https://doi.org/10.1099/mic.0.037143-0

Ghosh P, Mandal S, Chattopadhyay BD, Pal S (2005) Use of microorganism to improve the strength of cement mortar. Cem Concrete Res 35:1980–1983. https://doi.org/10.1016/j.cemconres.2005.03.005

Ghosh S, Kebaara BW, Atkin AL, Nickerson KW (2008) Regulation of aromatic alcohol production in Candida albicans. Appl Environ Microbiol 74(23):7211–7218. https://doi.org/10.1128/AEM.01614-08

Glazer AN, Nikaido H (2007) Microbial biotechnology: fundamentals of applied, 2nd edn. Cambridge University Press, New York, p 576

Grogan DW (1990) Physiology of prokaryotic cells, 4th edn. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-12-387738-3.00050-0

Gross T, Faull J, Ketteridge S, Springham D (1995) Eukaryotic microorganisms. In: Introductury microbiology. Springer-Science+Business Media, Berlin, pp 241–286. https://doi.org/10.1007/978-1-4899-7194-4

Gundogan N (2014) Definition and general physiological properties of Klebsiella spp. Encycl Food Microbiol 2:383–388. https://doi.org/10.1016/B978-0-12-384730-0.00172-5

Gupta V, Sengupta M, Prakash J, Tripathy BC (2016) An introduction to biotechnology. In: Basic and applied aspects of biotechnology. Springer, Berlin, pp 1–21. https://doi.org/10.1007/978-981-10-0875-7

Gupta C, Prakash D, Gupta S (2017) Microbes: "a tribute " to clean environment. In: Paradigms in pollution prevention, Springer briefs in environmental science. Springer, Berlin, pp 17–34. https://doi.org/10.1007/978-3-319-58415-7

Haq IU, Chaudhry WN, Akhtar MN, Andleeb S, Qadri I (2012) Bacteriophages and their implications on future biotechnology: a review. Virol J 9(1):9. https://doi.org/10.1186/1743-422X-9-9

Hara KY (2014) Development of bio-based fine chemical production through synthetic bioengineering. Microb Cell Factories 13(1):173. https://doi.org/10.1186/s12934-014-0173-5

Harvey L, Arnold B, Lawrence Z, Matsudaira P, Baltimore D, Darnell J (2000) The life cycle of cells. In: Molecular cell biology. McMillan Learning, New York

Hasan F, Shah AA, Javed S, Hameed A (2010) Enzymes used in detergents: lipases. Afr J Biotechnol 9(31):4836–4844. https://doi.org/10.5897/AJBx09.026

Herrero-Uribe L (2011) Viruses, definitions and reality. Rev Biol Trop 59(3):993–998

Ingale S, Joshi SJ, Gupte A (2014) Production of bioethanol using agricultural waste: banana pseudo stem. Braz J Microbiol 45(3):885–892. https://doi.org/10.1590/S1517-83822014000300018

Ivanov V, Stabnikov V (2017) Biotechnological admixtures for cement and mortars. In: Construction biotechnology. Springer Science+Business Media, Singapore, pp 41–50. https://doi.org/10.1007/978-981-10-1445-1_3

Jeong Y-S, Choi S-L, Kyeong H-H, Kim J-H, Kim E-J, Pan JG, Rha E, Song JJ, Lee S-G, Kim H-S (2012) High-throughput screening system based on phenolics-responsive transcription activator for directed evolution of organophosphate-degrading enzymes. Protein 25:725–731. https://doi.org/10.1093/protein/gzs071

Jonkers HM, Thijssen A, Muyzer G, Copuroglu O, Schlangen E (2010) Application of bacteria as self-healing agent for the development of sustainable concrete. Ecol Eng 36:230–235. https://doi.org/10.1016/j.ecoleng.2008.12.036

Jousset A (2017) Aplicación of protists to improve plant growth in sustainable agriculture. In: Rhizotrophs: plant growth promotion to bioremediation. Springer Nature, Springer, pp 263–273. https://doi.org/10.1007/978-981-10-4862-3_13

Jung WY, Jung JY, Lee HJ, Jeon CO (2016) Functional characterization of bacterial communities responsible for fermentation of Doenjang: a traditional Korean fermented soybean paste. Front Microbiol 7:827. https://doi.org/10.3389/fmicb.2016.00827

Karpiński TM, Szkaradkiewicz A (2016) Bacteriocins. In: Encylopedia of food and health. Elsevier, Amsterdam, pp 312–319. https://doi.org/10.1016/B978-0-12-384947-2.00053-2

Khan S, Ullah MW, Siddique R et al (2016) Role of recombinant DNA technology to improve life. Int J Genomics 2016:2405954. https://doi.org/10.1155/2016/2405954
Kim YJ, Park SY, Lee HC, Yoo SS, Oh SJ, Kim HS, Chin KB (2014) Evaluation of fermented sausages manufactured with reduced-fat and functional starter cultures on physicochemical. Funct Flavor Charact 34(3):346–354
Kobayashi H, Rittmann BE (1982) Microbial removal of hazardous organic compounds. Environ Sci Technol 16(3):170A–183A. https://doi.org/10.1021/es00097a002
Koch MS, Ward JM, Levine SL, Baum JA, Vicini JL, Hammond B (2015) The food and environmental safety of Bt crops. Front Plant Sci 6:1–22. https://doi.org/10.3389/fpls.2015.00283
Köhl J, Kolnaar R, Ravensberg WJ (2019) Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Front Plant Sci 10:845. https://doi.org/10.3389/fpls.2019.00845
Kouzuma A, Watanabe K (2014) Microbial ecology pushes frontiers in biotechnology. Microbes Environ 29(1):1–3. https://doi.org/10.1264/jsme2.ME2901rh
Krebs W, Brombacher C, Bosshard PP, Bachofen R, Brandl H (1997) Microbial recovery of metals from solids. FEMS Microbiol Rev 20(3–4):605–617. https://doi.org/10.1111/j.1574-6976.1997.tb00341.x
Krebs JE, Goldstein ES, Kilpatrick ST (2014) Extrachromosomal replicons. In: Lewin' s GENES XI. Jones and Bartlett, Sudbury, pp 328–353
Kumar R, Kumar P (2017) Future microbial applications for bioenergy production: a perspective. Front Microbiol 8:450. https://doi.org/10.3389/fmicb.2017.00450
Kumar A, Pal D (2017) Microorganisms in environmental biotechnology application. In: Advances in biotechnology. Springer, New Delhi, pp 1–259. https://doi.org/10.1007/978-81-322-1554-7
Kumar A, Sharma S (2019) Microbes and enzymes in soil health and bioremediation. Springer, Singapore
Kumar A, Bisht BS, Joshi V, Dhewa T (2011) Review on bioremediation of polluted environment: a management tool. Int J Environ Sci 1(6):1079–1093
Kumar G, Cho SK, Sivagurunathan P, Anburajan P, Mahapatra DM, Park JH, Pugazhendhi A (2018) Insights into evolutionary trends in molecular biology tools in microbial screening for biohydrogen production through dark fermentation. Int J Hydrogen Energy 43(43):19885–19901. https://doi.org/10.1016/j.ijhydene.2018.09.040
Kwak CS, Park SC, Song KY (2012) Doenjang, a fermented soybean paste, decreased visceral fat accumulation and adipocyte size in rats fed with high fat diet more effectively than nonfermented soybeans. J Med Food 15(1):1–9. https://doi.org/10.1089/jmf.2010.1224
Leavell MD, Singh AH, Kaufmann-Malaga BB (2020) High-throughput screening for improved microbial cell factories, perspective and promise. Curr Opin Biotechnol 62:22–28. https://doi.org/10.1016/j.copbio.2019.07.002
Lodish H, Berk H, Zipursky SL et al (2000) Molecular cell biology, 4th edn. W. H. Freeman, New York
Maeda K, Kikuchi T, Kasajima I, Li C, Yamagishi N (2020) Virus-induced flowering by apple latent spherical virus vector: effective use to accelerate breeding of grapevine. Viruses 12(1):70
Manosathiyadevan M, Bhuvaneshwari V, Latha R (2017) Impact of insects and pests in loss of crop production: a review. Springer, Singapore, pp 57–67
Mateo S, Gonzalez del Campo A, Cañizarez P, Lobato J, Rodrigo MA, Fernandez FJ (2014) Bioelectricity generation in a self-sustainable microbial solar cell. Bioresour Technol 159:451–454. https://doi.org/10.1016/j.biortech.2014.03.059
Mishra S, Panda S, Pradhan N, Biswal SK, Sukla LS, Mishra B (2015) Microbe–mineral interactions: exploring avenues towards development of a sustainable microbial technology for coal beneficiation. Environ Microbial Biotechnol 44:33–52. https://doi.org/10.1007/978-3-319-19018-1_2
Mitchell AL, Finn RD (2020) Microbial composition of Kombucha determined using amplicon sequencing and shotgun metagenomics. J Food Sci 85(2):455–464. https://doi.org/10.1111/1750-3841.14992

Moat AG, Foster JW, Spector MP (2002) Microbial physiology. Wiley-Liss, New York

Monciardini P, Iorio M, Maffioli S, Sosio M, Donadio S (2014) Discovering new bioactive molecules from microbial sources. Microb Biotechnol 3:209–220. https://doi.org/10.1111/1751-7915.12123

Mondal S, Palit D, Mondal S, Palit D (2019) Effective role of microorganism in waste management and environmental sustainability. In: Jhariya M, Banerjee A, Meena R, Yadav D (eds) Sustainable agriculture, forest and environmental management. Springer, Singapore, pp 487–516. https://doi.org/10.1007/978-981-13-6830-1_14

Morgan AG, McCauley TJ, Stanaitis ML, Mathrubutham M, Millis SZ (2004) Development and validation of a fluorescence technology for both primary and secondary screening of kinases that facilitates compound selectivity and site-specific inhibitor determination. Assay Drug Dev 2:171–181. https://doi.org/10.1089/154065804323056l2

Moss SMA, Wang T, Voogd C, Brian LA, Wu R, Hellens RP, Allan AC, Putterill J, Varkonyi-Gasic E (2018) AcFT promotes kiwifruit in vitro flowering when overexpressed and arabidopsis flowering when expressed in the vasculature under its own promoter. Plant Direct 2(7):e00068. https://doi.org/10.1002/pld3.68

Mostafiz S (2012) Biotechnology: role of microbes in sustainable agriculture and environmental health biotechnology. Internet J Microbiol 10(1):1937–1828. https://doi.org/10.5580/2b91

Murat D, Byrne M, Komeili A (2010) Cell biology of prokaryotic organelles, vol 2. Cold Spring Harbor Laboratory Press, New York. https://doi.org/10.1101/cshperspect.a000422

Murooka Y (1993) Recombinant microbes for industrial and agricultural applications, 1st edn. CRC Press, Boca Raton, p 896

Myhr A, Frida R, Brandtsegg AS, Bjerkseter C (2019) Towards a low CO2 emission building material employing bacterial metabolism (2/2): prospects for global warming potential reduction in the concrete industry. PLoS One 14(4):e0208643

Nakagawa H, Sakai M et al (eds) (2011; Ch. 30) Biomethanol production from forage grasses, trees, and crop residues. IntechOpen, Rijeka. https://doi.org/10.5772/18168

Nakamura CE, Whited GM (2003) Metabolic engineering for the microbial production of 1,3-propanediol. Curr Opin Biotechnol 14(5):454–459. https://doi.org/10.1016/j.copbio.2003.08.005

Nigam PS (2013) Microbial enzymes with special characteristics for biotechnological applications. Biomolecules 3(3):597–611. https://doi.org/10.3390/biom3030597

Ogawa J (1999) Microbial enzymes: new industrial applications from traditional screening methods. Trends Biotechnol 17:13–20. https://doi.org/10.1016/s0167-7799(98)01227-x

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14(12):1504. https://doi.org/10.3390/ijerph14121504

Omolara BO (2014) Cyanide content of commercial gari from different areas of Ekiti State, Nigeria. World J Nutr Health 2(4):58–60

Pan I, Dam B, Sen SK (2012) Composting of common organic wastes using microbial inoculants. 3 Biotech 2(2):127–134. https://doi.org/10.1007/s13205-011-0033-5

Panikov NS (1995) Microbial growth kinetics, 1st edn. Springer, Berlin, p 378

Partanen P, Hultman J, Paulin L, Auvinen P, Romantschuk M (2010) Bacterial diversity at different stages of the composting process. BMC Microbiol 10(1):94. https://doi.org/10.1186/1471-2180-10-94

Patra JK, Das G, Paramithiotis S, Shin H (2016) Kimchi and other widely consumed traditional fermented foods of Korea: a review. Front Microbiol 7:1493. https://doi.org/10.3389/fmicb.2016.01493

Petrova P, Petrova K (2020) Lactic acid fermentation of cereals and pseudocereals: ancient nutritional biotechnologies. Nutrients 12(4):1118

Piel J (2010) The chemistry of symbiotic interactions. In: Comprehensive natural products II: chemistry and biology. Elsevier Science, Oxford, pp 475–509. https://doi.org/10.1016/B978-008045382-8.00049-6

Pikkemaat MG, Dijk SO, Schouten J, Rapallini M, Van Egmond HJ (2008) A new microbial screening method for the detection of antimicrobial residues in slaughter animals: the Nouws antibiotic test (NAT-screening). Food Control 19:781–789. https://doi.org/10.1016/j.foodcont.2007.08.002

Plotkin SA, Orenstein WA, Offit PA (2008) Vaccines. Saunders/Elsevier, Philadelphia, pp 399–434

Prasad RK, Chatterjee S, Mazumder PB, Gupta SK, Sharma S, Vairale MG, Datta S, Dwivedi SK, Gupta DK (2019) Bioethanol production from waste lignocelluloses: a review on microbial degradation potential. Chemosphere 231:588–606. https://doi.org/10.1016/j.chemosphere.2019.05.142

Ram Y, Dellus-Gur E, Bibi M, Karkare K, Obolski U, Feldman MW, Cooper TF, Berman J, Hadany L (2019) Predicting microbial growth in a mixed culture from growth curve data. Proc Natl Acad Sci 116:14698–14707. https://doi.org/10.1073/pnas.1902217116

Rani, N.; Sangwan, P.; Joshi, M.; Sagar, A.; Bala, K. Chapter 5—Microbes: a key player in industrial wastewater treatment; Microbial wastewater treatment MP Shah, S Rodriguez-Couto Elsevier, Amsterdam 2019; pp. 83–102. doi: https://doi.org/10.1016/B978-0-12-816809-7.00005-1

Rastogi M, Nandal M, Khosla B (2020) Microbes as vital additives for solid waste composting. Heliyon 6(2):e03343. https://doi.org/10.1016/j.heliyon.2020.e03343

Raveendran S, Kuruvilla A, Rebello S (2018) Applications of microbial enzymes in food industry. Food Technol Biotechnol 56(1):16–30. https://doi.org/10.17113/ftb.56.01.18.5491

Rawlings DE (2002) Heavy metal mining using microbes. Annu Rev Microbiol 56(1):65–91. https://doi.org/10.1146/annurev.micro.56.012302.161052

Ray RC, Behera SS (2017) Solid state fermentation. Biotechnology of microbial enzymes. Elsevier, Amsterdam

Reineke W (2005) Aerobic and anaerobic biodegradation potentials of microorganisms. Biodegrad Persistance 2:1–161. https://doi.org/10.1007/10508767_1

Retra K, Geitmann M, Kool J, Smit AB, de Esch IJP, Danielson UH, Irth H (2010) Development of surface plasmon resonance biosensor assays for primary and secondary screening of acetylcholine binding protein ligands. Analy Biochem 407:58–64. https://doi.org/10.1016/j.ab.2010.06.021

Robinson HL (1997) Nucleic acid vaccines: an overview. Vaccine 15(8):785–787. https://doi.org/10.1016/S0264-410X(96)00249-6

Rogers K (2011) Bacterial morphology and reproduction. In: Bacteria and viruses. Britannica Educational Publishing, Chicago, pp 3–28. Retrieved from https://eb.pdn.ipublishcentral.com/product/bacteria-viruses

Rohe P, Venkanna D, Kleine B, Freudkl R, Oldiges M (2012) An automated workflow for enhancing microbial bioprocess optimization on a novel microbioreactor platform. Microb Cell Fact 11:144. https://doi.org/10.1186/1475-2859-11-144

Sarkar BC, Pandey S, Kumbhar B, Agrawal YC (2004) Aqueous oil extraction from enzyme pretreated sesame seed and process parameters optimization. J Food Sci Technol 41:604–608

Sarnaik A, Liu A, Nielsen D, Varman AM (2020) High-throughput screening for efficient microbial biotechnology. Curr Opin Biotechnol 64:141–150. https://doi.org/10.1016/j.copbio.2020.02.019

Schrader J (2005) Microbial flavour production. In: Berger RG (ed) Flavours and fragrances. Springer, Berlin, pp 507–574. https://doi.org/10.1007/978-3-540-49339-6_23

Schütz L, Gattinger A, Meier M, Müller A, Boller T, Mäder P, Mathimaran N, Scotti R (2018) Improving crop yield and nutrient use efficiency via biofertilization—a global meta-analysis. Front Plant Sci 8:2204. https://doi.org/10.3389/fpls.2017.02204

Schwan RF, Wheals AE (2010) The microbiology of cocoa fermentation and its role in chocolate quality the microbiology of cocoa fermentation and its role in chocolate quality. Food Sci Nutr 44:205–221. https://doi.org/10.1080/10408690490464104

Sciarria TP, Arioli S, Gargari G, Mora D, Adani F (2019) Monitoring microbial communities' dynamics during the start-up of microbial fuel cells by high-throughput screening techniques. Appl Biotechnol Rep 21:e00310. https://doi.org/10.1016/j.btre.2019.e00310
Seiter JA, Jay JM (1980) Comparison of direct serial dilution and most-probable number methods for determining endotoxins in meats by the Limulus amoebocyte lysate test. Appl Environ Microbiol 40:177–178. https://doi.org/10.1128/AEM.40.1.177-178.1980
Sen T, Barrow C, Deshmukh SK (2019) Microbial pigments in the food industry—challenges and the way forward. Front Nutr 6:7. https://doi.org/10.3389/fnut.2019.00007
Sergio A, De Araujo F, Mendes LW, Lemos LN, Emanuel J, Antunes L, Evando J, Beserra A, Catanho C, De Lyra P, Barreto V, Celis Â, Lopes DA, Lucia R, Gomes F, Bezerra WM, Maria V, Melo M, De Araujo FF, Geisen S (2018) Protist species richness and soil microbiome complexity increase towards climax vegetation in the Brazilian Cerrado. Commun Biol 1:135. https://doi.org/10.1038/s42003-018-0129-0
Shalaby EA (2011) Prospects of effective microorganisms technology in wastes treatment in Egypt. Asian Pac J Trop Biomed 1(3):243–248. https://doi.org/10.1016/S2221-1691(11)60035-X
Silva CCG, Silva SPM, Ribeiro SC (2018) Application of bacteriocins and protective cultures in dairy food preservation. Front Microbiol 9:594. https://doi.org/10.3389/fmicb.2018.00594
Sindhu R, Binod P, Pandey A, Ankaram S, Duan Y, Awasthi MK (2019) Chapter 5—Biofuel production from biomass: toward sustainable development. In: Kumar S, Kumar R, Pandey A (eds) Current development in biotechnology and bioengineering. Elsevier, Amsterdam, pp 79–92. https://doi.org/10.1016/B978-0-444-64083-3.00005-1
Singh A, Nigam P (2014) Microbial biofuels production. In: Microbial biotechnology progress and trends. CRC Press, Boca Raton, pp 155–168. https://doi.org/10.1201/b17587-8
Singh JS, Abhilas PC, Singh HB, Singh RP, Singh DP (2011) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480 (1–2):1–9. https://doi.org/10.1016/j.gene.2011.03.001
Singh R, Singh P, Sharma R (2014) Microorganism as a tool of bioremediation technology for cleaning environment: a review. Proc Int Acad Ecol Environ Sci 4(1):1–6
Snyder L, Peters JE, Henkin TM, Champness W (2013) Molecular genetics of bacteria, 4th edn. ASM Press, Washington, DC
Song G, Gao X (2017) Transcriptomic changes reveal gene networks responding to the overexpression of a blueberry DWARF AND DELAYED FLOWERING 1 gene in transgenic blueberry plants. BMC Plant Biol 17(1):106. https://doi.org/10.1186/s12870-017-1053-z
Speight JG (2011) Chapter 3—Fuels for fuel cells. In: Shekhawat D, Spivey JJ, Berry DA (eds) Fuel cells: technologies for fuel processing. Elsevier, Amsterdam, pp 29–48. https://doi.org/10.1016/B978-0-444-53563-4.10003-3
Spök A (2009) Environmental, health and legal aspects of cleaners containing living microbes as active ingredients. IFZ, Graz
Stabnikov V, Ivanov V, Chu J (2015) Construction biotechnology: a new area of biotechnological research and applications. World J Microbiol Biotechnol 31(9):1303–1314. https://doi.org/10.1007/s11274-015-1881-7
Straub CT, Counts JA, Nguyen DMN, Wu C, Zeldes BM, Crosby JR, Conway JM, Otten JK, Lipscomb GL, Schut GJ, Adams MWW, Kelly RM, Kelly RM (2018) Biotechnology of extremely thermophilic archaea. FEMS Microbiol Rev 42(5):543–578. https://doi.org/10.1093/femsre/fuy012
Tahernia M, Mohammadifar M, Gao Y, Panmanee W, Hassett DJ, Choi S (2020) A 96-well high-throughput, rapid-screening platform of extracellular electron transfer in microbial fuel cells. Biosens Bioelectron 162:112259. https://doi.org/10.1016/j.bios.2020.112259
Tamang JP, Shin D, Jung S, Chae S (2016) Functional properties of microorganisms in fermented foods. Front Microbiol 7:578. https://doi.org/10.3389/fmicb.2016.00578
Thayer PS, Gordon HL, Macdonald M (1971) In vitro growth inhibition by 3837 compounds tested for antitumor activity: comparison of tumor cell culture and microbial assays. Cancer Chemother Rep 2(1):27–55

Timmis K, de Vos WM, Ramos JL et al (2017) The contribution of microbial biotechnology to sustainable development goals. Microb Biotechnol 10:984–987. https://doi.org/10.1111/1751-7915.12818

Tortora GJ, Funke BR, Case CL (2019) Microbiology—an introduction. Pearson, London, pp 2–6

Venter JC, Remington K, Heidelberg JF et al (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66–74. https://doi.org/10.1126/science.1093857

Vitorino LC, Bessa LA (2017) Technological microbiology: development and applications. Front Microbiol 8:1–23. https://doi.org/10.3389/fmicb.2017.00827

Wagner M, Loy A, Nogueira R, Purkhold U, Lee N, Daims H (2002) Microbial community composition and function in wastewater treatment plants. Antonie Van Leeuwenhoek 81 (1):665–680. https://doi.org/10.1023/A:1020586312170

Walter V, Syldatk C, Hausmann R (2010) Screening concepts for the isolation of biosurfactant producing microorganisms. Biosurfactants 672:1–13. https://doi.org/10.1007/978-1-4419-5979-9_1

Walters GI, Burge PS, Moore VC, Thomas MO, Robertson AS (2019) Occupational asthma caused by peracetic acid-hydrogen peroxide mixture. Occup Med (Lond) 69(4):294–297. https://doi.org/10.1093/occmed/kqz032

Wang P, Wang H, Qiu Y, Ren L, Jiang B (2018) Microbial characteristics in anaerobic digestion process of food waste for methane production—a review. Bioresour Technol 248:29–36. https://doi.org/10.1016/j.biortech.2017.06.152

Ward BB (2002) How many species of prokaryotes are there? Proc Natl Acad Sci USA 99:10234–10236

Wassenaar TM (2008) Safety aspects and implications of regulation of probiotic bacteria in food and food supplements. J Food Prot 71(8):1734–1741. https://doi.org/10.4315/0362-028x-71.8.1734

Wen Z, Li Q, Liu J, Jin M, Yang S (2019) Consolidated bioprocessing for butanol production of cellulolytic Clostridia: development and optimization. Microb Biotechnol 13(2):410–422. https://doi.org/10.1111/1751-7915.13478

Xiaobo L (2020) Microbial technology for the sustainable development of energy and environment. Biotechnol Rep (Amst) 27:e00486. https://doi.org/10.1016/j.btre.2020.e00486

Yoshikawa N, Mizuno S, Ohta K, Suzuki M (1990) Microbial production of cis, cis-muconic acid. J Biotechnol 14:203–210. https://doi.org/10.1016/0168-1656(90)90009-Z

Yuan-Kun L (2013) Microbial biotechnology principles and applications, 3rd edn. World Scientific Publishing, Singapore

Zeikus MJG (1999) Biotechnology of succinic acid production and markets for derived industrial products. Appl Microbiol Biotechnol 51:545–552

Zouboulis AI, Moussas PA (2011) Groundwater and soil pollution: bioremediation. In: Encyclopedia of environmental health. Elsevier, Amsterdam, pp 1037–1044. https://doi.org/10.1016/B978-0-444-52272-6.00035-0

Applications of Microbes for Energy

5

Felipe M. de Souza, Tenzin Ingsel, and Ram K. Gupta

Abstract

This chapter discusses in detail the use of microbes as affordable and sustainable options to produce energy as well as chemicals for industrial applications. Several areas of applications such as fuel cells, methanol, ethanol, methane, hydrogen, solar cell, biodiesel, electrosynthesis, and energy storage are discussed demonstrating the recent progress in the field of microbes, biochemical mechanisms, and the challenges required to overcome for future works. Extensive use of petrochemicals for chemicals and energy has caused an increase in greenhouse gasses and environmental concerns. The use of microbes for these applications is a solution to these issues as they consume methane, carbon dioxide, and organic wastes to generate clean energy and chemicals. The recent advances in technology provide opportunities to couple microbial fuel cells with other processes such as microbial electrolysis, microbial electrosynthesis so that the hybrid device can simultaneously consume wastes to produce substances for industrial applications and generate clean energy. The application of the microbial process for clean energy and chemicals through the consumption of organic wastes is a promising green approach for a sustainable future.

Keywords

Microbes · Fuel cells · Biodiesel · Hydrogen · Bioalcohol

F. M. de Souza · T. Ingsel · R. K. Gupta (✉)
Department of Chemistry, Kansas Polymer Research Center, Pittsburg State University, Pittsburg, KS, USA
e-mail: rgupta@pittstate.edu

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5.1 Introduction

The microbes or microorganisms are the smallest life-forms known, usually composed of one cell or multi-cells to form a colony. Their size ranges between 1 and 200 μm. Due to their relatively simple structure, their mutation rates are much faster than other living beings. Because of that, microbes are extremely adaptable and are present in nearly every type of environment, even those considered fatal for most creatures, making them the most abundant type of life on the planet. Besides, their large variety enabled them to be among several kingdoms simultaneously such as Monera as bacteria, Protista as protozoans, and Fungi as fungus and mold, etc. Even though some microorganisms are harmful, there are a large number of microbes which are very important for life maintenance as well as industrial applications. Out of around 4000 cataloged enzymes, 200 enzymes are used for commercial applications (Liu and Kokare 2017). Microbes possess unique enzymes that allow them to use in specific enzymatic synthetic routes for stereoselective products (Gurung et al. 2013). The source of enzymes is abundant, for example, fungi and yeast are responsible for the production of about half of the known enzymes whereas bacteria and plants produce around 35% and 15% (Liu and Kokare 2017). The enzymes originating from fungi, yeast, and bacteria are mostly used for commercial applications due to their activeness and stability compared with the ones originating from animals and plants.

The use of microbes for producing high-value products is constantly increasing. The microbes market was valued at around USD 10 billion in 2019 and is expected to grow at 7.1% per year up to 2027 (Grand View Research 2020). Industries such as food and beverage, biofuel, animal feed, and home cleaning are mostly responsible for the increased demand of the market. With the advancement in technology, microbes are capable of producing several new products that were not possible in the past. For example, the advanced techniques allow microbial recombination for genetic manipulation to produce new products/enzymes (Adrio and Demain 2014). Metagenome mining helps to find functional genes and correlate them with other microbial members (Delmont et al. 2011). Fermentation, a widely used bioprocess, converts organic substrates like polysaccharides, sugars, or organic matter into ethanol and carbon dioxide in the absence of oxygen. Fermentation is the main process being used to generate ethanol as fuel. Other routes of fermentation process also enable the production of lactic, citric, and acetic acid, acetone, vitamin B_{12}, beer, wine, bread among many others (Stanbury et al. 2013). The recovery process is another important technique which thrives microbes for enzymatic synthesis and large-scale applications. This process reestablishes their use to guarantee satisfactory levels of production after their exposure to an aggressive environment like high or low temperature, pH, antibiotics, heavy metals, etc. (Wu 2008).

An important aspect of effective work with microbes is the controlled environment to optimize the process, which means proper temperature, pH, nutrients, and usually the absence of oxygen to allow the enzymatic fermentation processes to occur. Thus, some specific instruments may be required to carry out the procedures (Li et al. 2012). Despite those requirements, microbes are used in many sectors. In food and beverage, for example, the process of baking and brewing is made using mostly amylase, which is an enzyme able to convert sugar into ethanol and carbon dioxide and protease which is an enzyme able to chemically break protein (Collar et al. 2000). Detergents make good use of enzymes employing a wide variety of it to improve efficacy. Since they can break molecules into smaller ones, making stains easier to remove while adding an eco-friendly aspect to them. Commonly used enzymes for this end are amylase, protease, lipase, and cellulose (Bisgaard-Frantzen et al. 1999). Microbial enzymes are also employed in the textile market for the production of eco-friendly fibers. The wide application in the textile industry is due to the low cost and green approach that reduces the use of organic solvents and aggressive chemical processes (O'Neill et al. 2007). One of the most notable applications of microbial enzymes is in the production of ethanol. It provides a sustainable way to use bio-renewable resources for the production of energy materials. Ethanol is mostly obtained through the fermentation of starch present in corn kernels, sugarcane, or agro-residues (Gupta and Verma 2015). Other approaches such as using them in fuel cells have been deeply studied by researchers to develop sustainable routes to harvest energy. Microbes based fuel cells are called microbial fuel cells (MFCs) which is attending a significant amount of scientific interest for green energy production. A microbial fuel cell consists of the extraction of the energy from chemical bonds in organic components and turning it into electricity using microorganisms such as electrogenic bacteria or enzymes (Deval et al. 2017). Figure 5.1 shows the possible applications of microbes for energy. Eco-friendliness is one of the main advantages of using microbes for energy applications as microbes can consume organic wastes with a high concentration of polysaccharides, sugars, proteins, lipids, and so on without a need of purification as microbes only attack the organic matter which they can digest, thus making this process cost-effective too.

Another important aspect of microbial energy plants is that they can be installed in nearby cities to minimize energy loss as they don't impose any hazard or pollution. Despite the advantages of being used for a viable energy source for the future, microbes have some concerns that need to be addressed to make the process more efficient. One of the concerns is the removal of the catabolic products as their high concentrations inside the reactor make the process less efficient. Also, a microbial plant that produces hydrogen requires an oxygen-free environment to avoid the risk of an accident (Buckley 2006). Despite these concerns, microbes have huge potential for future energy generation by establishing a sustainable cycle with renewable and nearly zero cost sources along with waste management. Microbes are directly applied in fuel cells, production of hydrogen, ethanol, methane, biodiesel, solar cells, and electrosynthesis which are explained and discussed in the following sections.

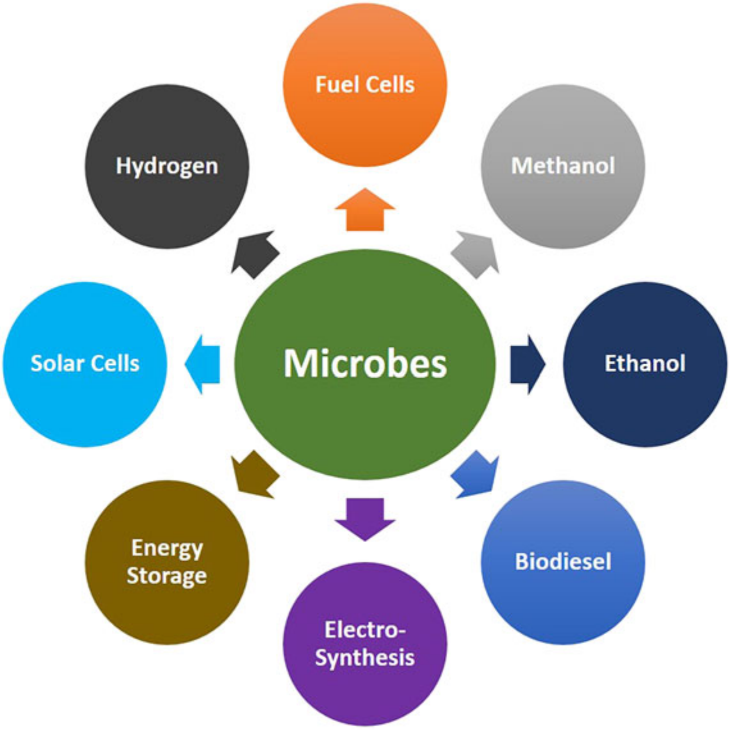


Fig. 5.1 Application of microbes for energy generation and storage

5.2 Microbes for Energy Applications

5.2.1 Microbes for Fuel Cells

Microbial fuel cells have been receiving a great amount of attention due to their ability to generate green energy using organic wastes. MFCs consist of the extraction of the energy from organic components through the breakage of chemical bonds and turning it into electricity using microorganisms (Deval et al. 2017). An MFC device is a variant version of a battery where the anode is in an anaerobic chamber and the cathode is in an aerobic chamber separated by a proton exchange membrane (PEM). In the anode chamber, oxidation of an organic substrate takes place by microbes, usually bacteria or fungus that forms a biofilm. During the oxidative process, the generated electrons are transferred to the cathode through an external circuit. Hence, the anode is composed of two elements. First is the microorganism that functions as biocatalyst due to its ability to metabolize organic matter through the produced enzyme. Second, the electrode that accepts the generated electrons during the oxidation of the organic materials by the microorganisms. The protons and electrons

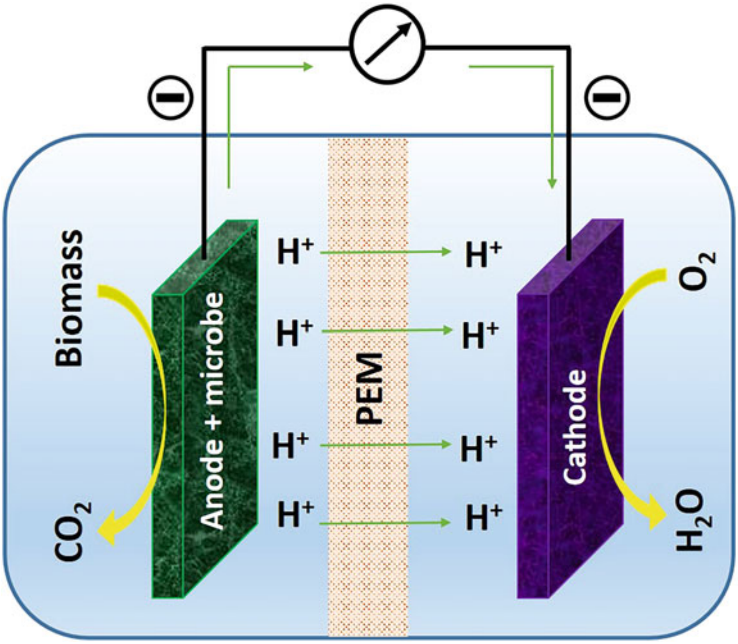


Fig. 5.2 A general schematic of a microbe fuel cell

obtained after the catabolism performed by the microbes are combined with oxygen at the cathode (aerobic) to produce water (Sonawane et al. 2020). Figure 5.2 describes the principle of a microbial fuel cell.

These microorganisms have a valuable impact on treating wastewater which enables the simultaneous production of electricity and cleaning wastewater (Pandit et al. 2017). The use of microorganisms and/or enzymatic catalysis for this end presents groundbreaking advances as it generates green energy in a cost-effective way along with environment managements as the substrates originate from either low-cost sources or even the wastes allowing them to use as consumable for energy production. Also, this process can be performed at room temperature and under ambient pressure making it a very viable industrial process (Du et al. 2007). One example to describe this process is the oxidation of acetate through the catabolism of microbes. The reactions below show that the process is spontaneous due to the negative value of the Gibbs free energy. Also, the generated voltage is the driving force for the usefulness of this process for energy applications.

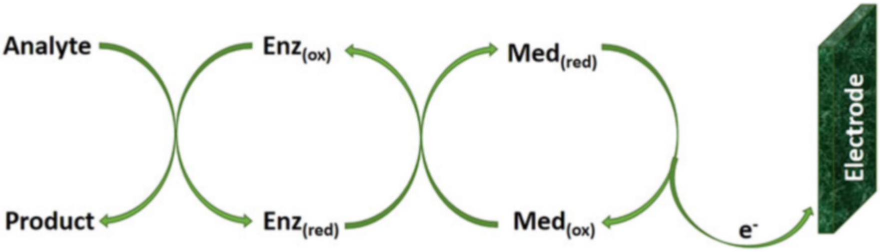


Fig. 5.3 Schematics of a redox mediator MFC system. (Adapted with permission, Chaubey and Malhotra (2002). Copyright (2002) Elsevier)

$$\text{Anode half-cell reaction}: CH_3COO^- + H_2O \overset{\text{Microbes}}{\rightarrow} 2CO_2 + 7H^+ + 8e^-$$
$$\text{Cathode half-cell reaction}: 2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O$$
$$\Delta G = -847.60\ \text{kJ/mol}$$
$$\text{EMF} = 1.10\ \text{V}$$

The MFCs receive two classifications based on the mechanism used to transfer electrons from the microorganism to the anode (Chaubey and Malhotra 2002). The first type is named Mediator MFC which uses a synthetic compound to aid the electron transfer process when it cannot be efficiently performed by the bacteria itself. The mediator forms an extra redox pair during the electrochemical process where it gets first reduced when accepts an electron generated by the bacteria. After that, it delivers an electron at the surface of the anode to an electron acceptor, oxidizing back to its original state enabling the recycling of the process. In the same way, the electron acceptor is also regenerated through oxidation by exposure to the oxygen present in the cathode. This type of MFC can be employed in situations where the bacteria present an insulator structure at its cell wall. Organometallic compounds can be employed as mediators because of their ability to extract the electron out of the bacteria and transfer it to the anode's surface. Some examples of mediators are ferrocene and its derivatives, tetracyanoquinodimethane (TCQN), 2,6-dichlorophenolindophenol, phenothiazines, phenazine ethosulfate, resorufin, benzylviologen, gallocyanine (Pandit et al. 2017; Lima Filho et al. 1996; Hendry et al. 1993). Some of the advantages of mediators are their good conductivity and low oxidation potential that causes less interference with undesirable species in the system (Chaubey and Malhotra 2002). The basic schematic of a Mediator MFC system is described in Fig. 5.3. In a microbial fuel cell glucose oxidase (GO_x) can be used as an enzyme to break glucose into gluconic acid (Fishilevich et al. 2009). The electrode for MFC was prepared by depositing GO*x* over the surface of the yeast (*Saccharomyces cerevisiae*). The prepared electrode was placed in an anaerobic anodic chamber in the presence of methylene blue (MB) as a mediator. In the cathodic compartment, laccase, an enzyme capable of reducing oxygen originated from the microbe *Trametes versicolor,* was placed with 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) as another mediator to improve the reaction rate

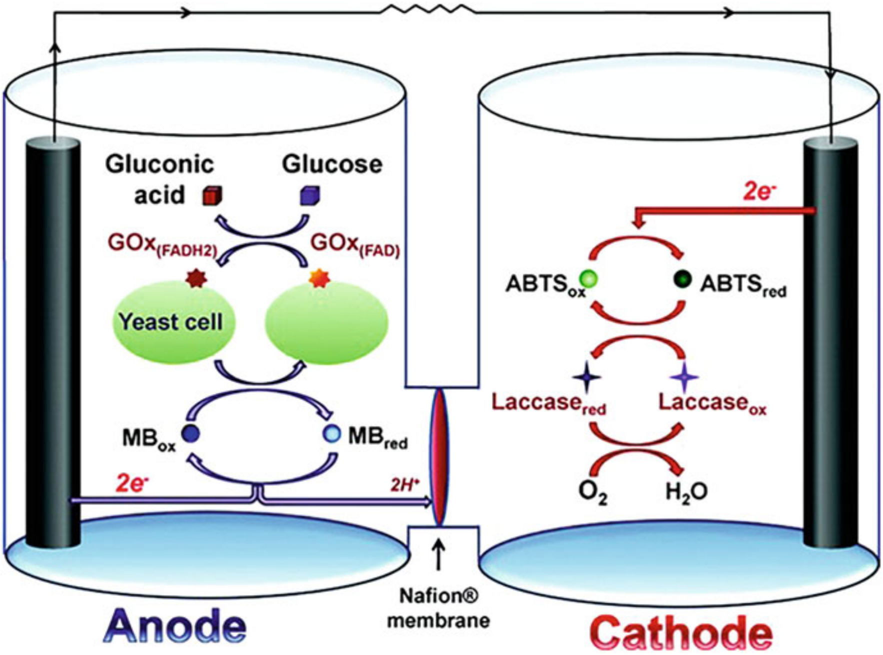
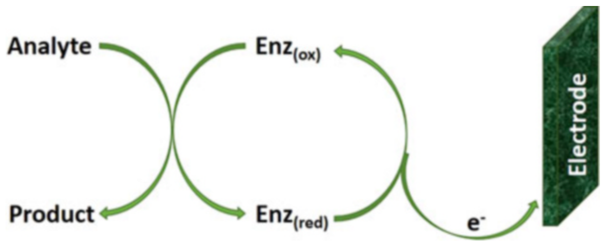


Fig. 5.4 Mediator MFC powered by glucose in the anode with yeast as a microbe, glucose oxidase as an enzyme to break glucose, and methylene blue as a mediator. At the cathode, the enzyme laccase was used with ABTS mediator to receive the electrons from the electrode and perform the formation of water. (Adapted with permission, Fishilevich et al. (2009). Copyright (2009) American Chemical Society)

Fig. 5.5 Schematics of an un-mediator (mediator-less) MFC system. (Adapted with permission, Chaubey and Malhotra (2002). Copyright (2002) Elsevier)



of the process. Such a microbe fuel cell was able to generate electric energy efficiently while using glucose as a substrate. The process is shown in Fig. 5.4.

The other type of MFC is an un-mediator (mediator-less) microbial fuel cell, where the microbe itself can transfer the electron to the anode surface either directly or through the self-synthesized precursor as shown in Fig. 5.5 (Chaubey and Malhotra 2002). To enhance the efficiency of an MFC, functionalization of the anode's surface to allow proper adhesion of the microbes and increasing the surface area to improve the electron transfer process is frequently used (He et al. 2012; Park et al. 2008). For example, a report described a functionalization process of a

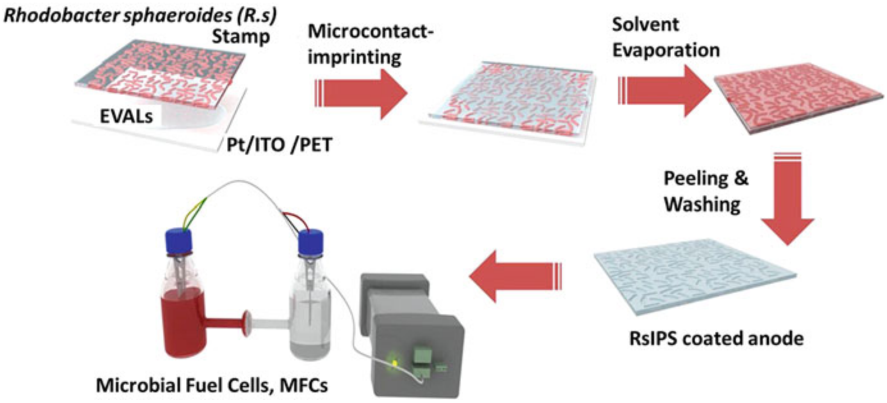


Fig. 5.6 Procedure for the fabrication of bacteria-imprinted polymer for enhancement of electron-transfer to the anode's surface for the development of a mediator-less MFC. (Adapted with permission, Lee et al. (2015). Copyright (2015) American Chemical Society)

cell-imprinted polymer (CIP) that increased the contact between the microbes and the anode, therefore, improving the electron transfer step to the surface of the anode (Lee et al. 2015). The process was performed on indium tin oxide with poly(ethylene terephthalate) (ITO/PET) based electrode. Platinum (Pt) was sputtered over the surface of the glass to form a thin conductive and stable film. After that, a solution containing a bacteria named *Rhodobacter sphaeroide* was added over the surface of the Pt/ITO/PET glass. After drying off the solution containing the bacteria from the glass a poly(ethylene-*co*-vinyl alcohol) (EVAL) solution was cast over it. The procedure is described in Fig. 5.6. The data acquired for the voltage of the system over time showed the highest open-circuit voltage of 0.62 V when a 5 wt% concentration of the *Rhodobacter sphaeroide* was used, however, an electrode without the bacteria provided a voltage of about 0.22 V. The thickness of the biofilm imprinted on the electrode played a major role in the performance. It was observed that an increase in the concentration of bacteria led to a decrease in generated voltage due to the diminishing of the electron transfer process at the electrode. The optimal thickness of the biofilm was around 1508 nm. Despite the decrease in performance due to excessive thickness of biofilm, the electrodes with the bacteria imprinted on its surface always showed better performance than bare electrodes (Lee et al. 2015).

Industries, cities, and farmlands produce a significant amount of biowaste and utilize a major portion of energy for their operations. Microbial fuel cells produce energy by consuming biowastes hence, providing a greener way to produce energy simultaneously handling waste management (Kiran Kumar et al. 2012; Chandrasekhar et al. 2015). Besides, microbes can be used for other applications such as biosensors, biochemical oxygen demand. As an example, an MFC device can be placed in contact with water contaminated with heavy metals such as mercury, chromium, cadmium, lead, or even organophosphorus-based compounds and can detect the contamination by providing different electrical response based on the concentration of the contamination. This method enables quick detection of the harmful component even allowing a quantitative analysis (Mook et al. 2013). The same principle works to detect spoil food. Microbes are also used in biomedical applications as an implant in the human body that generates low and stable power by harvesting energy through the consumption of glucose in the bloodstream (Chandrasekhar et al. 2015; Babauta et al. 2012). Studies have shown that the efficiency of such a process varies from 80 to 95% (Buckley 2006).

Slow electron transfer is one of the issues which need to be addressed in MFCs to improve their efficiency. Slow electron transfer reduces the oxygen reduction reaction (ORR) and hence enhances the overpotential (requirement of energy) of the process. To improve the electron transfer rate, other chemicals such as phenazine can be used. These compounds can be either naturally provided by the bacteria or introduced as a synthetic mediator. Their role is to shuttle the generated electrons to the surface of the anode to improve the performance of the fuel cell. However, some new approaches are still under study which can further improve the performance of MFCs. One of the approaches is the use of nanotechnology, such as nanowires and nanotubes that can mimic the microbial pili, which is an organelle of the bacteria responsible for the transfer of the electrons (Buckley 2006). Another approach to counter the slow step of oxygen reduction reaction at the cathode is improving catalytic properties of the cathode materials such as doping with nitrogen (Yang et al. 2019). The nitrogen-doped carbon aerogel improves the catalytic activity by creating more active sites into the structure of the electrode that improves the oxygen reduction process. Also, doping improves the conductivity and thus facilitate electron transport. The net effect is the reduction in the overpotential of the process which makes the process cost-effective. Such composite can be synthesized by a facile pyrolysis process of polyacrylonitrile. The nitrogen-doped carbon improved the catalytic performance of the MFC reaching 1.048 W/m^2, which is comparable to Pt/C based MFC (1.05 W/m^2). Results show that doping could enhance the catalytic activity of low-cost materials thus making applicable as a catalyst for MFC applications (Fig. 5.7).

Another practical example demonstrated the multifunctionality of an MFC by producing green energy as well as recovering dissolved copper in water waste (Ter Heijne et al. 2010). The MFC consisted of a system where bacteria were fed with acetate at the anode and the cathodic chamber was containing copper solution. Both chambers were connected through a bipolar membrane (Fig. 5.8). The MFC could function with or without oxygen and deliver a power density of 0.43 W/m^2 with a current density of 1.7 A/m^2 in an anaerobic environment, while in the aerobic system, it provided a power of 0.80 W/m^2 and a current density of 3.2 A/m^2. Along with that, the MFC was capable of recovering nearly 99.9% of the copper ions from the solution and converting them into copper metal, providing an eco-friendly approach to recycle copper from metallurgic industries. In summary, MFCs present great efficiency for energy production and can be operated at room temperature and ambient pressure. It can be also installed in far distances due to the easy availability of the raw materials where other conventional sources of energy may not be able to reach or may lead to a waste of energy due to the long distance

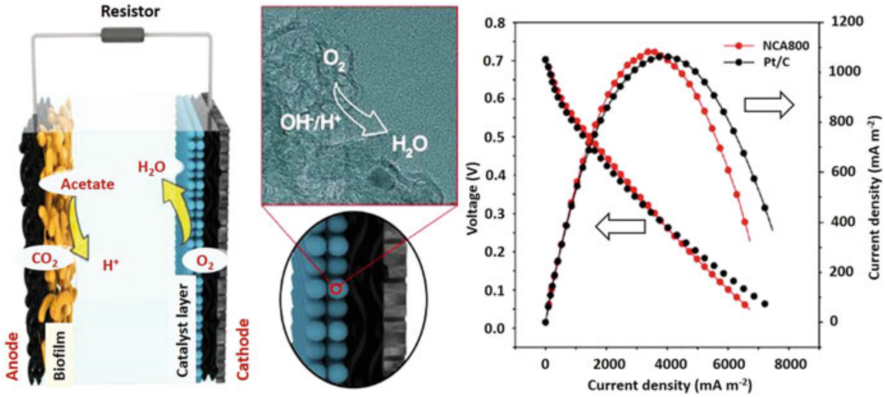


Fig. 5.7 Schematics of an MFC composite with carbon aerogels doped with nitrogen (NCA) for improvement of ORR performance. (Adapted with permission, Yang et al. (2019). Copyright (2019) American Chemical Society)

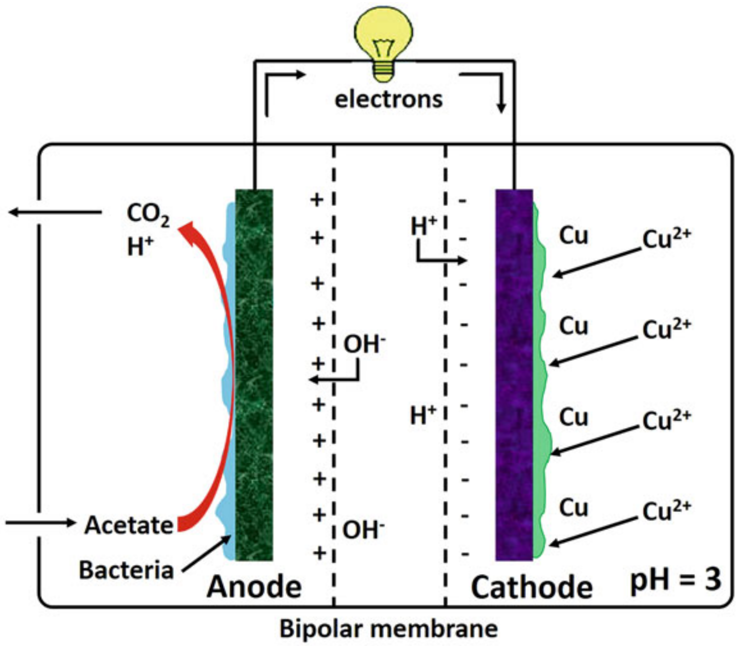


Fig. 5.8 Schematic for an MFC with a bipolar membrane for electric energy generation and reduction of copper. (Adapted with permission, Ter Heijne et al. (2010). Copyright (2010) American Chemical Society)

from the power plant. Therefore, the potential application of microbes for powerhouse is foreseeable soon that provides very interesting and unique possibilities while adding a sustainable aspect (Buckley 2006).

5.2.2 Microbes for Hydrogen Production

The extended use of fossil fuels pushed international authorities to come up with alternative strategies to diminish their use due to economic instability and environmental concerns such as the enhancement of the greenhouse effect. The Paris Agreement, in 2015, determined that the average temperature of the planet's surface should not increase more than to 2 °C as it was predicted to happen if the emission rate of greenhouse gases continued to increase. In practice, this demands a reduction of around 50% of fossil fuels such as oil, gas, and coal that are currently being used (Kadier et al. 2016). Hence, the development of alternative sources of energy is a challenging necessity. Hydrogen gas is a promising fuel due to its high-energy release (120 kJ/g) that is almost three times higher than energy from petroleum (43.4 kJ/g) and much higher than from coal (29 kJ/g) and ethanol (26.7 kJ/g) (Bartels et al. 2010; Njenga et al. 2014). Also, its combustion generates only water which makes it a clean source of energy. Hydrogen does not have a source like natural gases because of its low density which makes it ascend to outside the atmosphere. Therefore, hydrogen has to be produced and stored smartly. Hydrogen can be obtained during the extraction of natural gas or coal and by thermolysis of biomass. However, these processes are not sustainable to produce hydrogen as they introduce more carbon to the atmosphere and demand high input (Bartels et al. 2010; Njenga et al. 2014; Kadier et al. 2014). Therefore, sustainable and efficient methods are needed for the generation of hydrogen gas for energy applications.

Fortunately, some bacteria such as exoelectrogens can break down organic compounds into protons and electrons. The generated electrons can be absorbed by the anode and flow through an external circuit and the protons pass through a proton exchange membrane to the cathode to produce hydrogen through a process called hydrogen evolution reaction (HER) (Kumar et al. 2017). The scheme of a general microbial fuel cell and a microbial electrolysis cell (MEC) showing their differences are given in Fig. 5.9 (Escapa et al. 2016). At the anode, most of the organic compounds can be oxidized in a potential range of −0.5 to −0.2 V. The most common substrates are acetate (−0.29 V), wastewater and ethanol (−0.33 V), lactate (−0.34 V), pyruvate (−0.37 V), and glucose (−0.43 V) (Kadier et al. 2020). However, under standard thermodynamic conditions, the potential required at the cathode to perform the HER is around −0.41 V (Rozendal et al. 2006). As a consequence, theoretically, only glucose could be oxidized spontaneously. But, even under these conditions, it would not be fully converted into carbon dioxide as the process is taking place in an anaerobic condition. Thus, glucose would be converted into glutamic acid or other derivatives (Wünschiers and Lindblad 2002). At the cathode, the proper control of some parameters can influence the production of hydrogen. The increase of temperature, partial pressure, and pH makes cathode

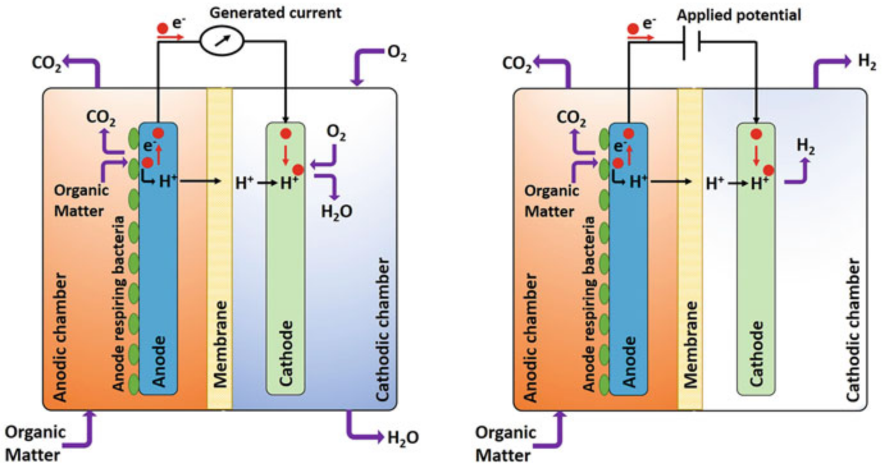


Fig. 5.9 General scheme of a microbial fuel cell for generation of electricity (left) and a microbial electrolysis cell for generation of hydrogen gas (right). (Adapted with permission, Escapa et al. (2016). Copyright (2016) Elsevier)

potential more negative which means an increase of energy input will be required for hydrogen production. Low pH, low partial H_2 pressure, and low temperature are the thermodynamic requirements for the optimum condition which will require less energy input for hydrogen production (Rozendal et al. 2006). Among these three parameters, pH has the most influence on the potential. However, in practical terms, there are some limitations to these requirements. First, the decrease of pH is the most reasonable approach to increase the cell potential toward a less negative value, however, if the pH is too low it can become an aggressive environment for microbes. Second, maintaining a low partial H_2 pressure requires special techniques that will add cost to the process (Kadier et al. 2020).

Microbial electrolysis requires a low energy input of 0.11 V when acetate is used as a substrate which is 10 times lower than hydrogen production via water electrolysis which requires over 1.23 V (Kadier et al. 2016; Liu et al. 2005). Also, compared with conventional fermentation processes both yield and purity of H_2 produced in microbial electrolysis are higher. These differences occur due to the incomplete oxidation reaction of the substrate during the fermentation process which leads to a mixture of by-product gases and hydrogen. Additionally, an increase of partial pressure has a huge influence on the conventional fermentation process than in MEC, which also diminishes the yield. In MECs, hydrogen gas is generated at the cathode, which is a separated compartment from the anode where the digestion of the substrate takes place. Hence, leading to higher purity of H_2 along with less influence of H_2 partial pressure (Kadier et al. 2016). The current challenge for large-scale generation of hydrogen is the production rate which drops from 50 to 3 m^3 H_2/m^3/day moving from a laboratory to an industrial scale (Escapa et al. 2016; Call and Logan 2008; Li et al. 2018).

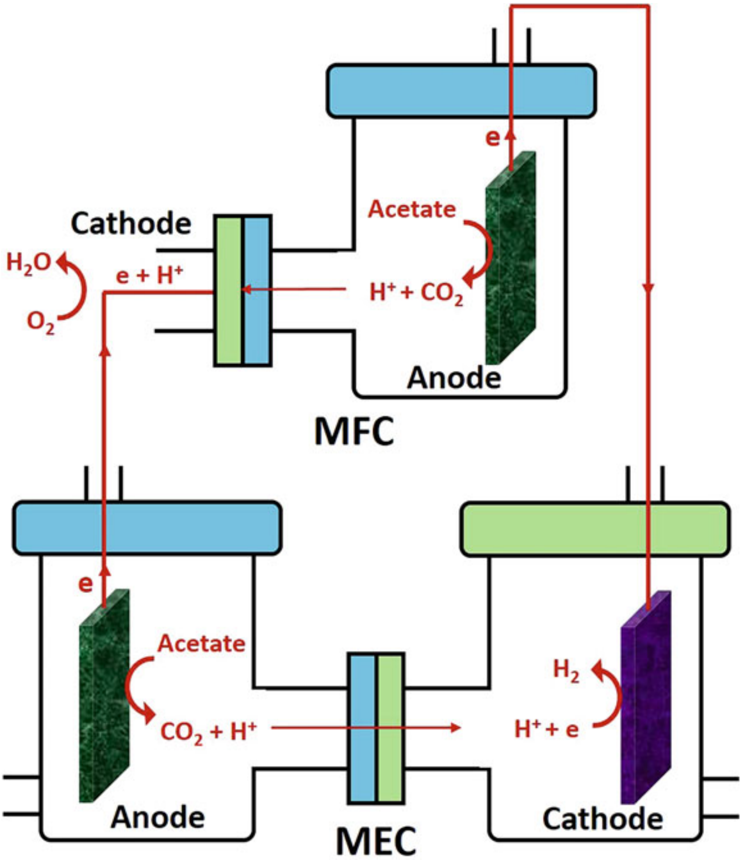


Fig. 5.10 Microbial fuel cell acetate-fed is used to generate electricity to activate a microbial electrolysis cell (MEC) to produce hydrogen. (Adapted with permission, Sun et al. (2008). Copyright (2008) American Chemical Society)

One sustainable approach to design a closed cycle of electricity and hydrogen production consists of the use of an MFC that consumes organic wastes to generate electricity and the generated energy can power a microbial electrolysis cell to produce H_2. Figure 5.10 shows a schematic of such concept where acetate is used as a substrate to produce hydrogen (Sun et al. 2008). Both anodes for the MFC and microbial electrolysis cell were in the compartments in where acetate was oxidized to H^+. The electricity generated by the MFC's anode served as an energy supply to overcome the thermodynamic barrier for the microbial electrolysis cell's cathode to reduce H^+ to H_2 (anaerobic process). Likewise, the microbial electrolysis cell's anode through the oxidation of acetate provided energy for the MFC's cathode to reduce H^+ into H_2O (aerobic process) (Sun et al. 2008). The main advantage of this coupling system is the production of H_2 without the expense of external energy input. The in situ electricity generated by the MFC is more efficient as there is no need to store the energy externally which causes power loss. The coupled cell has the potential to generate hydrogen efficiently and economically from organic wastes, however, further developments are required to optimize the hydrogen production as some parameters can greatly influence the efficiency of the cell.

Photovoltaic cells are another way to generate green energy by utilizing solar light. Photovoltaic cells (solar cells) can be also coupled with microbial electrolysis cells to provide the power for their operation. The coupling of a solar cell with a microbial electrolysis cell can generate several fuels besides hydrogen such as methane and ethanol, which offers a reasonable approach to generate green energy and biofuels (Zhang and Angelidaki 2011). A previous report demonstrated the application of a dye sensitive solar cell (DSSC) to provide energy to a microbial electrolysis cell that did not require Pt as a catalyst at the cathode (Chae et al. 2009). Microbial electrolysis cells powered by the solar cell provided a hydrogen conversion efficiency of 77%. The solar cell was designed by using an FTO glass that was platinized as an electrode and I^-/I_3^- as an electrolyte that functioned as a redox pair for electron transfer (a mediator) to the anode's surface, as described by the schematic in Fig. 5.11.

As discussed in this session, the production of hydrogen through microbial electrolysis cell is a promising way to harvest energy due to its versatility that enables it to be coupled with other systems. Some key factors are important to scale up the production of hydrogen via microbes. For example, the anode needs to be biocompatible with the microbes to allow an efficient electron transfer as well as a biofilm formation under a non-aggressive environment, accompanied by an exoelectrogen metabolism of the bacteria/fungus. These factors aid to reduce the ohmic resistance of the media. Also, the cathode may require a proper catalyst to improve efficiency and decrease ohmic resistance in the solution. Besides, the electrolyte must present high ionic strength, proper mass transfer to permeate through the proton exchange membrane along with the capability to form a buffer to create a less-aggressive environment for the microbe. Furthermore, the membrane has to provide minimum energy loss during mass transfer and prevent the permeation of hydrogen as well as keep the pH gradient (Kadier et al. 2016, 2020).

5.2.3 Microbes for Methane Production

Methane is the main component of natural gas and it is a useful source of energy for many applications such as fuel for rockets and steam turbines to generate electricity during its combustion. Methane presents the lowest emission of carbon dioxide among the other fuel gases along with the highest production of heat per mass compared with other hydrocarbons, reaching around 55 kJ/g (Schmidt-Rohr 2015). The extraction of natural gas is costly and needs an effective transport system to power urban regions such as gas pipelines that could have leakage creating safety issues and environmental concerns. A promising way to improve this scenario is to use methanogen microbes that can effectively generate methane by breaking down organic materials (Ding et al. 2017). This is a smart approach as microbes can use a

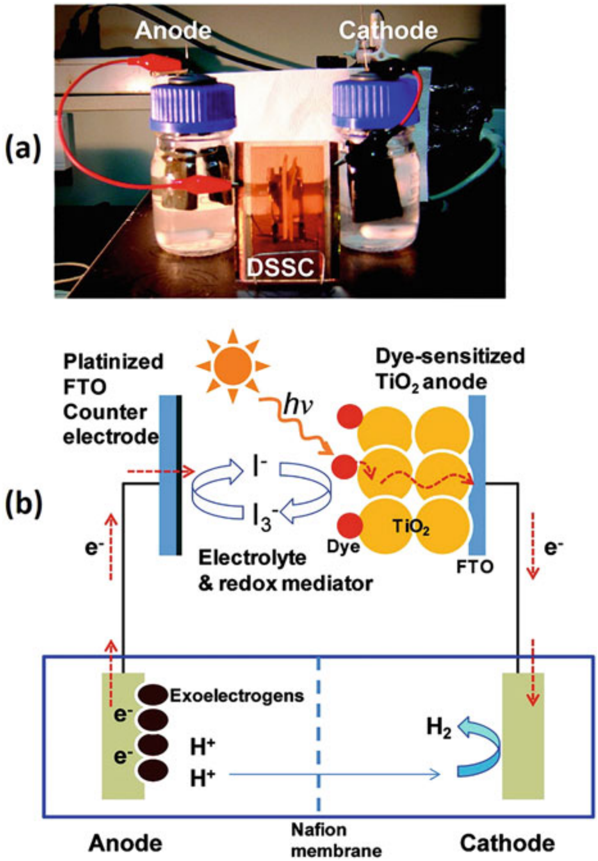


Fig. 5.11 (**a**) Dye-sensitized solar cell coupled with a MEC to generate hydrogen, (**b**) schematics for the system of a coupled DSSC-MEC. (Adapted with permission, Chae et al. (2009). Copyright (2009) American Chemical Society)

variety of organic wastes for methane production which provides efficient waste management and economic benefits.

Methane producing microbes are called methanogenic microbes and go through a complex mechanism to convert biomass into methane. The scientific community and researchers need to understand the conversion mechanism to design an improved process. Among the possible mechanisms for the methane formation, two of them provide detail of the process as shown in Fig. 5.12 and discussed as follows. First, the enzyme known as Methyl coenzyme M reductase (CoM-S-Methyl) presents an active site with a Ni atom at the center, where a redox pair is formed to bio-catalyze the reaction. The Ni atom is present as a bio-complex in the form of nickel tetrapyrrole cofactor, which is part of the CoM-S-Methyl. The mechanism can go into two routes: (1) formation of an organometallic intermediate, methyl-Ni^{3+} (*Mechanism 1*) and (2) generation of a methyl radical ($CH_3^{\bullet}$) due to the linkage between a sulfur atom with Ni^{+}, which forms a Ni^{2+}-S-CoM specie while the radical methyl is released as a leaving group (*Mechanism 2*) which is the most accepted mechanism among the scientific community (Borman 2016; Wongnate et al. 2016; Li et al.

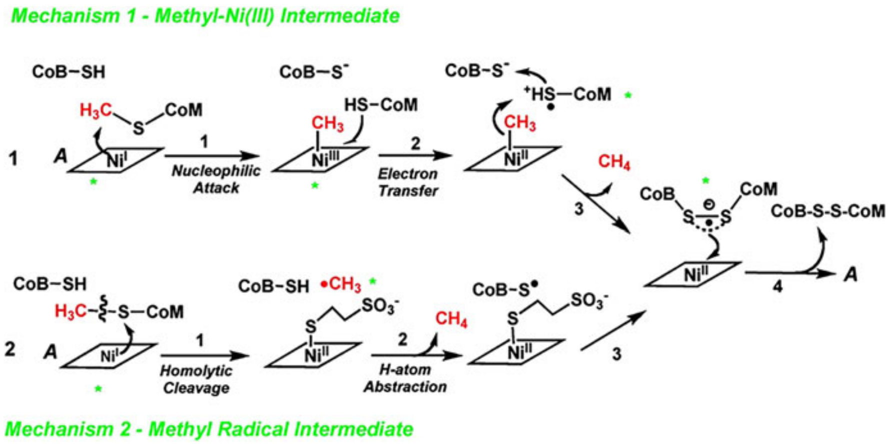


Fig. 5.12 Possible mechanisms for the methanogenesis through the feedback enzymatic reaction between Methyl coenzyme M reductase (CoM) with the coenzyme B (CoB) while mechanism 2 is more prompt to occur. The asterisk indicates the species that can be identified by electron paramagnetic resonance (EPR). (Adapted with permission, Dey et al. (2010). Copyright (2010) American Chemical Society)

2010). As a follow-up, the methyl radical, a highly unstable specie, can abstract an H atom from coenzyme B (H-S-CoB), hence releasing methane. Finally, to reestablish the cycle the coenzyme B (H-S-CoB) that was converted to $^{\bullet}$S-CoB forms a disulfide linkage with $^{\bullet}$S-CoB.

Besides the production of methane, the methanogenic microbes are also capable of generating electricity. A previous study demonstrated that this process can be influenced by the type of substrate used for the process and observed that sewage sludge can improve the voltage generated by the MFC system from 0.576 to 0.6 V along with improvement in the Coulombic efficiency of the conversion process (Xiao et al. 2014). Following this process, a report described an efficient way to harvest electric energy and methane through a system that utilizes the synergy between a microbial fuel cell along with an anaerobic digester (AD) (Vu and Min 2019). Anaerobic digestion is a promising way to convert industrial or domestic wastes with high loads of organic matter into important substances such as nutrients, hydrogen, and methane. Methane is produced by the methanogenic microbes; however, it requires certain conditions to function properly such as the low concentration of volatile fatty acids, stable pH, controlled organic load rates, and proper carbon/nitrogen ratio (C/N) (Vu and Min 2019). The production of methane can be affected by the formation of acidic by-products as they can alter the pH of the system. To avoid this scenario and keep the process continuously producing methane some techniques such as pretreatment of the substrate and integration with other systems are used.

One of the possibilities includes the integration of microbial electrochemical systems (MESs). It is a device that can target a specific substrate under the presence of electroactive microorganisms with the introduction of low voltage to generating a specific product (Lee et al. 2019; Kondaveeti and Min 2015; Amin et al. 2017; Min et al. 2012; Nagendranatha Reddy and Venkata Mohan 2016). These microorganisms act as an extra aid by oxidizing organic matter at the anode, which is converted into methane and carbon dioxide at the cathode using the applied voltage. Also, it diminishes overpotential and resistance that could appear otherwise (Lee et al. 2019; Czajczyńska et al. 2017). The key aspect of contribution of an MES is the consumption of volatile fatty acids, which prevents the drop of pH, consequently improving the production of methane as mentioned by previous studies (Vu and Min 2019; Liu et al. 2016; Villano et al. 2016; Xafenias and Mapelli 2014; Yin et al. 2016). Since a high concentration of H^+ is toxic to microbes, the integration of an MES within an AD is a convenient approach to guarantee a stable microenvironment as the MES can properly control the media to reach the optimal condition. For this to take place proper bacteria should be selected, usually in the form of a biofilm, to target volatile fatty acids, which helps to adjust the system's pH to prevent the methanogenic microbes from degrading. The rate of addition of the substrate can also influence the methane generation as the increase of substrate load increases the volatile fatty acid content (Lee et al. 2019; Sun et al. 2017; Zhao et al. 2014). Nevertheless, many other parameters influence the overall production such as substrate, applied potential, buffer, temperature, etc. (Xiao et al. 2014; Moreno et al. 2018; Ren et al. 2007; Ding et al. 2016; Parkin and Owen 1986; Liu et al. 2013a; Choi et al. 2017). Thus, an MES-AD system presents the advantage of higher yields of methane production with the cost of external input of voltage. On the other hand, microbial fuel cells integrated with anaerobic digester (MFC-AD) do not require energy input, which brings economical value for its use. To target this scenario, a submersible microbial fuel cell (SMFC) into an AD was configured to obtain electricity without energy input requirements using various concentrations of glucose as a substrate to define the optimum amount for methane production (Vu and Min 2019). The scheme of the system is shown in Fig. 5.13. First, to make the system stable, a substrate of low electrical potential was added, in this case, acetate was added with wastewater. After the stabilization of the system, the actual substrate such as an anaerobic sludge with different concentrations of glucose can be added. The system of an MFC-AD works in a feedback process where the anaerobic digester consumes the glucose sludge producing mainly methane but also H_2 and CO_2 along with other by-products such as acetate, butyrate, and propionate acids, which are the main components of the volatile fatty acids (Ren et al. 2007). The microbial fuel cell then plays a role of consuming these volatile fatty acids to properly control the pH with the simultaneous release of energy. In this manner, the synergetic system of an MFC-AD provides a stable production of methane and electricity even at higher concentrations of substrate, which could lead to an inhibition of microbial activity due to the fast conversion of products including the volatile fatty acids that become toxic to microbes. However, in short periods the MFC-AD system can recover from the adverse situation and continue to produce until it reaches the optimal condition again. Therefore, the MFC-AD system is a valuable tool for the production of methane and energy while it treats residues in a self-contained microenvironment (Maspolim et al. 2015).

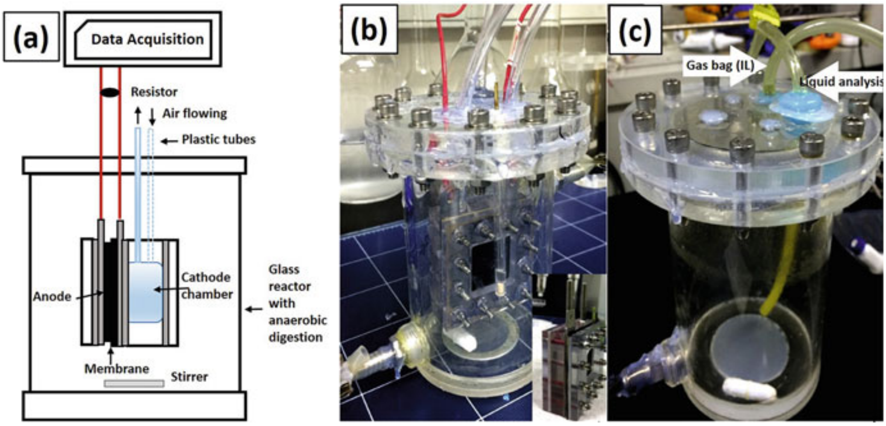


Fig. 5.13 Schematics of (**a**) coupling between the submersible microbial fuel cell (SMFC) with an anaerobic digestion (AD) reactor, (**b**) image of an SMFC, and (**c**) Reactor to carry on the anaerobic digestion reaction. (Adapted with permission, Vu and Min (2019). Copyright (2019) Elsevier)

Methane can be also produced during the digestion of cellulose by ruminants, decomposition of organic waste through bacterial or fungal action, and eutrophication process that can occur in swamps. However, this process can create an issue of the greenhouse effect as methane can reflect up to 25–38 times more infra-red radiation back to the planet's surface when it ascends to the atmosphere compared with carbon dioxide. Therefore, the emission of methane to the atmosphere is highly undesirable. However, there are ways to extract energy from this process and turn it into a sustainable way to harvest electric energy. An electrical system that can consume methane and convert it into electricity can be designed which usually has three steps (Soo et al. 2016). In the first step, bacteria named *Methanosarcina acetivorans* can consume methane (methanotroph) due to the presence of an enzyme, methyl-coenzyme M reductase (Mcr) that could yield electrons and acetate as products. This step of the process can alone produce electricity that can be harvested by the ferricyanide/ferrocyanide redox pair at the cathode (Soo et al. 2016). However, to enhance the energy production two extra microbes were added into the system. For this, an intermediate type of bacteria colony (*Paracocccus denitrificans*) was used to produce humic acids that acted as a natural electron shuttle to improve electron transfer (Scheller et al. 2016). Finally, a bacteria, *Geobacter sulfurreducens*, capable of digesting the acetate to produce electrons was used to close the electrical system to build a fuel cell (McAnulty et al. 2017). It has proven to be an efficient method to harvest electricity from microbes reaching around 90%, which was comparable to previous reports that obtained around 90 and 85% (Scheller et al. 2016; Zhang et al. 2015). The process has been described in Fig. 5.14 (McAnulty et al. 2017). Even though methane is a greenhouse gas, it holds great potential as an energy source due to its high calorific energy and abundance. The biosynthesis of this fuel through microbial route is a greener way to produce methane which can be

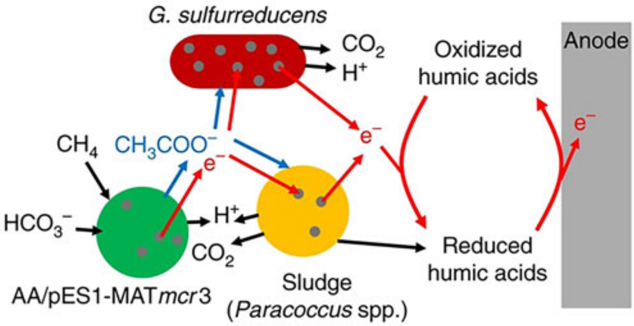


Fig. 5.14 Schematics of the microbial fuel cell. The *Methanosarcina Acetivorans* (AA/pES1-MATmcr3) feds on the methane to convert it into acetate and electrons through the catabolic process of the enzyme methyl-coenzyme M reductase (Mcr). The recently generated acetate is further oxidized into carbon dioxide by *Geobacter sulfurreducens* to generate more electrons. Then the Paracoccus denitrificans produces natural electron shuttles, which are humic acids capable of transferring the electrons to the anode to close the system (McAnulty et al. 2017)

used as a fuel along with the eco-friendly conversion of methane into electrical energy.

5.2.4 Microbes for Ethanol Production

Non-renewable resources are still a popular method of current energy sources; however, with the increased demand for energy and sustainability issues, new approaches need to develop which can use renewable resources to produce energy (Balat and Balat 2009; Bozell and Petersen 2010; Sarkar et al. 2012; Sarris and Papanikolaou 2016). Ethanol can be produced using bio-renewal resources in an eco-friendly way. Ethanol comes as an alternative automobile fuel despite its lower efficiency compared to gasoline due to its lower price (Sarris and Papanikolaou 2016). Ethanol is the most produced biofuel used in transportation. The largest producers of ethanol are the USA that uses corn starch and Brazil that uses saccharose from sugarcane for the production of ethanol (Wang et al. 2012). Most ethanol production occurs through the fermentation process that breaks down bio-based materials such as starch, sugar, and glycerol into ethanol, carbon dioxide, and water. This production led to two main research lines. One focused on finding new strains of microorganisms that can produce ethanol using other substrates such as pentoses, xyloses, or other types of sugars. This line is directed to bioengineering viable microbe candidates, the most known ones being *Saccharomyces cerevisiae* and *Zymomonas mobilis*. It can then allow these microbes to produce more ethanol and catabolize other types of sugars like arabinose or xylose, which are found in biowastes (Lin and Tanaka 2006; Zhang et al. 1995; Hahn-Hägerdal et al. 2006). The other focus is on the optimization of ethanol production by finding novel fermentation processes along with utilizing secondary products that could be converted into

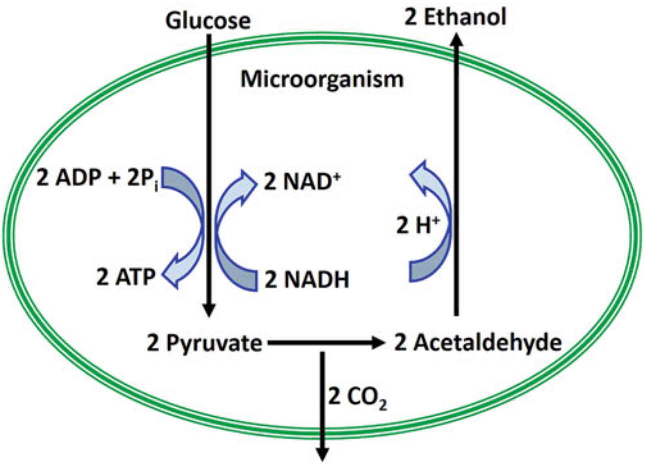


Fig. 5.15 The anaerobic fermentation process for the synthesis of ethanol (Gunawardena et al. 2008)

ethanol (Hahn-Hägerdal et al. 2006; Philbrook et al. 2013; Sanchez and Demain 2008). By improving the overall production and efficiency of ethanol a reduction of emission of greenhouse gases is expected due to the cleaner combustion reaction (McMillan 1997; Cardona Alzate and Sánchez Toro 2006; Marchetti et al. 2007). Also, the implementation of production in no aseptic environments aids to reduce cost and therefore making it a viable option for green fuel (McMillan 1997).

The production of bioethanol starts with some organic substrates such as glucose, disaccharides, xylose, and glycerol that are convertible to ethanol through the use of microbes. For each case, a specific type of microbe can be used. For example, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are the most commonly used microbes for the conversion of glucose into ethanol (Lin and Tanaka 2006; Hahn-Hägerdal et al. 2006; Petre 2013). For the conversion of xylose into ethanol, *Pachysolen tannophilus*, *Pichia stipis,* and *Candida shehatae* are found to be very effective (Hahn-HäGerdal et al. 1991). Other routes of conversion of saccharose into ethanol are evolving and require further investigation to become more applicable (Nwachukwu et al. 2012; Choi et al. 2011; Ito et al. 2005). The biochemical route for the conversion of glucose into ethanol is a complex process, which depends on the microbes, conditions (aerobic or anaerobic), and substrates. Many studies have been performed to describe the biochemical mechanism involved in these processes. Besides the metabolic pathways, there are other possible routes (Sarris and Papanikolaou 2016). However, the main aim of this chapter is the production of energy through microbes, therefore, the discussion is focused on how the microbes' metabolism aids in harvesting energy. The key elements for these processes are the biochemical transformations of glucose (1 mol) into ethanal (2 mol) which converts to ethanol (2 mol) through enzymatic catalysis process (Fig. 5.15) (Gunawardena et al. 2008). However, scientists have observed that the process can be halted during

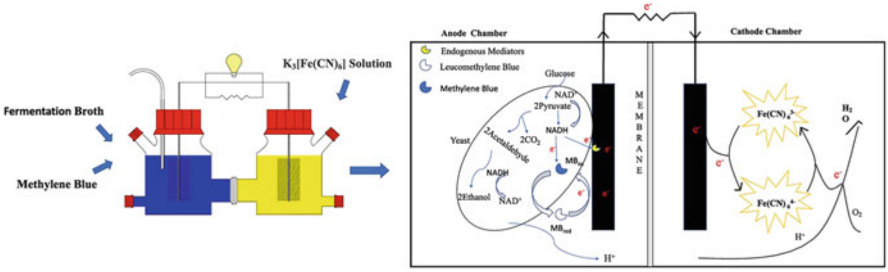


Fig. 5.16 Schematics of a microbial fuel cell to produce ethanol and electricity. (Adapted with permission, Yuan et al. (2020). Copyright (2020) American Chemical Society)

the conversion of ethanal into ethanol due to the excess production of nicotinamide adenine dinucleotide-hydrogen (NADH). It occurs due to the dependence of alcohol dehydrogenase (the enzyme responsible for converting ethanal into ethanol) toward NADH (Gunawardena et al. 2008; Panagiotou and Christakopoulos 2004; Walker and Stewart 2016; Zaunmüller et al. 2006).

To counter this issue, electrons must be removed from the system to adjust the redox pair of NADH/NAD^+ to reestablish the microbe's metabolic activities. This is the part where MFC can help to absorb these electrons to generate electricity (Yuan et al. 2020). An MFC can also contribute to generating energy by harvesting the heat released through biomass and converts it into electricity (Yuan et al. 2020). Such an approach can provide both bioethanol and electricity from renewable resources (Christwardana and Kwon 2017; Christwardana et al. 2019). This is achieved by constructing a two-chambered MFC where the chambers are connected through a proton exchange membrane (Yuan et al. 2020). Carbon fiber was used as an electrode and electrodes were connected through an external circuit. The system in the anolyte was composed of a mix of sugars, NaH_2PO_3 as a buffer, methylene blue (MB) as a chemical mediator, and neutral pH. At the catholyte side, the solution was also prepared using NaH_2PO_3 as a buffer, $K_3[Fe(CN)_6]$ as the electron acceptor, and pH close to 6.5. The yeast, *Saccharomyces cerevisiae* was inoculated into the anolyte. The scheme for the ethanol/electricity production through an MFC is shown in Fig. 5.16 (Yuan et al. 2020). Methylene blue as a mediator plays an important role in improving the energy output which is an important factor for yeast-MFCs since their energy production tends to be relatively lower compared to traditional MFCs (Sarris and Papanikolaou 2016). An MFC without mediator showed an open-circuit voltage of about 655 mV that improved to about 750 mV with the mediator. The measurement of open-circuit voltage is an important parameter as it describes the microbial growth at the beginning of the operation (Christwardana et al. 2018). Also, MB facilitates to track the behavior of the yeast-MFC. Figure 5.17 describes the electrochemical behavior of the anolyte with MB when external resistance is connected to the system (a) and when there is no external resistance (b). The process is governed by the reactions given below:

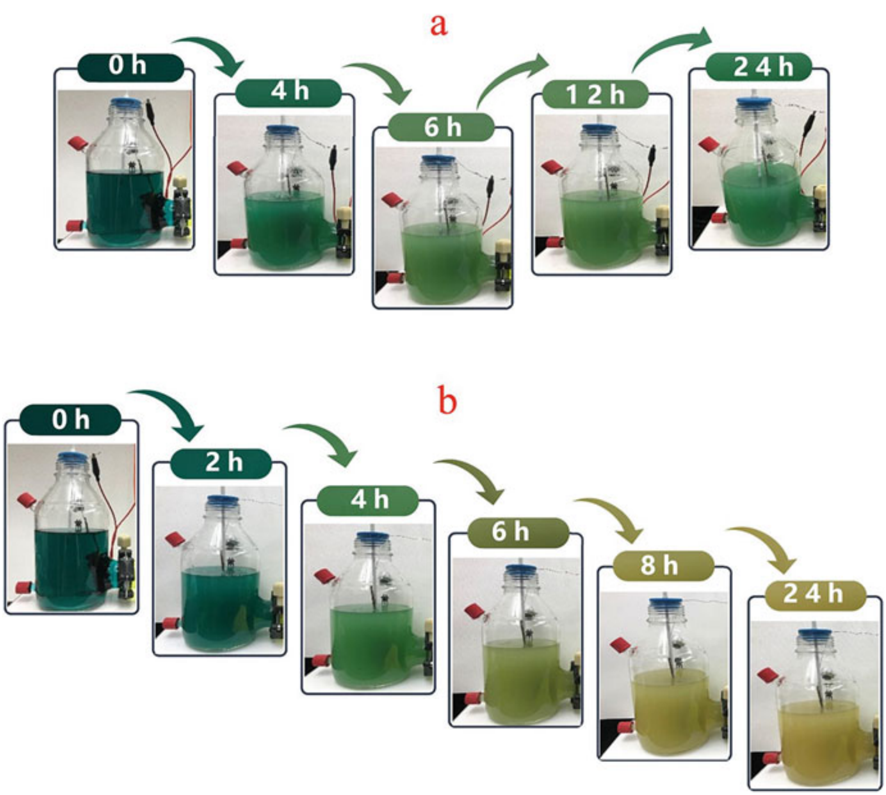


Fig. 5.17 Color effect on the anode of the yeast-MFC using MB as a chemical mediator showing the (**a**) anode without connection to an external circuit and (**b**) with connection to an external circuit. (Adapted with permission, Yuan et al. (2020). Copyright (2020) American Chemical Society)

$$MB + NADH + H^+ \rightarrow MBH_2 + NAD^+$$
$$MBH_2 \xrightarrow{\text{Electrode}} MB + H^+ + 2e^-$$

These reactions take place when the MB permeates through the cell wall of the microbes and get reduced to MBH_2 (leucomethylene blue) while NADH oxidized to NAD^+ (1). Since MBH_2 is colorless, the solution loses its color as seen in Fig. 5.17a. As time passes, the color of the anode becomes lighter, therefore, fading of the color indicates that MB is partially converted to MBH_2. In the absence of an external circuit, the electron transfer is hindered, therefore reaction (2) does not occur. However, in the presence of an external circuit the electron transfer occurs which leads to the formation of MB (Fig. 5.17b).

The empirical effect of MB shows that the implementation of a chemical mediator and a connection with external circuit aid to the production of electrical current. However, to improve the production of ethanol, it is also important to address issues with the chemical redox pairs that take place within the microbe. NAD^+ is the specie responsible for reducing catabolism of substrates such as glucose to generate NADH (Vemuri et al. 2007). However, it has been observed that an excess of NADH can be produced during alcoholic fermentation, which ceases the metabolism of the microbe (Walker and Stewart 2016). Hence, to properly control the ratio of $NADH/NAD^+$ some strategies such as the use of different strains of microbes and chemical electron acceptors can be adopted (Liu et al. 2012, 2013b). As described above, MB can react with NADH to increase the concentration of NAD^+, which is responsible for breaking down the carbon source into ethanol. However, it was observed that higher concentrations of MB inhibit the production of electricity as well as ethanol. Therefore, an optimal quantity of chemical mediators should be added to achieve the highest yield.

New approaches for the production of ethanol are being used to associate different types of processes where the catabolic products can serve as a substrate for the other, hence, creating a symbiotic effect between them. A previous report demonstrated an interesting approach to explore this effect by using *Clostridium cellulolyticum*, which a microbe able to break cellulose into acetate, hydrogen, and ethanol (Desvaux et al. 2000). This scenario sets the environment for the bacteria *Geobacter sulfurreducens*, which can use these products as substrates to convert them into acetate, hydrogen, carbon dioxide, ethanol, and electrons that can be absorbed by the electrode (Bond and Lovley 2003; Caccavo et al. 1994). This is an important approach as it manages to consume biomass in the form of cellulose, which is an abundant source while producing fuels and electricity without the use of exogenous catalyst (Ren et al. 2007). The results for this microbial fuel cell regarding power density were around 143 mV/m^2 and open-circuit voltage around 430 mV. The MFCs which can produce electric energy while consuming biowaste are a viable tool for energy harvesting. Nevertheless, further, improvement can be performed by increasing the capability of the buffer, along with the use of a solid substrate that may impart different kinetics into the MFCs. Thus, these technologies show an applicable way to acquire energy; however, it still demands clearance of some issues such as improving overall efficiency (Ren et al. 2007).

5.2.5 Microbes for Biodiesel Production

Biodiesel is a bio-based fuel that is produced through transesterification reaction yielding fatty acid methyl esters (FAME). It can be performed using many types of fats both from vegetal or animal-derived and waste oils. They are placed to react with short-chain alcohol such as methanol, ethanol, or butanol, which can be produced by microbes, like bacteria, fungus, and microalgae (Ratledge and Cohen 2008; Papanikolaou and Aggelis 2011). The interest in this type of fuel resides in its performance in comparison to petroleum-based diesel. The biodiesel presents similar power compared to its non-renewable counterpart, with the environmentally-friendly pros of lower greenhouse gas emissions (Wahlen et al. 2013). One of the sources that can be used for the production of biodiesel is wastewater having a high ratio of C/N

(Mondala et al. 2012). A ratio of 70 is considered as the optimal ratio to promote the production of lipidic products from the microbial which can be converted to biodiesel through transesterification reaction (Mondala et al. 2013; Revellame et al. 2010). The production of biodiesel through microbes using biomass can be enhanced by regulating the carbon source which has a higher C/N ratio such as glucose, xylose, and acetic acid. This feature allows the use of organic wastes for the production of biodiesel and thus providing waste management and eco-friendly ways to convert them into value-added fuel (Fortela et al. 2016a). In addition to C/N ratio, there are many other parameters such as types of microbes, type and concentration of substrates, and operational conditions which can influence the quality of the produced biodiesel (Fortela et al. 2016b). These parameters can affect properties such as viscosity, volatility, and calorific power of the produced biodiesel. Incomplete combustion of biodiesel is another issue that creates carbon deposition in the engine. Some of the issues can be addressed by using low molecular weight biodiesel.

The production of short-chain alcohols by the microbes through transesterification of fats is a way to create low molecular weight biodiesel. The alcohols also act as solvents as well as fuel which reduces the viscosity and increases the volatile content of the biodiesel. The presence of lipases, enzymes responsible for breaking down fat, helps the biochemical process more efficient which requires less energy input, mild operational conditions, and a lower number of by-products compared to diesel produced via chemical routes (Park and Mori 2005; Du et al. 2006; Wang et al. 2006; Nie et al. 2006). The latter case presents some drawbacks such as the formation of soap requiring purification along with the removal of glycerol which is an inherent by-product of the process. Also, a high load of methanol/vegetable oil of around 6:1 is required to obtain an appreciable yield (Marchetti et al. 2007; Srivastava et al. 2006). The microbial process for the synthesis of biodiesel requires further development of immobilized enzymes or whole cells, which currently implies in higher overall cost than chemical catalysis (Akoh et al. 2007). The tools that are being employed to counter these issues are genetic engineering on the strains of microbes to improve stereoselectivity and synthesizing efficient and thermostable catalysts (Yang et al. 2007). Thus, even though biodiesel derived from microbes are a reasonable and green source of fuel it still demands effort from the scientific community to establish a definitive and effective route of production.

5.2.6 Microbes for Electrosynthesis

The microbial electrosynthesis (MES) technology presented itself as a valuable tool to reduce carbon footprint while enabling the production of short-chain fatty acids. The process consists of the chemical reduction of CO_2 to obtain acetic acid with the help of acetogenic microbes (Nevin et al. 2011). The fuel cell for this process can be assembled by using a water-split system at the anode where H^+, $O_{2,}$ and electrons can be produced which can use to power the cathode where microbial system

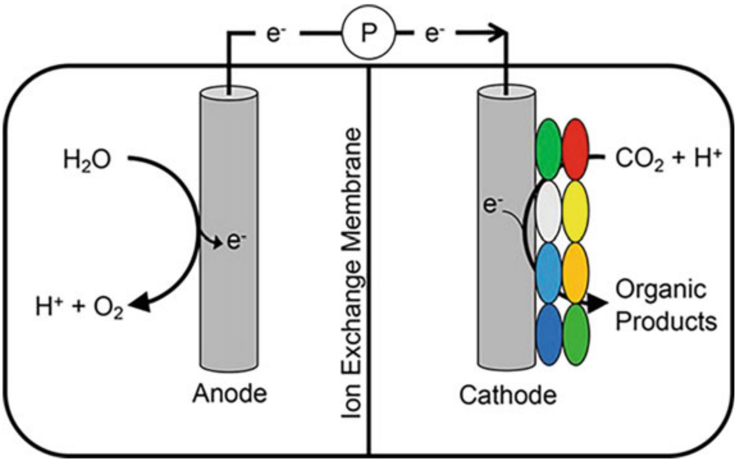


Fig. 5.18 General scheme of a microbial electrosynthesis cell.(Adapted with permission, LaBelle et al. (2020). Copyright (2020) American Chemical Society)

catabolizes CO_2 to produce acetic acid. A general scheme for this process is described in Fig. 5.18 (Christodoulou and Velasquez-Orta 2016). Although this technique enables a green approach to produce important organic acid for the industry through consumption of CO_2, however the low productivity and efficiency hinder its application for the industrial process (Christodoulou and Velasquez-Orta 2016; LaBelle et al. 2020). Recently, there has been significant progress to handle such issues. For example, a facile and efficient method was used to produce acetic acid using wastewater as a substrate and porous graphitic rod as a cathode (Marshall et al. 2012, 2013; LaBelle et al. 2014). The metabolic products obtained through this process were mostly formate, acetate, and methane along with lesser amounts of isobutyrate, butyrate, and propionate (Marshall et al. 2012, 2013; LaBelle et al. 2014). The synthesis of these metabolic products demonstrates that this technique can be used to convert CO_2 into other useful chemicals under mild conditions, which is one of the main advantages of this process. Despite the low production rate of this process, the condition to synthesize organic substances using low energy input brings many possibilities for industrial applications (Richter et al. 2013; Conrado et al. 2013).

The selection of microbiome (types of microbes that constitute the environment) and control of undesirable methanogenesis plays an important role in optimizing the biosynthesis process. In one study, the methanogenesis was inhibited by the addition of 2-bromoethanesulfonate which improved the production of acetate (Liu et al. 2011). Another way to improve the efficiency of the biosynthesis process is to reduce the pH so that the acidic environment can kill the bacteria that do not produce acetate (LaBelle et al. 2014). It can be performed either by flushing CO_2 into the system or adding a buffer such as carbonate of phosphate (LaBelle et al. 2014). However, a pH around 6.5 was observed to be optimal for acetate production. A pH below 5 tends to

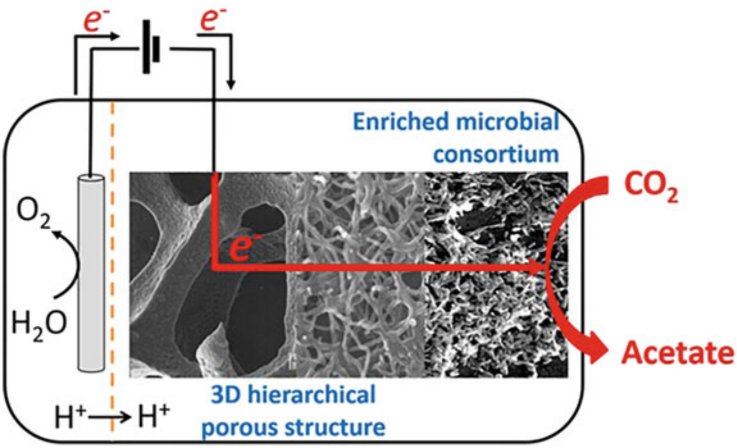


Fig. 5.19 Scheme of a microbial electrolysis cell for the production of acetate through enhanced cathode for improvement of microbial activity. (Adapted with permission, Flexer and Jourdin (2020). Copyright (2020) American Chemical Society)

produce more hydrogen, hence, pH can alter the major product formation and efficiency of the microbial electrosynthesis (Marshall et al. 2012, 2013; LaBelle et al. 2014). It was seen that cell design and cathode surface area can also affect the performance of a microbial electrosynthesis process (LaBelle et al. 2020; Flexer and Jourdin 2020). The performance of a microbial electrosynthesis depends on the electrode used. Some of the electrode characteristics such as biocompatibility, 3D structure, conductivity, and porosity are very important for an efficient conversion process as these properties facilitate the electrons transfer from the cathode to the microbes, which consequently improves the rate of production. Also, the high surface area allows better growth of microbes which improves their catalytic activities. The system accompanied by a constant injection of CO_2 and energy supply through anode promotes the biosynthesis of acetate. A general scheme of such a system is shown in Fig. 5.19 (Flexer and Jourdin 2020).

The studies so far showed that microbial electrolysis is a promising technology for the production of a variety of value-added materials like acetate, butyric, isobutyric, caproic, and poly(3-hydroxybutyrate) under mild conditions of reactions (Vassilev et al. 2018; Chen et al. 2018). Further improvement in the process, cell design, and microbes is needed to establish a more stable and efficient biosynthetic pathway to produce industrially important chemicals (LaBelle et al. 2020).

5.2.7 Microbes for Energy Storage

As discussed so far, the level of technology regarding the use of microbes is pushing new ways to obtain sustainable energy. Although this technology is not being widely used for industrial applications, the development of this applied science is crucial for

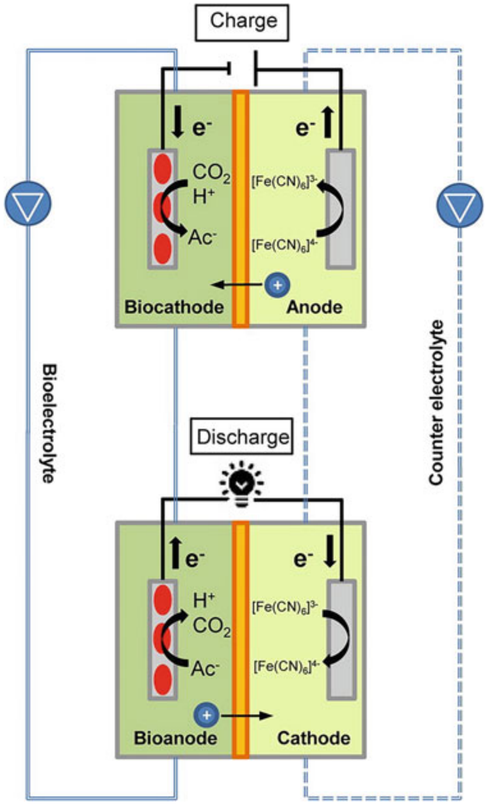


Fig. 5.20 Schematics of a microbial rechargeable battery (MRB) coupled with a microbial electrosynthesis (MES), at the top, that uses electric energy to produce acetate and a microbial fuel cell (MFC), at the bottom, that uses the acetate to produce electricity. (Adapted with permission, Molenaar et al. (2016). Copyright (2016) American Chemical Society)

the future as it uses renewable resources for energy production. One of the new applications of microbes is in energy storage devices. Microbial electrosynthesis process can be coupled with microbial fuel cells to develop microbial rechargeable batteries (MRB). The concept of this device works through the synergy of these two bioelectrochemical processes. The microbial electrosynthesis process uses electrical energy to produce acetate, simultaneously, the microbial fuel cell uses the acetate to produce electricity. This scenario can enable a stable and uninterrupted system of a biocathode at the microbial electrosynthesis and a bioanode at the microbial fuel cell, with their respective counter electrodes (Molenaar et al. 2016). A general scheme for microbial rechargeable batteries coupled with a microbial electrosynthesis and the microbial fuel cell is described in Fig. 5.20.

A similar approach was demonstrated by building a microbial battery that used organic wastes as a carbon source to produce electricity and CO_2 (Dubrawski et al. 2019). Both electrons and CO_2 were used to obtain either H_2 or $HCOO^-$ (formate) through enzymatic electrosynthesis (EES). One of the issues with this technology is the generation of electrons from water which can release oxygen. The poor electron transfer capability of water accompanied by the presence of oxygen can deactivate the enzyme imposing a challenge for this process. To counter this situation an oxygen-free redox cathode such as Prussian Blue can be used for microbial battery (Dubrawski et al. 2019). Its structure is generally composed of $A_xTM(CN)_6.nH_2O$ where A is an alkali cation metal (mostly Na^+ or K^+), T is a transition metal, and the fraction $M(CN)_6$ is an anionic portion where M is a trivalent metal like Fe^{3+}, Mn^{3+}, or Cr^{3+}. The key aspect of these cathodes is their structure with an open framework, which allows rapid interactions with cations such as H^+, $Na^{+,}$ and K^+. Also, these cathodes after going through the reduction process can quickly oxidize back after exposure to oxygen (Kong et al. 2017; Xie et al. 2015). Several microbial batteries can be connected in series to provide higher voltage for practical applications such as to power other as microbial electrolysis, electrosynthesis, and enzymatic electrosynthesis (ElMekawy et al. 2016; Rozendal et al. 2009). The latter is one of the latest technologies that focus on the straight use of enzymes (ElMekawy et al. 2016; Rozendal et al. 2009; Milton et al. 2016; Sakai et al. 2016; Amao 2017). Different enzymes can be used to synthesize specific products such as methane, ammonia, hydrogen, acetate, or formate (Milton et al. 2016, ; Milton et al. 2017; Chica et al. 2017; Deutzmann et al. 2015; Zheng et al. 2018). Henceforth, a recent report described the coupling of stacked microbial batteries and enzymatic electrosynthesis for a self-sustainable system. The dilute organic matter was used to produce CO_2 and electrons in the oxygen-free cathode, with stacked microbial batteries to generate a higher voltage. This cathode is used as a power source to donate electrons to the enzymatic electrosynthesis process through a mediator. Hydrogenase and formate dehydrogenase were used as enzymes to produce H_2 and $HCOO^-$ (Dubrawski et al. 2019). The schematics of the system is described in Fig. 5.21. This technology presents an applicable approach to use microbial electrochemical systems to store energy and to couple it with the production of several products through electrolysis, which simultaneous work on waste management, synthesize products, and store electrical energy.

5.3 Conclusion and Future Remarks

As described throughout this chapter the use of microbes for energy purposes is an extremely vast and versatile technology that enables many different approaches. Despite the challenge of improving efficiency and turning this technology more applicable for large-scale production, it is a highly sustainable process that provides a proper end for waste management. The process can convert most of the organic wastes into useful products, electricity, or both under mild conditions such as neutral pH and room temperature. This is a valuable aspect of microbes based technology as the synthesis of similar products using chemical process demands higher energy. An enzymatic process is an important tool that must be explored to bring a sustainable way of producing new materials. Even though further research and development are still required to apply these technologies for industrial production, the results obtained so far are promising.

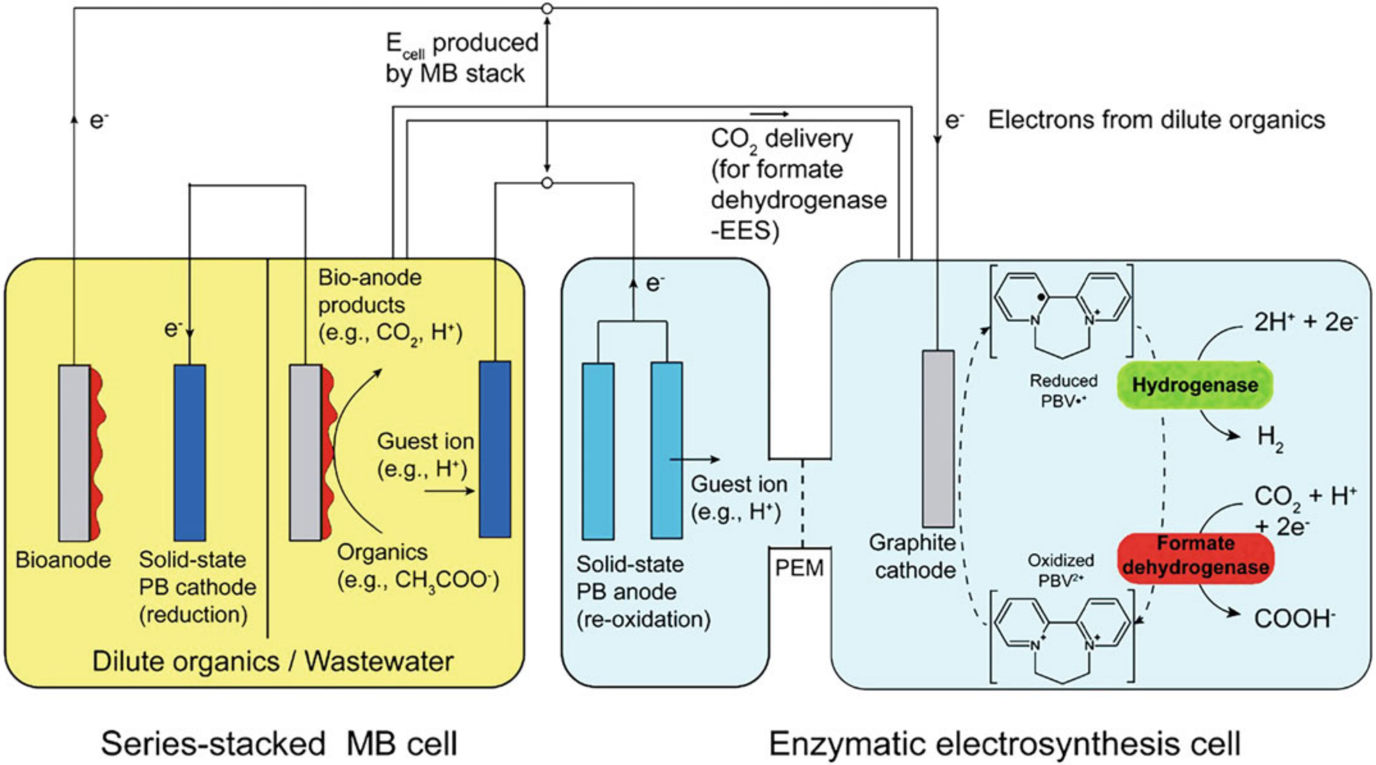


Fig. 5.21 The occurring process shows the stacked microbial battery (left) that is responsible for generating CO_2 and electrons to power the enzymatic electrosynthesis cell (EES) (right). At the EES the enzymes hydrogenase and formate dehydrogenase produce H_2 and COO^-, respectively. The redox pair of trimethylene-2-2′-bipyridinium dibromide (PBV) is responsible for the electron transfer to allow the enzymatic synthesis. Also, the solid-state Prussian Blue (PB) anode acts as an electron acceptor for that system. (Adapted with permission, Dubrawski et al. (2019). Copyright (2019) American Chemical Society)

References

Adrio JL, Demain AL (2014) Microbial enzymes: tools for biotechnological processes. Biomol Ther 4:117–139

Akoh CC, Chang S-W, Lee G-C, Shaw J-F (2007) Enzymatic approach to biodiesel production. J Agric Food Chem 55:8995–9005. https://doi.org/10.1021/jf071724y

Amao Y (2017) Viologens for coenzymes of biocatalysts with the function of CO2 reduction and utilization. Chem Lett 46:780–788. https://doi.org/10.1246/cl.161189

Amin FR, Khalid H, Zhang H, Rahman S, Zhang R, Liu G, Chen C (2017) Pretreatment methods of lignocellulosic biomass for anaerobic digestion. AMB Express 7:72. https://doi.org/10.1186/s13568-017-0375-4

Babauta J, Renslow R, Lewandowski Z, Beyenal H (2012) Electrochemically active biofilms: facts and fiction. A review. Biofouling 28:789–812. https://doi.org/10.1080/08927014.2012.710324

Balat M, Balat H (2009) Recent trends in global production and utilization of bio-ethanol fuel. Appl Energy 86:2273–2282. https://doi.org/10.1016/j.apenergy.2009.03.015

Bartels JR, Pate MB, Olson NK (2010) An economic survey of hydrogen production from conventional and alternative energy sources. Int J Hydrog Energy 35:8371–8384. https://doi.org/10.1016/j.ijhydene.2010.04.035

Bisgaard-Frantzen H, Svendsen A, Norman B, Pedersen S, Kjaerulff S, Outtrup H, Borchert TV (1999) Development of industrially important α-amylases. J Appl Glycosci 46:199–206. https://doi.org/10.5458/jag.46.199

Bond DR, Lovley DR (2003) Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl Environ Microbiol 69:1548–1555. https://doi.org/10.1128/AEM.69.3.1548-1555.2003

Borman S (2016) Mechanism confirmed for methane-making enzyme. C&EN Glob Enterp 94:7. https://doi.org/10.1021/cen-09421-notw6

Buckley M, Wal J (2006) Microbial energy conversion. American academy of microbiology, Washington, DC, United States

Bozell JJ, Petersen GR (2010) Technology development for the production of biobased products from biorefinery carbohydrates—the US Department of Energy's "top 10" revisited. Green Chem 12:539–554. https://doi.org/10.1039/B922014C

Caccavo F, Lonergan DJ, Lovley DR, Davis M, Stolz JF, McInerney MJ (1994) Geobacter sulfurreducens sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl Environ Microbiol 60:3752–3759

Call D, Logan BE (2008) Hydrogen production in a single chamber microbial electrolysis cell lacking a membrane. Environ Sci Technol 42:3401–3406. https://doi.org/10.1021/es8001822

Cardona Alzate CA, Sánchez Toro OJ (2006) Energy consumption analysis of integrated flowsheets for production of fuel ethanol from lignocellulosic biomass. Energy 31:2447–2459. https://doi.org/10.1016/j.energy.2005.10.020

Chae K-J, Choi M-J, Kim K-Y, Ajayi FF, Chang I-S, Kim IS (2009) A solar-powered microbial electrolysis cell with a platinum catalyst-free cathode to produce hydrogen. Environ Sci Technol 43:9525–9530. https://doi.org/10.1021/es9022317

Chandrasekhar K, Amulya K, Venkata Mohan S (2015) Solid phase bio-electrofermentation of food waste to harvest value-added products associated with waste remediation. Waste Manag 45:57–65. https://doi.org/10.1016/j.wasman.2015.06.001

Chaubey A, Malhotra BD (2002) Mediated biosensors. Biosens Bioelectron 17:441–456. https://doi.org/10.1016/S0956-5663(01)00313-X

Chen X, Cao Y, Li F, Tian Y, Song H (2018) Enzyme-assisted microbial electrosynthesis of poly(3-hydroxybutyrate) via CO2 bioreduction by engineered Ralstonia eutropha. ACS Catal 8:4429–4437. https://doi.org/10.1021/acscatal.8b00226

Chica B, Wu C-H, Liu Y, Adams MWW, Lian T, Dyer RB (2017) Balancing electron transfer rate and driving force for efficient photocatalytic hydrogen production in CdSe/CdS nanorod–[NiFe]

hydrogenase assemblies. Energy Environ Sci 10:2245–2255. https://doi.org/10.1039/C7EE01738C
Choi WJ, Hartono MR, Chan WH, Yeo SS (2011) Ethanol production from biodiesel-derived crude glycerol by newly isolated Kluyvera cryocrescens. Appl Microbiol Biotechnol 89:1255–1264. https://doi.org/10.1007/s00253-010-3076-3
Choi K-S, Kondaveeti S, Min B (2017) Bioelectrochemical methane (CH4) production in anaerobic digestion at different supplemental voltages. Bioresour Technol 245:826–832. https://doi.org/10.1016/j.biortech.2017.09.057
Christodoulou X, Velasquez-Orta SB (2016) Microbial electrosynthesis and anaerobic fermentation: an economic evaluation for acetic acid production from CO2 and CO. Environ Sci Technol 50:11234–11242. https://doi.org/10.1021/acs.est.6b02101
Christwardana M, Kwon Y (2017) Yeast and carbon nanotube based biocatalyst developed by synergetic effects of covalent bonding and hydrophobic interaction for performance enhancement of membraneless microbial fuel cell. Bioresour Technol 225:175–182. https://doi.org/10.1016/j.biortech.2016.11.051
Christwardana M, Frattini D, Accardo G, Yoon SP, Kwon Y (2018) Early-stage performance evaluation of flowing microbial fuel cells using chemically treated carbon felt and yeast biocatalyst. Appl Energy 222:369–382. https://doi.org/10.1016/j.apenergy.2018.03.193
Christwardana M, Frattini D, Duarte KDZ, Accardo G, Kwon Y (2019) Carbon felt molecular modification and biofilm augmentation via quorum sensing approach in yeast-based microbial fuel cells. Appl Energy 238:239–248. https://doi.org/10.1016/j.apenergy.2019.01.078
Collar C, Martinez JC, Andreu P, Armero E (2000) Effects of enzyme associations on bread dough performance. A response surface analysis/Efectos de las asociaciones enzimáticas sobre la calidad funcional de masas panarias. Análisis de superficies de respuesta. Food Sci Technol Int 6:217–226. https://doi.org/10.1177/108201320000600304
Conrado RJ, Haynes CA, Haendler BE, Toone EJ (2013) Electrofuels: a new paradigm for renewable fuels. In: Advanced biofuels and bioproducts. Springer, Berlin, pp 1037–1064
Czajczyńska D, Anguilano L, Ghazal H, Krzyżyńska R, Reynolds AJ, Spencer N, Jouhara H (2017) Potential of pyrolysis processes in the waste management sector. Therm Sci Eng Prog 3:171–197. https://doi.org/10.1016/j.tsep.2017.06.003
Delmont TO, Malandain C, Prestat E, Larose C, Monier J-M, Simonet P, Vogel TM (2011) Metagenomic mining for microbiologists. ISME J 5:1837–1843. https://doi.org/10.1038/ismej.2011.61
Desvaux M, Guedon E, Petitdemange H (2000) Cellulose catabolism by *Clostridium cellulolyticum* growing in batch culture on defined medium. Appl Environ Microbiol 66:2461–2470. https://doi.org/10.1128/AEM.66.6.2461-2470.2000
Deutzmann JS, Sahin M, Spormann AM (2015) Extracellular enzymes facilitate electron uptake in biocorrosion and bioelectrosynthesis. MBio 6:e00496–e00415. https://doi.org/10.1128/mBio.00496-15
Deval AS, Parikh HA, Kadier A, Chandrasekhar K, Bhagwat AM, Dikshit AK (2017) Sequential microbial activities mediated bioelectricity production from distillery wastewater using bio-electrochemical system with simultaneous waste remediation. Int J Hydrog Energy 42:1130–1141. https://doi.org/10.1016/j.ijhydene.2016.11.114
Dey M, Li X, Kunz RC, Ragsdale SW (2010) Detection of organometallic and radical intermediates in the catalytic mechanism of methyl-coenzyme M reductase using the natural substrate methyl-coenzyme M and a coenzyme B substrate analogue. Biochemistry 49:10902–10911. https://doi.org/10.1021/bi101562m
Ding A, Yang Y, Sun G, Wu D (2016) Impact of applied voltage on methane generation and microbial activities in an anaerobic microbial electrolysis cell (MEC). Chem Eng J 283:260–265. https://doi.org/10.1016/j.cej.2015.07.054
Ding J, Lu Y-Z, Fu L, Ding Z-W, Mu Y, Cheng SH, Zeng RJ (2017) Decoupling of DAMO archaea from DAMO bacteria in a methane-driven microbial fuel cell. Water Res 110:112–119. https://doi.org/10.1016/j.watres.2016.12.006

Du D, Sato M, Mori M, Park EY (2006) Repeated production of fatty acid methyl ester with activated bleaching earth in solvent-free system. Process Biochem 41:1849–1853. https://doi.org/10.1016/j.procbio.2006.03.042

Du Z, Li H, Gu T (2007) A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Adv 25:464–482. https://doi.org/10.1016/j.biotechadv.2007.05.004

Dubrawski KL, Shao X, Milton RD, Deutzmann JS, Spormann AM, Criddle CS (2019) Microbial battery powered enzymatic electrosynthesis for carbon capture and generation of hydrogen and formate from dilute organics. ACS Energy Lett 4:2929–2936. https://doi.org/10.1021/acsenergylett.9b02203

ElMekawy A, Hegab HM, Mohanakrishna G, Elbaz AF, Bulut M, Pant D (2016) Technological advances in CO2 conversion electro-biorefinery: a step toward commercialization. Bioresour Technol 215:357–370. https://doi.org/10.1016/j.biortech.2016.03.023

Escapa A, Mateos R, Martínez EJ, Blanes J (2016) Microbial electrolysis cells: an emerging technology for wastewater treatment and energy recovery. From laboratory to pilot plant and beyond. Renew Sust Energ Rev 55:942–956. https://doi.org/10.1016/j.rser.2015.11.029

Fishilevich S, Amir L, Fridman Y, Aharoni A, Alfonta L (2009) Surface display of redox enzymes in microbial fuel cells. J Am Chem Soc 131:12052–12053. https://doi.org/10.1021/ja9042017

Flexer V, Jourdin L (2020) Purposely designed hierarchical porous electrodes for high rate microbial electrosynthesis of acetate from carbon dioxide. Acc Chem Res 53:311–321. https://doi.org/10.1021/acs.accounts.9b00523

Fortela DL, Hernandez R, Zappi M, French TW, Bajpai R, Chistoserdov A, Revellame E, Holmes W (2016a) Microbial lipid accumulation capability of activated sludge feeding on short chain fatty acids as carbon sources through fed-batch cultivation. J Bioprocess Biotechnol 6:2

Fortela DL, Hernandez R, Chistoserdov A, Zappi M, Bajpai R, Gang D, Revellame E, Holmes W (2016b) Biodiesel profile stabilization and microbial community selection of activated sludge feeding on acetic acid as a carbon source. ACS Sustain Chem Eng 4:6427–6434. https://doi.org/10.1021/acssuschemeng.6b01140

Grand View Research (2020) Enzymes market size, share & trends analysis report, San Francisco, CA, United States

Gunawardena A, Fernando S, To F (2008) Performance of a yeast-mediated biological fuel cell. Int J Mol Sci 9:1893–1907. https://doi.org/10.3390/ijms9101893

Gupta A, Verma JP (2015) Sustainable bio-ethanol production from agro-residues: a review. Renew Sust Energ Rev 41:550–567. https://doi.org/10.1016/j.rser.2014.08.032

Gurung N, Ray S, Bose S, Rai V (2013) A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. Biomed Res Int 2013:329121. https://doi.org/10.1155/2013/329121

Hahn-HäGerdal B, Lindén T, Senac T, Skoog K (1991) Ethanolic fermentation of pentoses in lignocellulose hydrolysates. Appl Biochem Biotechnol 28:131–144. https://doi.org/10.1007/BF02922595

Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund MF, Lidén G, Zacchi G (2006) Bio-ethanol—the fuel of tomorrow from the residues of today. Trends Biotechnol 24:549–556. https://doi.org/10.1016/j.tibtech.2006.10.004

He Z, Liu J, Qiao Y, Li CM, Tan TTY (2012) Architecture engineering of hierarchically porous chitosan/vacuum-stripped graphene scaffold as bioanode for high performance microbial fuel cell. Nano Lett 12:4738–4741. https://doi.org/10.1021/nl302175j

Hendry SP, Cardosi MF, Turner APF, Neuse EW (1993) Polyferrocenes as mediators in amperometric biosensors for glucose. Anal Chim Acta 281:453–459. https://doi.org/10.1016/0003-2670(93)85002-2

Ito T, Nakashimada Y, Senba K, Matsui T, Nishio N (2005) Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process. J Biosci Bioeng 100:260–265. https://doi.org/10.1263/jbb.100.260

Kadier A, Simayi Y, Kalil MS, Abdeshahian P, Hamid AA (2014) A review of the substrates used in microbial electrolysis cells (MECs) for producing sustainable and clean hydrogen gas. Renew Energy 71:466–472. https://doi.org/10.1016/j.renene.2014.05.052

Kadier A, Kalil MS, Abdeshahian P, Chandrasekhar K, Mohamed A, Azman NF, Logroño W, Simayi Y, Hamid AA (2016) Recent advances and emerging challenges in microbial electrolysis cells (MECs) for microbial production of hydrogen and value-added chemicals. Renew Sust Energ Rev 61:501–525. https://doi.org/10.1016/j.rser.2016.04.017

Kadier A, Jain P, Lai B, Kalil MS, Kondaveeti S, Alabbosh KFS, Abu-Reesh IM, Mohanakrishna G (2020) Biorefinery perspectives of microbial electrolysis cells (MECs) for hydrogen and valuable chemicals production through wastewater treatment. Biofuel Res J 7:1128–1142. https://doi.org/10.18331/BRJ2020.7.1.5

Kiran Kumar A, Venkateswar Reddy M, Chandrasekhar K, Srikanth S, Venkata Mohan S (2012) Endocrine disruptive estrogens role in electron transfer: bio-electrochemical remediation with microbial mediated electrogenesis. Bioresour Technol 104:547–556. https://doi.org/10.1016/j.biortech.2011.10.037

Kondaveeti S, Min B (2015) Bioelectrochemical reduction of volatile fatty acids in anaerobic digestion effluent for the production of biofuels. Water Res 87:137–144. https://doi.org/10.1016/j.watres.2015.09.011

Kong D, Xie X, Lu Z, Ye M, Lu Z, Zhao J, Criddle CS, Cui Y (2017) Use of an intermediate solid-state electrode to enable efficient hydrogen production from dilute organic matter. Nano Energy 39:499–505. https://doi.org/10.1016/j.nanoen.2017.07.024

Kumar G, Saratale RG, Kadier A, Sivagurunathan P, Zhen G, Kim S-H, Saratale GD (2017) A review on bio-electrochemical systems (BESs) for the syngas and value added biochemicals production. Chemosphere 177:84–92. https://doi.org/10.1016/j.chemosphere.2017.02.135

LaBelle EV, Marshall CW, Gilbert JA, May HD (2014) Influence of acidic pH on hydrogen and acetate production by an electrosynthetic microbiome. PLoS One 9:e109935

LaBelle EV, Marshall CW, May HD (2020) Microbiome for the electrosynthesis of chemicals from carbon dioxide. Acc Chem Res 53:62–71. https://doi.org/10.1021/acs.accounts.9b00522

Lee M-H, Thomas JL, Chen W-J, Li M-H, Shih C-P, Lin H-Y (2015) Fabrication of bacteria-imprinted polymer coated electrodes for microbial fuel cells. ACS Sustain Chem Eng 3:1190–1196. https://doi.org/10.1021/acssuschemeng.5b00138

Lee M, Nagendranatha Reddy C, Min B (2019) In situ integration of microbial electrochemical systems into anaerobic digestion to improve methane fermentation at different substrate concentrations. Int J Hydrog Energy 44:2380–2389. https://doi.org/10.1016/j.ijhydene.2018.08.051

Li X, Telser J, Kunz RC, Hoffman BM, Gerfen G, Ragsdale SW (2010) Observation of organometallic and radical intermediates formed during the reaction of methyl-coenzyme M reductase with bromoethanesulfonate. Biochemistry 49:6866–6876. https://doi.org/10.1021/bi100650m

Li S, Yang X, Yang S, Zhu M, Wang X (2012) Technology prospecting on enzymes: application, marketing and engineering. Comput Struct Biotechnol J 2:e201209017. https://doi.org/10.5936/csbj.201209017

Li S, Chen G, Anandhi A (2018) Applications of emerging bioelectrochemical technologies in agricultural systems: a current review. Energies 11:2951

Lima Filho JL, Pandey PC, Weetall HH (1996) An amperometric flow injection analysis enzyme sensor for sucrose using a tetracyanoquinodimethane modified graphite paste electrode. Biosens Bioelectron 11:719–723. https://doi.org/10.1016/0956-5663(96)85922-7

Lin Y, Tanaka S (2006) Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol 69:627–642. https://doi.org/10.1007/s00253-005-0229-x

Liu X, Kokare C (2017) Chapter 11—Microbial enzymes of use in industry. In: Brahmachari G, Demain AL, Adrio JL (eds) Biotechnology of microbial enzymes: production, biocatalysis and industrial applications. Academic Press, New York, pp 267–298

Liu H, Grot S, Logan BE (2005) Electrochemically assisted microbial production of hydrogen from acetate. Environ Sci Technol 39:4317–4320. https://doi.org/10.1021/es050244p

Liu H, Wang J, Wang A, Chen J (2011) Chemical inhibitors of methanogenesis and putative applications. Appl Microbiol Biotechnol 89:1333–1340. https://doi.org/10.1007/s00253-010-3066-5

Liu C-G, Wang N, Lin Y-H, Bai F-W (2012) Very high gravity ethanol fermentation by flocculating yeast under redox potential-controlled conditions. Biotechnol Biofuels 5:61. https://doi.org/10.1186/1754-6834-5-61

Liu H, Hu TJ, Zeng GM, Yuan XZ, Wu JJ, Shen Y, Yin L (2013a) Electricity generation using p-nitrophenol as substrate in microbial fuel cell. Int Biodeterior Biodegradation 76:108–111. https://doi.org/10.1016/j.ibiod.2012.06.015

Liu C-G, Xue C, Lin Y-H, Bai F-W (2013b) Redox potential control and applications in microaerobic and anaerobic fermentations. Biotechnol Adv 31:257–265. https://doi.org/10.1016/j.biotechadv.2012.11.005

Liu D, Zhang L, Chen S, Buisman C, ter Heijne A (2016) Bioelectrochemical enhancement of methane production in low temperature anaerobic digestion at 10 °C. Water Res 99:281–287. https://doi.org/10.1016/j.watres.2016.04.020

Marchetti JM, Miguel VU, Errazu AF (2007) Possible methods for biodiesel production. Renew Sust Energ Rev 11:1300–1311. https://doi.org/10.1016/j.rser.2005.08.006

Marshall CW, Ross DE, Fichot EB, Norman RS, May HD (2012) Electrosynthesis of commodity chemicals by an autotrophic microbial community. Appl Environ Microbiol 78:8412–8420. https://doi.org/10.1128/AEM.02401-12

Marshall CW, Ross DE, Fichot EB, Norman RS, May HD (2013) Long-term operation of microbial electrosynthesis systems improves acetate production by autotrophic microbiomes. Environ Sci Technol 47:6023–6029. https://doi.org/10.1021/es400341b

Maspolim Y, Zhou Y, Guo C, Xiao K, Ng WJ (2015) The effect of pH on solubilization of organic matter and microbial community structures in sludge fermentation. Bioresour Technol 190:289–298. https://doi.org/10.1016/j.biortech.2015.04.087

McAnulty MJ, Poosarla VG, Kim K-Y, Jasso-Chávez R, Logan BE, Wood TK (2017) Electricity from methane by reversing methanogenesis. Nat Commun 8:15419. https://doi.org/10.1038/ncomms15419

McMillan JD (1997) Bioethanol production: status and prospects. Renew Energy 10:295–302. https://doi.org/10.1016/0960-1481(96)00081-X

Milton RD, Abdellaoui S, Khadka N, Dean DR, Leech D, Seefeldt LC, Minteer SD (2016) Nitrogenase bioelectrocatalysis: heterogeneous ammonia and hydrogen production by MoFe protein. Energy Environ Sci 9:2550–2554. https://doi.org/10.1039/C6EE01432A

Milton RD, Cai R, Abdellaoui S, Leech D, De Lacey AL, Pita M, Minteer SD (2017) Bioelectrochemical Haber–Bosch process: an ammonia-producing H2/N2 fuel cell. Angew Chemie Int Ed 56:2680–2683. https://doi.org/10.1002/anie.201612500

Min B, Poulsen FW, Thygesen A, Angelidaki I (2012) Electric power generation by a submersible microbial fuel cell equipped with a membrane electrode assembly. Bioresour Technol 118:412–417. https://doi.org/10.1016/j.biortech.2012.04.097

Molenaar SD, Mol AR, Sleutels THJA, ter Heijne A, Buisman CJN (2016) Microbial rechargeable battery: energy storage and recovery through acetate. Environ Sci Technol Lett 3:144–149. https://doi.org/10.1021/acs.estlett.6b00051

Mondala AH, Hernandez R, French T, McFarland L, Santo Domingo JW, Meckes M, Ryu H, Iker B (2012) Enhanced lipid and biodiesel production from glucose-fed activated sludge: kinetics and microbial community analysis. AICHE J 58:1279–1290. https://doi.org/10.1002/aic.12655

Mondala A, Hernandez R, Holmes W, French T, McFarland L, Sparks D, Haque M (2013) Enhanced microbial oil production by activated sludge microorganisms via co-fermentation of glucose and xylose. AICHE J 59:4036–4044. https://doi.org/10.1002/aic.14169

Mook WT, Aroua MKT, Chakrabarti MH, Noor IM, Irfan MF, Low CTJ (2013) A review on the effect of bio-electrodes on denitrification and organic matter removal processes in bio-electrochemical systems. J Ind Eng Chem 19:1–13. https://doi.org/10.1016/j.jiec.2012.07.004

Moreno R, Martínez EJ, Escapa A, Martínez O, Díez-Antolínez R, Gómez X (2018) Mitigation of volatile fatty acid build-up by the use of soft carbon felt electrodes: evaluation of anaerobic digestion in acidic conditions. Fermentation 4(1):2

Nagendranatha Reddy C, Venkata Mohan S (2016) Integrated bio-electrogenic process for bioelectricity production and cathodic nutrient recovery from azo dye wastewater. Renew Energy 98:188–196. https://doi.org/10.1016/j.renene.2016.03.047

Nevin KP, Hensley SA, Franks AE, Summers ZM, Ou J, Woodard TL, Snoeyenbos-West OL, Lovley DR (2011) Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of Acetogenic microorganisms. Appl Environ Microbiol 77:2882–2886. https://doi.org/10.1128/AEM.02642-10

Nie K, Xie F, Wang F, Tan T (2006) Lipase catalyzed methanolysis to produce biodiesel: optimization of the biodiesel production. J Mol Catal B Enzym 43:142–147. https://doi.org/10.1016/j.molcatb.2006.07.016

Njenga M, Karanja N, Karlsson H, Jamnadass R, Iiyama M, Kithinji J, Sundberg C (2014) Additional cooking fuel supply and reduced global warming potential from recycling charcoal dust into charcoal briquette in Kenya. J Clean Prod 81:81–88. https://doi.org/10.1016/j.jclepro.2014.06.002

Nwachukwu RES, Shahbazi A, Wang L, Ibrahim S, Worku M, Schimmel K (2012) Bioconversion of glycerol to ethanol by a mutant Enterobacter aerogenes. AMB Express 2:20. https://doi.org/10.1186/2191-0855-2-20

O'Neill A, Araújo R, Casal M, Guebitz G, Cavaco-Paulo A (2007) Effect of the agitation on the adsorption and hydrolytic efficiency of cutinases on polyethylene terephthalate fibres. Enzym Microb Technol 40:1801–1805. https://doi.org/10.1016/j.enzmictec.2007.02.012

Panagiotou G, Christakopoulos P (2004) NADPH-dependent D-aldose reductases and xylose fermentation in Fusarium oxysporum. J Biosci Bioeng 97:299–304. https://doi.org/10.1016/S1389-1723(04)70209-1

Pandit S, Chandrasekhar K, Kakarla R, Kadier A, Jeevitha V (2017) Basic principles of microbial fuel cell: technical challenges and economic feasibility. In: Microbial applications, vol 1. Springer, Berlin, pp 165–188

Papanikolaou S, Aggelis G (2011) Lipids of oleaginous yeasts. Part II: technology and potential applications. Eur J Lipid Sci Technol 113:1052–1073. https://doi.org/10.1002/ejlt.201100015

Park EY, Mori M (2005) Kinetic study of esterification of rapeseed oil contained in waste activated bleaching earth using Candida rugosa lipase in organic solvent system. J Mol Catal B Enzym 37:95–100. https://doi.org/10.1016/j.molcatb.2005.10.001

Park HI, Sanchez D, Cho SK, Yun M (2008) Bacterial communities on electron-beam Pt-deposited electrodes in a mediator-less microbial fuel cell. Environ Sci Technol 42:6243–6249. https://doi.org/10.1021/es8006468

Parkin GF, Owen WF (1986) Fundamentals of anaerobic digestion of wastewater sludges. J Environ Eng 112:867–920. https://doi.org/10.1061/(ASCE)0733-9372(1986)112:5(867)

Petre M (2013) Environmental biotechnology: new approaches and prospective applications. IntechOpen, London

Philbrook A, Alissandratos A, Easton CJ (2013) Biochemical processes for generating fuels and commodity chemicals from lignocellulosic biomass. BoD – Books on Demand, Norderstedt

Ratledge C, Cohen Z (2008) Microbial and algal oils: do they have a future for biodiesel or as commodity oils? Lipid Technol 20:155–160. https://doi.org/10.1002/lite.200800044

Ren Z, Ward TE, Regan JM (2007) Electricity production from cellulose in a microbial fuel cell using a defined binary culture. Environ Sci Technol 41:4781–4786. https://doi.org/10.1021/es070577h

Revellame E, Hernandez R, French W, Holmes W, Alley E (2010) Biodiesel from activated sludge through in situ transesterification. J Chem Technol Biotechnol 85:614–620. https://doi.org/10.1002/jctb.2317

Richter H, Martin ME, Angenent LT (2013) A two-stage continuous fermentation system for conversion of syngas into ethanol. Energies 6:3987–4000

Rozendal RA, Hamelers HVM, Euverink GJW, Metz SJ, Buisman CJN (2006) Principle and perspectives of hydrogen production through biocatalyzed electrolysis. Int J Hydrog Energy 31:1632–1640. https://doi.org/10.1016/j.ijhydene.2005.12.006

Rozendal RA, Leone E, Keller J, Rabaey K (2009) Efficient hydrogen peroxide generation from organic matter in a bioelectrochemical system. Electrochem Commun 11:1752–1755. https://doi.org/10.1016/j.elecom.2009.07.008

Sakai K, Kitazumi Y, Shirai O, Takagi K, Kano K (2016) Efficient bioelectrocatalytic CO2 reduction on gas-diffusion-type biocathode with tungsten-containing formate dehydrogenase. Electrochem Commun 73:85–88. https://doi.org/10.1016/j.elecom.2016.11.008

Sanchez S, Demain AL (2008) Metabolic regulation and overproduction of primary metabolites. Microb Biotechnol 1:283–319. https://doi.org/10.1111/j.1751-7915.2007.00015.x

Sarkar N, Ghosh SK, Bannerjee S, Aikat K (2012) Bioethanol production from agricultural wastes: an overview. Renew Energy 37:19–27. https://doi.org/10.1016/j.renene.2011.06.045

Sarris D, Papanikolaou S (2016) Biotechnological production of ethanol: biochemistry, processes and technologies. Eng Life Sci 16:307–329. https://doi.org/10.1002/elsc.201400199

Scheller S, Yu H, Chadwick GL, McGlynn SE, Orphan VJ (2016) Artificial electron acceptors decouple archaeal methane oxidation from sulfate reduction. Science (80-) 351:703–707. https://doi.org/10.1126/science.aad7154

Schmidt-Rohr K (2015) Why combustions are always exothermic, yielding about 418 kJ per mole of O2. J Chem Educ 92:2094–2099. https://doi.org/10.1021/acs.jchemed.5b00333

Sonawane J, Ezugwu CI, Ghosh PC (2020) Microbial fuel cells-based biological oxygen demand sensors for monitoring wastewater: state-of-the-art and practical applications. ACS Sensors 5 (8):2297–2316. https://doi.org/10.1021/acssensors.0c01299

Soo VWC, McAnulty MJ, Tripathi A, Zhu F, Zhang L, Hatzakis E, Smith PB, Agrawal S, Nazem-Bokaee H, Gopalakrishnan S, Salis HM, Ferry JG, Maranas CD, Patterson AD, Wood TK (2016) Reversing methanogenesis to capture methane for liquid biofuel precursors. Microb Cell Factories 15:11. https://doi.org/10.1186/s12934-015-0397-z

Srivastava A, Akoh CC, Chang S-W, Lee G-C, Shaw J-F (2006) Candida rugosa lipase LIP1-catalyzed transesterification to produce human milk fat substitute. J Agric Food Chem 54:5175–5181. https://doi.org/10.1021/jf060623h

Stanbury PF, Whitaker A, Hall SJ (2013) Principles of fermentation technology. Elsevier Science, Amsterdam

Sun M, Sheng G-P, Zhang L, Xia C-R, Mu Z-X, Liu X-W, Wang H-L, Yu H-Q, Qi R, Yu T, Yang M (2008) An MEC-MFC-coupled system for biohydrogen production from acetate. Environ Sci Technol 42:8095–8100. https://doi.org/10.1021/es801513c

Sun M-T, Fan X-L, Zhao X-X, Fu S-F, He S, Manasa MRK, Guo R-B (2017) Effects of organic loading rate on biogas production from macroalgae: performance and microbial community structure. Bioresour Technol 235:292–300. https://doi.org/10.1016/j.biortech.2017.03.075

Ter Heijne A, Liu F, van der Weijden R, Weijma J, Buisman CJN, Hamelers HVM (2010) Copper recovery combined with electricity production in a microbial fuel cell. Environ Sci Technol 44:4376–4381. https://doi.org/10.1021/es100526g

Vassilev I, Hernandez PA, Batlle-Vilanova P, Freguia S, Krömer JO, Keller J, Ledezma P, Virdis B (2018) Microbial electrosynthesis of isobutyric, butyric, caproic acids, and corresponding alcohols from carbon dioxide. ACS Sustain Chem Eng 6:8485–8493. https://doi.org/10.1021/acssuschemeng.8b00739

Vemuri GN, Eiteman MA, McEwen JE, Olsson L, Nielsen J (2007) Increasing NADH oxidation reduces overflow metabolism in *Saccharomyces cerevisiae*. Proc Natl Acad Sci 104:2402–2407. https://doi.org/10.1073/pnas.0607469104

Villano M, Ralo C, Zeppilli M, Aulenta F, Majone M (2016) Influence of the set anode potential on the performance and internal energy losses of a methane-producing microbial electrolysis cell. Bioelectrochemistry 107:1–6. https://doi.org/10.1016/j.bioelechem.2015.07.008

Vu HT, Min B (2019) Integration of submersible microbial fuel cell in anaerobic digestion for enhanced production of methane and current at varying glucose levels. Int J Hydrog Energy 44:7574–7582. https://doi.org/10.1016/j.ijhydene.2019.01.091

Wahlen BD, Morgan MR, McCurdy AT, Willis RM, Morgan MD, Dye DJ, Bugbee B, Wood BD, Seefeldt LC (2013) Biodiesel from microalgae, yeast, and bacteria: engine performance and exhaust emissions. Energy Fuel 27:220–228. https://doi.org/10.1021/ef3012382

Walker GM, Stewart GG (2016) Saccharomyces cerevisiae in the production of fermented beverages. Beverages 2(4):30

Wang L, Du W, Liu D, Li L, Dai N (2006) Lipase-catalyzed biodiesel production from soybean oil deodorizer distillate with absorbent present in tert-butanol system. J Mol Catal B Enzym 43:29–32. https://doi.org/10.1016/j.molcatb.2006.03.005

Wang M, Han J, Dunn JB, Cai H, Elgowainy A (2012) Well-to-wheels energy use and greenhouse gas emissions of ethanol from corn, sugarcane and cellulosic biomass for US use. Environ Res Lett 7:45905. https://doi.org/10.1088/1748-9326/7/4/045905

Wongnate T, Sliwa D, Ginovska B, Smith D, Wolf MW, Lehnert N, Raugei S, Ragsdale SW (2016) The radical mechanism of biological methane synthesis by methyl-coenzyme M reductase. Science (80-) 352:953–958. https://doi.org/10.1126/science.aaf0616

Wu VCH (2008) A review of microbial injury and recovery methods in food. Food Microbiol 25:735–744. https://doi.org/10.1016/j.fm.2008.04.011

Wünschiers R, Lindblad P (2002) Hydrogen in education—a biological approach. Int J Hydrog Energy 27:1131–1140. https://doi.org/10.1016/S0360-3199(02)00098-8

Xafenias N, Mapelli V (2014) Performance and bacterial enrichment of bioelectrochemical systems during methane and acetate production. Int J Hydrog Energy 39:21864–21875. https://doi.org/10.1016/j.ijhydene.2014.05.038

Xiao B, Han Y, Liu X, Liu J (2014) Relationship of methane and electricity production in two-chamber microbial fuel cell using sewage sludge as substrate. Int J Hydrog Energy 39:16419–16425. https://doi.org/10.1016/j.ijhydene.2014.08.024

Xie X, Ye M, Liu C, Hsu P-C, Criddle CS, Cui Y (2015) Use of low cost and easily regenerated Prussian Blue cathodes for efficient electrical energy recovery in a microbial battery. Energy Environ Sci 8:546–551. https://doi.org/10.1039/C4EE03268C

Yang J, Guo D, Yan Y (2007) Cloning, expression and characterization of a novel thermal stable and short-chain alcohol tolerant lipase from Burkholderia cepacia strain G63. J Mol Catal B Enzym 45:91–96. https://doi.org/10.1016/j.molcatb.2006.12.007

Yang W, Peng Y, Zhang Y, Lu JE, Li J, Chen S (2019) Air cathode catalysts of microbial fuel cell by nitrogen-doped carbon aerogels. ACS Sustain Chem Eng 7:3917–3924. https://doi.org/10.1021/acssuschemeng.8b05000

Yin Q, Zhu X, Zhan G, Bo T, Yang Y, Tao Y, He X, Li D, Yan Z (2016) Enhanced methane production in an anaerobic digestion and microbial electrolysis cell coupled system with co-cultivation of Geobacter and Methanosarcina. J Environ Sci 42:210–214. https://doi.org/10.1016/j.jes.2015.07.006

Yuan J, Liu S, Jia L, Ji A, Chatterjee SG (2020) Co-generation system of bioethanol and electricity with microbial fuel cell technology. Energy Fuel 34:6414–6422. https://doi.org/10.1021/acs.energyfuels.0c00749

Zaunmüller T, Eichert M, Richter H, Unden G (2006) Variations in the energy metabolism of biotechnologically relevant heterofermentative lactic acid bacteria during growth on sugars and organic acids. Appl Microbiol Biotechnol 72:421–429. https://doi.org/10.1007/s00253-006-0514-3

Zhang Y, Angelidaki I (2011) Submersible microbial fuel cell sensor for monitoring microbial activity and BOD in groundwater: focusing on impact of anodic biofilm on sensor applicability. Biotechnol Bioeng 108:2339–2347. https://doi.org/10.1002/bit.23204

Zhang M, Eddy C, Deanda K, Finkelstein M, Picataggio S (1995) Metabolic engineering of a pentose metabolism pathway in ethanologenic Zymomonas mobilis. Science (80-) 267:240–243. https://doi.org/10.1126/science.267.5195.240

Zhang X, He W, Ren L, Stager J, Evans PJ, Logan BE (2015) COD removal characteristics in air-cathode microbial fuel cells. Bioresour Technol 176:23–31. https://doi.org/10.1016/j.biortech.2014.11.001

Zhao Z, Zhang Y, Chen S, Quan X, Yu Q (2014) Bioelectrochemical enhancement of anaerobic methanogenesis for high organic load rate wastewater treatment in a up-flow anaerobic sludge blanket (UASB) reactor. Sci Rep 4:6658. https://doi.org/10.1038/srep06658

Zheng Y, Harris DF, Yu Z, Fu Y, Poudel S, Ledbetter RN, Fixen KR, Yang Z-Y, Boyd ES, Lidstrom ME, Seefeldt LC, Harwood CS (2018) A pathway for biological methane production using bacterial iron-only nitrogenase. Nat Microbiol 3:281–286. https://doi.org/10.1038/s41564-017-0091-5

6 Applications of Microbes in Electric Generation

Shichang Cai and Meng Zhang

Abstract

With the aggravation of environmental pollution and energy crisis, the society demands for green and ecological economics in the future. In recent years, the progress of nanotechnologies has provided technical support for the development of new energy. Under this background, microbial fuel cell, as a new kind of energy conversion and storage devices, can convert chemical energy in the organic matters into electrical energy with the assistance of microorganisms. Microorganisms are ubiquitous in nature. During the electricity generation process conducted by microorganisms, pollutants can be treated at the same time. For example, the synergistic effect between nanoirons and microorganisms can improve the treatment effect of nitrogen and phosphorus existed in wastewater. In addition, microbial fuel cell also has other applications in life, such as waste degradation, human self-powered sensors, and so on. Nanotechnologies have provided a new strategy for the preparation of cathode and anode electrode materials with controllable structure and performance to enhance the performance and stability of microbial fuel cell. However, there are still several shortcomings in the development of microbial fuel cell, such as low power density and poor stability, which need to be solved in the future. This chapter will focus on the working principle and classification of microbial fuel cell, the construction and application of nanomaterials in the microbial fuel cell electrodes, and introduce the research hotspots and development tendency in this field systematically.

S. Cai (✉) · M. Zhang
School of Material Science and Engineering, Henan University of Technology, Zhengzhou, Henan, China

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Keywords

New energy · Microbial fuel cell · Electricity generation · Nanomaterials

6.1 Introduction

Animals, plants, and microbes cover almost all the life forms in nature on earth. Compared with animals and plants, microbes generally are tiny and invisible to the naked eye in common life. However, they exist almost everywhere and reproduce rapidly under suitable conditions in fact. Microbes can be divided into eight categories, i.e. bacteria, viruses, fungi, actinomycetes, rickettsia, mycoplasma, chlamydia, and spirochete. There is a close connection between microbes and human beings, such as yeasts and other fungi can be used in brewing beer, penicillin can be extracted from penicillium, and lactobacillus can be used to produce yogurt. It is worth noting that the chemical reaction occurring in microbes involves the electron transfer process, which can convert the chemical energy to electric energy. Biofuel cell (BFC) is one kind of novel devices for electricity production (Du et al. 2007; Habermann and Pommer 1991). BFC utilizes the microbes or enzymes in nature as the catalysts and convert the chemical energy stored in the fuel (such as glucose) to electric energy (Li et al. 2018; Santoro et al. 2017; Xu et al. 2018). In 1911, Potter, a botanist from Britain, for the first time, produced electric current using yeast and *Escherichia coli* (Potter 1911). Since then, the study about BFC has attracted tremendous attention of the researchers from all over the world.

With the rapid development of chemistry and technology, the human society entered industrial age since 1760s. Traditional fossil fuels, such as coal, petroleum, and natural gas, usually emit pollutant gases when consuming, e.g. SO_2, NO_2, NO, and CO_2, which do harm to the environment and human health. BFC as one kind of environmentally friendly energy conversion and storage devices has shown great application potential in water purification (Jiang et al. 2018; Chakraborty et al. 2020; Khajeh et al. 2020; Rabaey and Verstraete 2005) and electric generation (Raad et al. 2020; Sambavi et al. 2020). However, since 1911, BFC has suffered from low output power density, low battery stability, expensive electrode materials, and unclear electron transfer mechanism, thus leading to very slow development (Davis and Higson 2007). In 1990s, nanomaterials and biotechnology have experienced significant breakthrough and provided profound technology and knowledge support for the development of BFC.

In 2005, Reguera et al. found that some fimbriae of *G. sulfureducens* DL1 had the ability to conduct electron. Because of its nanometer scale diameter and naturally generated property, it was named "microbial nanowire" (Reguera et al. 2005). This work immediately attracted the attention of international scholars: why do microorganisms consume energy to produce fimbriae with electron conduction ability? What are their functions? Gorby et al. confirmed that microbes such as *S. oneidensis* MR-1, *Synechocystis* PCC6803, and *Pelotomaculum thermopropionicum* could also produce electron transfer nanowires, and their length was usually more than 10 μm (Gorby et al. 2009). The presence of wires made it possible for microbes to acquire energy from a long distance without directly contacting the electron acceptor. Metal reducing bacteria, such as *Geobacter* and *Shewanella*, can transfer the intracellular electrons to the extracellular oxidized substances (such as Cr (VI), Mn (IV), U (VI), polyhalogenated pollutants, and nitro aromatic compounds, etc.) through nanowires. Then the reduced substances show low toxicity and mobility. Considering this, how to effectively exploit the electron in the microbe system to generate electricity becomes especially important for the development of microbe fuel cell.

BFC, as one kind of novel battery devices, belongs to fuel cells. Compared with the other types of traditional fuel cells, BFC has some unique advantages as follows:

1. Environment friendly. The main reaction products in BFC are CO_2 and H_2O without any toxic pollutants emission. So BFC belongs to a real sense of green energy and are environment friendly in reality.
2. Mild reaction condition. Because of the presence of microbes in BFC cell chamber, appropriate temperature (usually room temperature), atmospheric pressure, and neutral pH medium are significantly necessary and important for the successful operation of BFC. Considering this, it is relatively facile and convenient for the operation and maintenance of BFC device without any other additional equipment in the manufacture.
3. Good biocompatibility. For instance, BFC is expected to be planted in human body working as the electricity supply for artificial organs, such as pacemaker, molecular robot, and microsensor. The glucose and oxygen in the blood can provide the fuel for BFC.
4. Abundant battery materials. Glucose, alcohols, and starch are widely existed in nature, which can be used as the fuel of BFC. Especially, BFC can be applied to purify sewage and produce electricity at the same time, realizing the sustainable development of society. Meanwhile, there are a large amount of microbes or enzymes in nature, thus supplying abundant catalytic materials for BFC.

6.2 Different BFC Types

According to the mode of electron transfer between microbe and external terminal electron acceptor, BFC can be classified to two types by electronic transfer way, i.e. direct electronic transfer BFC and indirect electron transfer BFC. On the one hand, the electrode and biocatalyst of direct electronic transfer (DET) BFC contacts with each other tightly, the electron stemming from the oxidation of fuel will be transferred to the electrode surface directly. On the other hand, the electron stemming from the oxidation of fuel requires the assistance of electron mediator in order to be transferred to the electrode surface. Hence, this type of BFC is called mediated electron transfer (MET) BFC. Besides, BFC also can be classified to microbial fuel cell and enzyme biofuel cell according to different biocatalyst types.

6.2.1 DET-BFC

The intracellular electron is transferred to extracellular terminal electron acceptor or extracellular electronic donor transfers the electron to the interior of the cell directly for DET-BFC. The direct electronic transfer is achieved with the help of the membrane protein (cytochrome c) and nanowires produced by microbes. Cytochrome c, as one kind of electron transfer proteins, presents ubiquitously in living organisms and contains several closely arranged hemosiderins. Furthermore, it has significant relationship with electron transfer in metabolism process. CX_2CH is the common group of cytochrome c and $CX_{3\sim4}CH$, CX_2CK, and A/FX_2CH are also existed in cytochrome c. The electron can be transferred from inner membrane to periplasm, then to outer membrane and finally to extracellular metallic oxide, such as Fe (III) and Mn (IV) oxide. CymA protein belongs to inner membrane cytochrome c and it is the initiation point of the electron transfer from quinone pool to periplasm. MtrA protein existed in periplasm is soluble cytochrome c and contains ten hemoglobin, which can facilitate the electron getting through periplasm.

6.2.2 MET-BFC

The intracellular electron is transferred to extracellular terminal electron acceptor with the assistance of microbe secretion or exogenous substance. During the mediated electron transfer process, the redox active material, acting as the electron carrier, is called electron medium. The electron medium in a reduction state is oxidized by electron acceptor and the electron medium in an oxidation state is reduced by cell. Such oxidation and reduction process recycles between microbe and electron medium, realizing the electron transfer in MET-BFC. Besides, the outward materials can exchange electronics without entering microbe during the mediated electron transfer process and the redox reaction can rapidly degrade the heavy metals, organic materials in the environment.

The electron mediums not only include endogenous substances, such as flavin, phenazine, and quinone, but also exogenous substances, such as ferricyanide, neutral red. There are many microbes in nature can secrete flavin, such as riboflavin, flavin adenine dinucleotide, and flavin mononucleotide. They are involved in the oxidative metabolism of microbes and provide necessary nutrients for the growth of microbes. It was found that *S. oneidensis* could mediate the electron transfer between bacteria and the external electron acceptor through the secretion of extracellular flavin, and improve the energy output efficiency of microbial fuel cells (Canstein et al. 2008; Covington et al. 2010). Phenazine is also a common extracellular electron mediator, first class secondary metabolites produced by *Pseudomonas* include 1-formamide-phenazine, 1-hydroxy-phenazine, 1-carboxylic acid phenazine, and pyocyanin. Phenazine secreted by *Pseudomonas aeruginosa* KRP1 can act as an electron mediator to promote the extracellular electron transfer rate (Rabaey et al. 2005). Wang et al. found that phenazine secreted by *P. aeruginosa* PA14 could transfer electrons to the electrode surface in anaerobic environment and the bacteria could survive under anaerobic conditions (Wang et al. 2009). Humus is the main component of soil organic matter, mainly including fulvic acid which is soluble in acid and alkali, humic acid that is insoluble in acid but soluble in alkali, and humic substance that is neither soluble in acid nor alkali (Klüpfel et al. 2014). Shiwania, geobacterium, sulfate reducing bacteria, and methanogens can use humus as electron mediator to mediate extracellular electron transfer. For example, when *G. metallireducens* uses humic acid as an electron acceptor for metabolism, it can not only accelerate the metabolism of microorganisms, but also promote the reduction of extracellular iron oxides (Lovley et al. 1996). *S. putrefaciens* CN32 and *G. sulfurreducens* PCA can transfer the electron from the cell to the insoluble humus outside the cell, and then the electron is transferred to the solid iron oxide by the humus (Roden et al. 2010).

6.2.3 EBFC

According to the types of biocatalysts, BFC can be divided into enzyme biofuel cell (EBFC) and microbial fuel cell (MFC).

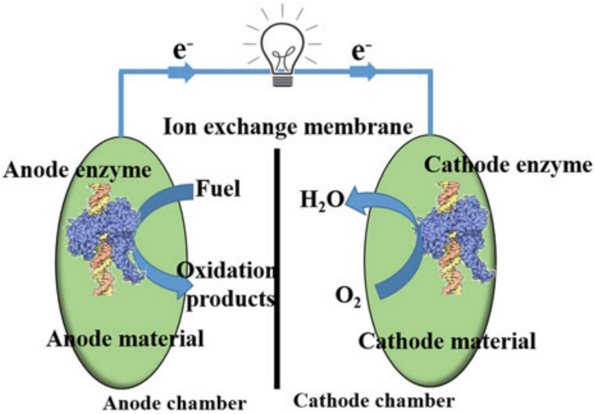
Enzyme biofuel cell is one kind of biofuel cell, which uses enzymes as the catalyst and carbohydrate, alcohol, and other substances widely existing in nature as biofuel. Under the catalysis of specific enzymes, the oxidation reaction takes place and the generated electrons reach the cathode through the external circuit, the cathodic oxidant (such as O_2, H_2O_2) receives the electrons under the catalysis effect of the corresponding enzyme to produce current (Babadi et al. 2019; Jeon et al. 2019; Mano and de Poulpiquet 2018). Using glucose-O_2 enzyme biofuel cell as an example, the reaction equations are as follows.

$$\text{Bioanode}: C_6H_{12}O_6 \rightarrow C_6H_{10}O_6 + 2H^+ + 2e^-$$

$$\text{Biocathode}: O_2 + 4H^+ + 4e^- \rightarrow H_2O$$

Its working principle is shown in Fig. 6.1. The research about EBFC began in the 1950s. At first, people intended to utilize human body fluids or metabolites to realize electric energy conversion, which could be used as a micro power supply for artificial organs in human body, or to deal with astronauts' garbage in space flight. However, it was not until 1964 that Kimble's team developed the first EBFC (Yahiro et al. 1964). They have constructed three different types of batteries, using glucose oxidase, amino acid oxidase, and alcohol dehydrogenase as anode catalysts, respectively. The results showed that the open circuit voltage of the battery with oxidase as catalyst could reach as high as 350 mV, while the battery with dehydrogenase as catalyst could not obtain positive open circuit voltage. Due to the incomplete electron transfer mechanism and poor battery stability, the research in this field has been stagnant from the 1960s to 1970s. Until the end of the twentieth century, Palmore and Whitesides made a breakthrough in the development of EBFC. They combined three kinds of dehydrogenase (methanol dehydrogenase, formaldehyde

Fig. 6.1 The working principle of EBFC



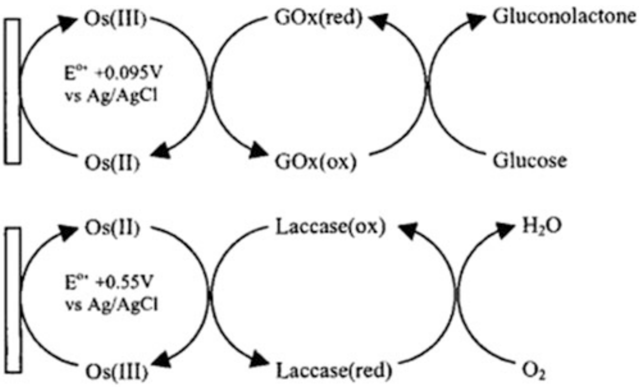


Fig. 6.2 Electron-transferring steps in the electrocatalytic oxidation of glucose (top) and in the electrocatalytic reduction of O_2 (bottom). (Figure adapted from Ref. Chen et al. (2001) with permission)

dehydrogenase, and formate dehydrogenase) to oxidize methanol into carbon dioxide completely (Palmore et al. 1998). Then Adam Heller's team reported the EBFC using glucose oxidase as the enzyme catalyst (Chen et al. 2001) and the electron-transfer steps underlying the electrocatalytic reactions were shown in Fig. 6.2 for the anode and at its bottom for the cathode. They used redox polymers to immobilize glucose oxidase on the anode of the biofuel cell. Compared with the methanol biofuel cell reported by Palmore (the enzyme was dispersed in the solution and the life was only 8 h), the BFC that immobilized the enzyme on the electrode surface could operate for 7–10 days and the battery life was improved significantly. After that, their team applied enzyme to both the anode and cathode for the first time to construct a membrane-free EBFC (Mano and Heller 2003). Since then, the research on EBFC has focused on battery performance, namely power output and stability (Armstrong et al. 1988; Ghindilis et al. 2010; Armstrong 2002).

At present, low power output and poor stability are the main factors restricting the development of EBFC. According to the working principle, the essential factors determining the performance of EBFC are the electron transfer efficiency between the enzyme, the electrode surface and the stability of the enzyme on the electrode surface. Therefore, the selection of substrate electrode materials is very important. In recent years, owing to the unique electrochemical properties of electrocatalytic nanomaterials, they have been widely used to accelerate the electron transfer efficiency between enzyme and electrode surface and promote the stability of enzyme on the electrode surface, thus improving the output performance of EBFC. In order to obtain EBFC with excellent performance, the electrocatalytic nanomaterials used to construct EBFC should have the following properties:

1. The conductivity of electrocatalytic nanomaterials is very important. Good conductivity can ensure that the electrons generated by enzyme catalysis can be transferred rapidly in the whole system, which do favor to the catalytic behavior of enzymes.
2. The biocompatibility of electrocatalytic nanomaterials is a critical factor, which can determine the stability of enzyme. The excellent biocompatibility can provide a good microenvironment for maintaining the activity of enzyme immobilized on the electrode surface, which can efficiently catalyze the reaction between the anode and the cathode.
3. The specific surface area of electrocatalytic nanomaterials directly affects the enzyme loading amount. Therefore, the electrocatalytic nanomaterials with large specific surface area will provide more active sites for the attachment of enzyme, which will do favor to increase the enzyme adhesion on the electrode surface and ultimately improve the performance of EBFC.

6.2.4 MFC

Microbial fuel cell (MFC) is one kind of energy conversion devices based on EBFC. According to the principle of electricity generation, it can be divided into three types:

1. Hydrogen MFC (Oh and Logan 2005; Kadier et al. 2020; Wan et al. 2015).. The device can combine hydrogen production with power generation, and microbes are used to produce hydrogen from organic matter. Meanwhile, hydrogen is oxidized by electrodes dispersed with chemical catalysts to generate electricity.
2. Photoautotrophic MFC (Cao et al. 2009; Kakarla and Min 2014; Mitra and Hill 2012).. Optical energy can be directly converted into electricity by photosynthesis of cyanobacterium or other photosensitive microbes.
3. Heterotrophic MFC (Ren et al. 2007; Rhoads et al. 2005; He et al. 2009; Jin et al. 2019; Liu and Choi 2017).. Anaerobic or facultative microorganisms are used to extract electrons from organic dyes. The electrons can be transferred to electrodes and electricity is generated during the process.

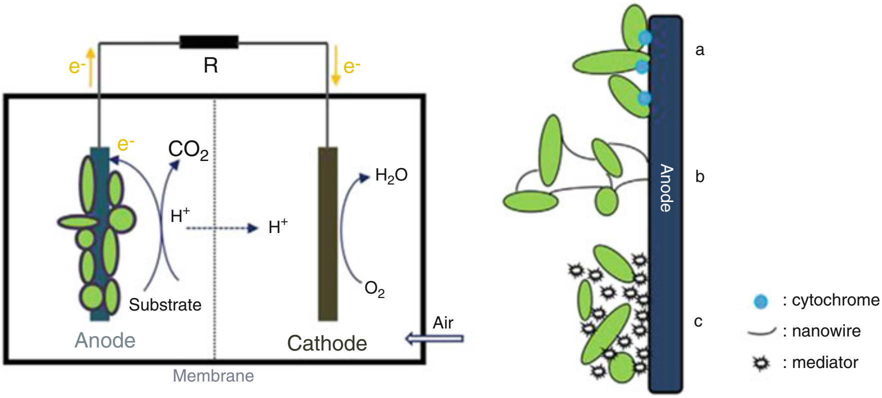


Fig. 6.3 Schematic illustration of MFC and the extracellular electron transfer through (**a**) outer-membrane bound cytochromes, (**b**) conductive nanowires (pili), (**c**) redox mediators. (Figure adapted from Ref. Li et al. (2018) with permission)

In the field of MFC, the heterotrophic type MFC has been the most widely studied, and its working principle is shown in Fig. 6.3 (Li et al. 2018).. In the anode chamber, microbes degrade or oxidize organic matter by respiration, and the generated electrons are transferred by respiratory enzymes in the cell, and provide energy for microbial growth in the form of ATP. And then the electrons are transferred to the anode directly or indirectly through electronic media. Next, through the external circuit containing the load, the electrons finally arrive at the cathode and react with the electrolyte to form a closed circuit. At the same time, the proton produced by the anode diffuses to the cathode through the ion exchange membrane, and reacts with oxygen and electrons at the cathode to generate water. Because oxygen entering into the anode chamber will hinder the generation of electric energy, it is necessary to place an ion exchange membrane between anode chamber and cathode chamber, for the purpose of guaranteeing the oxygen free environment and normal proton transfer in the anode chamber. In the laboratory, the common load is resistance, and the potential difference between the two ends of the resistance is measured by multimeter or potentiostat, so as to obtain the output current of MFC. Taking the MFC of S*hewanella oneidensis* MR-1 as an example, the reactions are as follows.

$$\text{Anodic reaction}: C_3H_5O_3{}^- + 2H_2O \rightarrow CH_3COO^- + HCO_3{}^- + 5H^+ + 4e^-$$

$$\text{Cathode reaction}: O_2 + 4H^+ + 4e^- \rightarrow H_2O$$

MFC is a major breakthrough in biological capacity. Since microbes can obtain energy for self-reproduction while generating electricity, catalyst failure can be avoided in this system. Theoretically, as long as the fuel is injected continuously, the system can work stably for a long time to generate electric energy. In EBFC system, the enzyme catalytic activity in vitro is an important factor limiting its life span. While in MFC, microbes exhibit better tolerance and can keep working under a variety of complex conditions. In addition to produce biological capacity, MFC can also be used for wastewater treatment. Compared with conventional wastewater treatment technology, MFC not only does not need to consume energy, but also generates electricity or hydrogen from wastewater treatment process. In the late 1990s, Kim et al. (1999a) discovered that *Shewanella putrefaciens* could be used in MFC, which could consume the lactic acid in wastewater. Then they used industrial wastewater containing starch to produce electricity, which opened up the application of MFC in wastewater treatment field (Kim et al. 1999b). However, the capacity of MFC in the above study was relatively low. In recent years, Logan and Regan (2006) have developed the scalable technology of MFC in the treatment of domestic sewage, industrial wastewater, and other wastewater, which has inspired the research of MFC and aroused great concern all over the world. Therefore, microbial fuel cells play a very important role in environmental fields, such as wastewater treatment (Min and Logan 2004) (Fig. 6.4), water self-purification, and so on.

Although MFC exhibits a bright application prospect, its practical performance still cannot meet the needs in real applications. In MFC system, anode and cathode play important roles in electron transfer. So the electrode materials can affect the performance of MFC directly. With the rapid development of nanotechnology, more and more electrocatalytic nanomaterials are used to construct MFCs with high performance. Similar to EBFC, the ideal electrocatalytic nanomaterials for the anode of MFC usually need to possess good conductivity, good biocompatibility (conducive to bacterial adhesion), and large specific surface area. Due to the corrosion of the electrode caused by bacteria inner the MFC, this kind of electrocatalytic nanomaterials should also have good stability. Compared with EBFC, the performance of MFC depends on the catalytic properties of cathode materials to a great extent. Therefore, the electrocatalytic nanomaterials used in MFC cathodes generally need to have a low overpotential to oxygen or other electron acceptors, which can reduce the activation energy of the reaction. Besides, the cathode material should also have good conductivity and large specific surface area in order to improve the electron transfer rate and increase the contact area between the electrode and the electron acceptor, which is beneficial for the improvement of MFC power density.

6.3 Electrocatalytic Nanomaterials for EBFC

The major characteristic of EBFC is that enzyme directly participates in energy conversion. The main factors determining its performance are the electron transfer rate between enzyme and electrode and the catalytic activity of enzyme on the electrode surface. So far, researchers have made some progress in the construction of EBFC based on the synthesis and design of electrocatalytic nanomaterials. The nanomaterials used to construct EBFC mainly include carbon materials, metal

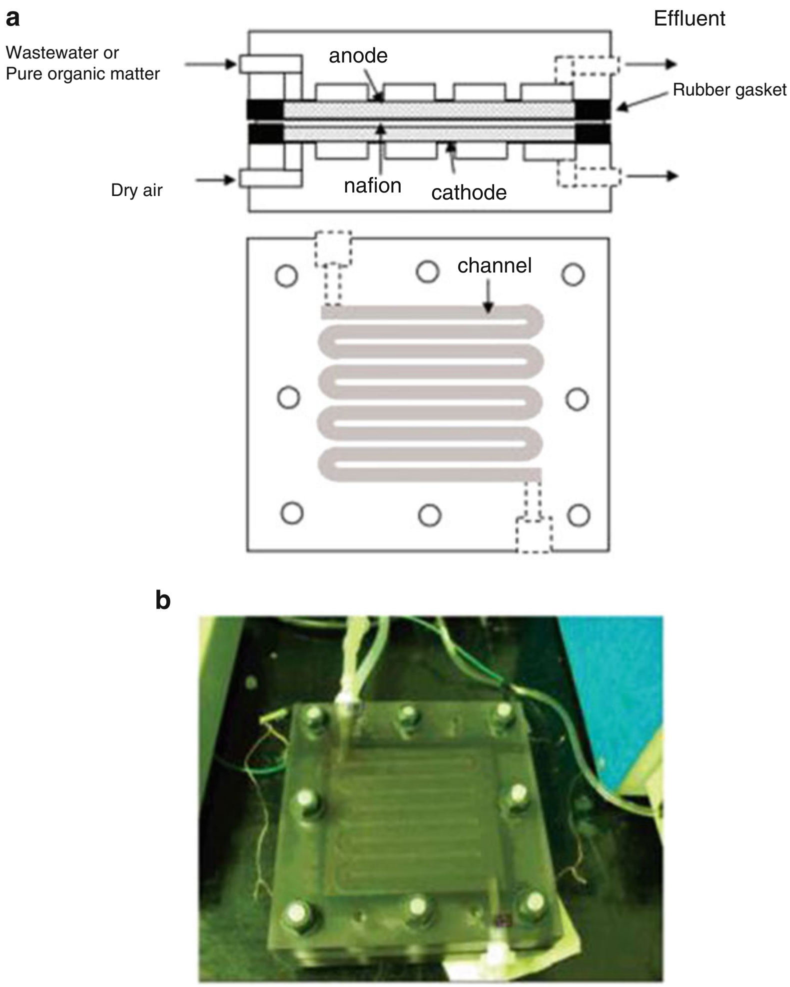


Fig. 6.4 Schematic (**a**) (upper, side view; lower, top view) and laboratory-scale prototype (**b**) of the flat plate microbial fuel cell (FPMFC). (Figure adapted from Ref. Min and Logan (2004) with permission)

nanoparticles, nanostructured conductive polymers, and the composites of the above materials.

6.3.1 Carbon Materials

Carbon materials have become one kind of attractive electrode substrate materials due to their good conductivity, large specific surface area, high chemical and thermal stability, and good biocompatibility (Banks et al. 2006; Xin et al. 2006; Tu et al.

2010; Noll 2011). So far, the carbon materials reported for the construction of enzyme biofuel cells include carbon nanotubes, graphene, carbon nanosheets, carbon nanodots, mesoporous carbon, carbon fibers, and so on.

In 1991, Japanese physicist Sumio Iijima discovered carbon nanotubes (CNTs) from carbon fibers produced by arc-discharge method for the first time (Iijima 1991). Since then, CNTs have aroused great interest of scientists and become the most popular nanomaterials. The main advantages of CNTs are shown in five aspects.

1. Large specific surface area. The specific surface area of CNTs can reach 1000 m^2 g^{-1}, which can increase the enzyme loading amount and enhance the catalytic performance of microbe fuel cell (Yang et al. 2013; Dai 2003).
2. The surface is easy to be functionalized. The highly delocalized π bonds of CNTs are greatly important for the non-covalent bonding of CNTs with some conjugated macromolecules. Besides, the surface of CNTs can be oxidized by strong oxidants, such as strong acid, and carboxyl functional groups can be formed on the surface of CNTs, which makes it easy to immobilize proteins, biological enzymes or coenzymes (Holzinger et al. 2012; Smart et al. 2006; Minteer et al. 2012).
3. Good conductivity. As an electronic pathway between ligase and electrode, CNTs with good electron transfer rate can ensure the effective contact between redox center and the electrode.
4. Diverse nanoscale. The diameter of single-walled carbon nanotubes is about 0.6–2 nm. The inner layer of multiwalled carbon nanotubes can reach 0.4 nm, and the coarsest can reach hundreds of nanometers. Therefore, CNTs with appropriate size can be used to approach the active site of enzyme, in order to realize direct electron transfer between enzyme and electrode (Willner et al. 2006).
5. Unique nanostructure. The unique nanostructure of CNTs makes it easy to form porous structure with other nanomaterials, which is beneficial to the rapid diffusion of reactants and products between electrode surface and solution (Yan et al. 2006; Qiu et al. 2009) (Fig. 6.5).

Based on the good performance of CNTs, Cosnier's team has constructed a new kind of nanomaterial bioelectrodes. They made CNTs and enzymes into a "vacuum" bioelectrode, which increased the effective contact between the enzyme and the electrode, thus facilitating electron transport. The glucose oxidase bioanode and laccase biocathode based on this method could realize direct electron transfer on the electrode surface. The open circuit voltage of the EBFC without electronic medium was 0.95 V and the maximum power output achieved 1.25 mW cm^{-2} (Zebda et al. 2011).

However, Reuillard's group found that there were still some defects in the compression type bioelectrode designed by Cosnier's group. The study showed that there was a weak oxidation peak of glucose at about 0.4 V through polarization scanning of the biological anode. This indicated that the biological anode prepared by the above method could only make a few CNTs closely contact with GOD, and

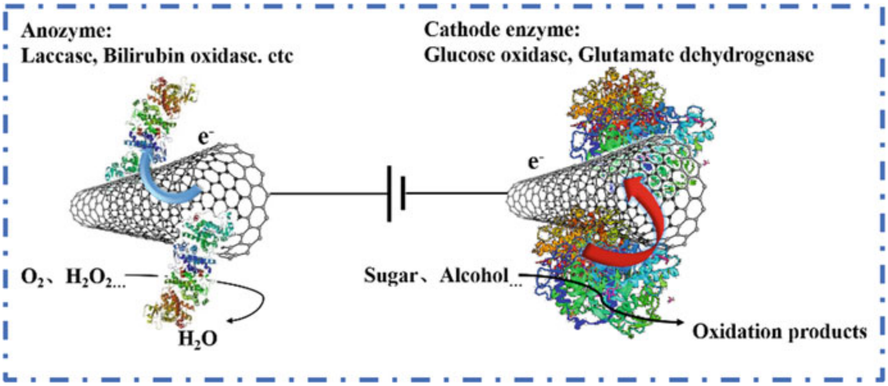


Fig. 6.5 The diagram of electronic transport and electrocatalytic reduction of EBFC based on CNTs

the effective connection between immobilized enzyme and electrode was low. Therefore, Reuillard's group has designed a new type of biological anode with a new structure, which combined direct electron transport and medium electron transport together (Reuillard et al. 2013). They added naphthoquinone, a redox electron mediator, into the GOD/CNTs mixture before making the tablet electrode. After compression, naphthoquinone was fixed in the electrode. Because naphthoquinone was a small organic molecule, it could capture electrons from GOD surface and transferred them to CNTs matrix. The power output density of as-fabricated EBFC increased to 1.54 mW cm^{-2}. This was ascribed to the addition of redox electron mediator to immobilize the electron link between enzyme and electrode surface.

In order to prove that CNTs have better performance in the construction of EBFC, Gao et al. constructed glucose/O_2 EBFC based on CNTs fiber and carbon fiber (CF), respectively (Gao et al. 2010). At the EBFC anode, glucose lose electrons under the catalysis effect of GOD, and the electrons were transferred to redox polymer (I), and then from redox polymer (I) to CNTs fiber. At the EBFC cathode, electron was transferred from CNTs fibers to redox polymer (II), and then electrons were transferred from polymer (II) to bilin oxidase (BOD) to catalyze oxygen reduction. Compared with EBFC based on CF, CNTs fiber electrode has a better performance than EBFC based on CF, which was mainly attributed to the fact that CNTs fiber electrode not only has the advantages of good conductivity, but also could realize the electrochemical connection between enzyme and electrode more effectively.

Owning to the improved free charge carrier density, p-bond electron binding ability, electron donor and acceptor capacity, nitrogen doped carbon materials show better performance than the original materials. In 2012, Wei's group used nitrogen doped CNTs (NCNTs) prepared by chemical vapor deposition (CVD) for the first time to construct glucose/O_2 EBFC (Wei et al. 2012). Under the same conditions, the biological electrode based on NCNTs had better catalytic performance than undoped CNTs. The reason was that NCNTs have better biocompatibility and conductivity

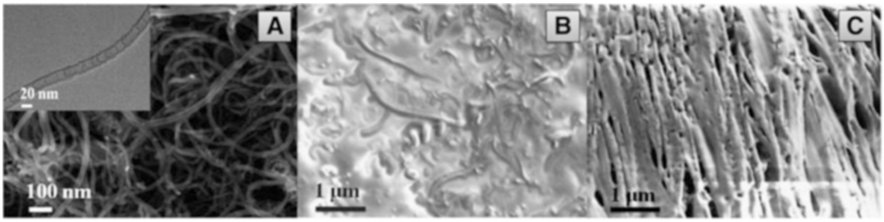


Fig. 6.6 SEM images of (**a**) NCNTs, (**b**) GCS/NCNTs, (**c**) Lac/GCS/NCNTs. Inset in (**a**) was the TEM image of NCNT. (Figure adapted from Ref. Wei et al. (2012) with permission)

than CNTs, and provided more active sites for enzyme immobilization. Thus, the performance of EBFC based on NCNTs has been greatly improved (Fig. 6.6).

Graphene is one kind of two-dimensional crystals composed of single or multi-layer carbon atoms. In 2004, Andre Geim and Konstantin Novoselov, as physicists from University of Manchester, successfully separated graphene from graphite and confirmed that graphene can exist alone, so they won the 2010 Nobel Prize in physics together. Graphene has many unique characteristics, such as monoatomic layer structure (Rao et al. 2009), large specific surface area (2630 m^2 g^{-1}) (Sun et al. 2011), good electrical conductivity (Zhou et al. 2013), high mechanical strength (about 1100 GPa) (Karaskiewicz et al. 2012; Lee et al. 2008), high thermal conductivity (5000 W m^{-1} K^{-1}) (Balandin et al. 2008), easy functionalization (Stankovich et al. 2006) and so on. Graphene has become a new dimension material in biological nanomaterials and plays an important role in the construction of biofuel cells based on nanomaterials. Similar to CNTs, graphene surface can be functionalized by covalent and non-covalent functional groups. It is worth noting that reasonable functionalization does not affect the excellent conductive channels of graphene materials (Malig et al. 2011).

In view of the excellent properties of graphene, the combination of graphene and biocatalysis has been widely studied by researchers. Cai's group fixed GOD directly on the surface of graphene and invested the influence of conductivity and biological activity of enzyme on graphene surface. The research showed that GOD still kept its original molecular structure, and the biological activity would not be affected (Wu et al. 2010). This novel method of constructing enzyme modified nanomaterials and the good response of electrode to fuel established the foundation for the construction of biological anode in EBFC (Fig. 6.7).

Besides, graphene can also be made into a thin film electrode, which is beneficial to the dispersion of biopolymers such as chitosan. Lin's group prepared graphene/chitosan nanocomposite as electrode in order to immobilize GOD, thus GOD/graphene/chitosan electrode could be constructed. The electrochemical performance of the electrode was measured and used to assemble EBFC. The results showed that GOD could be adsorbed on the surface of graphene/chitosan film and realize direct electron transfer (Kang et al. 2009). Li's group has constructed a graphene electrode based membrane-free EBFC, which further proved the excellent electrochemical performance of the graphene based bioelectrode. Compared with the

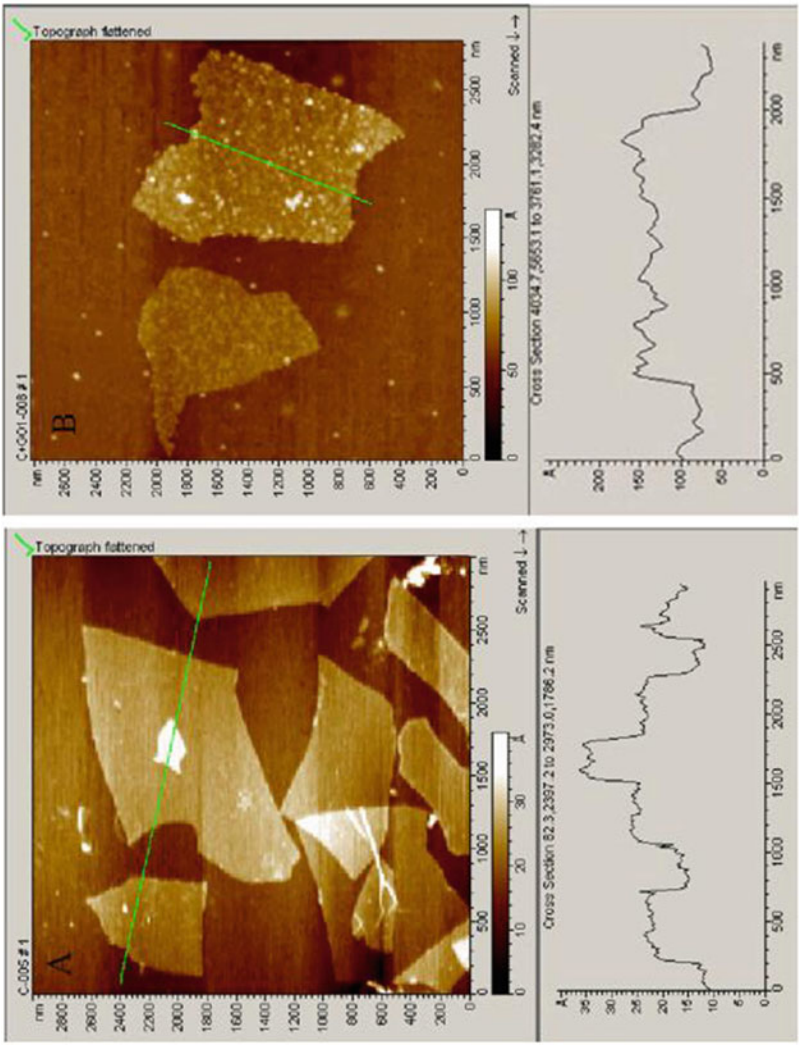


Fig. 6.7 Typical AFM images and the cross-sectional analysis of graphene sheets with (**b**) and without (**a**) assembly of GOx. (Figure adapted from Ref. Wu et al. (2010) with permission)

same redox catalysts, the current density and power output density of EBFC based on multiwalled CNTs increased by two times and three times, respectively. The results exhibited that graphene could be treated as the outstanding electrochemical catalysts for EBFC. Moreover, with the fast development of graphene research, three-dimensional graphene materials have been widely used in energy storage, catalysis, environmental protection, and flexible conductors owing to their unique properties (Niu et al. 2014; Huang et al. 2014; Sun et al. 2013). They have larger specific surface area, connected conductive network and special microenvironment, exhibiting better performance than two-dimensional graphene.

6.3.2 Metal Nanoparticles

Metal nanoparticles are metals and alloys, which form nanocrystallines. Metal nanoparticles, especially gold, silver, and copper nanoparticles, have been widely studied in the recent years due to their special electrical, optical, and catalytic properties. The properties of metal nanoparticles largely depend on their size, shape, distance between particles and the properties of stabilizers. The chemical stability of nanoparticles is very important and many nanoparticles lack sufficient stability, which limits their further practical application. Au nanoparticles play an important role in nanomaterials. This is mainly due to Au nanoparticles are the most stable metal nanoparticles in nanoscale, and its performance superior to silicon semiconductor, which is widely used in nanodevices. Besides, the gold surface has unique chemical properties and can bonded with other nanoparticles.

The Au NPs/glucose dehydrogenase (GDH) composite biological anode was constructed by electropolymerization of mercaptoaniline modified glucose dehydrogenase and Au NPs onto a single-layer mercaptoaniline modified gold electrode. The electrode exhibited good electrocatalytic activity for glucose oxidation (Yehezkeli et al. 2011). The researchers found that the presence of oxygen had little effect on the catalytic activity of glucose. They constructed a glucose/O_2 EBFC without membrane using Au NPs/GDH composite electrode as biological anode and BOD/CNTs as biological cathode. The open circuit voltage of the as-prepared EBFC is 0.5 V and the maximum output power is 32 μW cm^{-2}. Shleev et al. fabricated a new type of membrane-free glucose/O_2 EBFC using Au NPs. The anode enzyme was cellobiose dehydrogenase (CDH) and could not affected by oxygen when catalyzing. It could be used as an anode enzyme for glucose oxidation, and the cathode enzyme was BOD to reduce oxygen. The results showed that the open circuit voltage of glucose/O_2 EBFC was 0.68 V, the maximum power output was 3.3 μW cm^{-2} at 0.52 V, and the output stability of EBFC decreases only 20% after 12 h of continuous operation.

6.3.3 Composite Materials

Composite materials generally mean the combination of two or more different kinds of materials possessing obvious different physical and chemical properties, making full use of their unique characteristics to obtain nanomaterials, which can meet the target requirements. The structure and performance of composite materials generally are superior to single materials, and have multiple properties, such as conductivity, hydrophilicity, biocompatibility, electrocatalytic performance, etc. Therefore, researchers have widely used it to fabricate high-performance EBFC.

As mentioned above, carbon nanotubes can be used as "nanowires" to transfer electrons directly from the catalytic center of enzymes to the electrode surface due to their high conductivity, and electrochemical stability. Graphene, especially three-dimensional graphene, has also achieved excellent performance as an electrode material to construct EBFC. When graphene was compounded with CNTs, on the one hand, the good dispersion of graphene oxide and the excellent conductivity of CNTs could be utilized; on the other hand, CNTs and graphene may form a special structure, thus further improving their electrochemical performance (Woo et al. 2012; Yang et al. 2011). Besides, carbon nanoparticles/CNTs composites were also used to construct EBFC (La Rottaz and Gonzalez 2013). CNTs could act as "electronic wires" to connect more carbon nanoparticles. By optimizing the concentration of carbon nanoparticles and CNTs, the current density of the battery reached the maximum.

At the same time, CNTs/Au NPs composites can be prepared by combining carbon materials with metal nanoparticles. Because of the excellent conductivity of CNTs and the good biocompatibility of Au NPs, CNTs have been used to construct biological anode substrates (Neto et al. 2015). The good diffusion and electron transfer properties of the composites make contribution to the outstanding performance of EBFC. Furthermore, graphene can also be combined with Au NPs to assemble EBFC (Chen et al. 2015).

So far, there are few reports about the construction of EBFC based on ternary composite materials. On the basis of carbon nanotubes/nitrogen doped graphite carbon/gold nanoparticles ternary composite materials, Zhu etc. have constructed a kind of glucose/O_2 EBFC without membrane and electronic medium. The results exhibited that the EBFC based on ternary composites showed better performance than CNTs/Au NPs composite, its power output and stability could be significantly improved (Gai et al. 2015).

6.4 Electrocatalytic Nanomaterials for MFC

In recent years, due to the excellent electrocatalytic activity and physicochemical properties of nanomaterials, they are widely utilized to construct MFC, and its output power and coulomb efficiency have been greatly improved. In view of the different roles of electrocatalytic nanomaterials at the two poles of the fuel cell, the anode electrocatalytic nanomaterials and the cathode electrocatalytic nanomaterials will be introduced briefly.

6.4.1 Electrocatalytic Nanomaterials for MFC Anode

In MFC system, microbial catalyst is attached to the bioanode spontaneously and catalyzes the fuel to generate electrons. Therefore, the performance of bioanode directly restricts the adhesion rate and catalytic efficiency of microbial catalysts. The earliest used MFC anode materials are conductive materials, which are easy to obtain, such as stainless steel wire mesh, graphite rod, carbon cloth, etc. Next, electrocatalytic nanomaterials with large specific surface area were modified on the anode surface to improve the adhesion of bacteria on the electrode surface and the extracellular electron transfer efficiency. At present, in order to further promote the performance of MFC and reduce the cell volume, three-dimensional (3D) electrocatalytic nanomaterials are usually used for MFC anode. The effects of anode materials on MFC mainly include the following aspects: (1) the effective contact distance between the microbes and the anode, the number of microbes that transfer electrons to the anode. Both the two factors can affect the efficiency of electron transfer in MFC; (2) the anode electrode potential affects the redox potential of the intracellular electron of the microbes, thus influencing the metabolic pathway of the microbes; (3) the conductivity of electrode affects the power output of MFC. Therefore, the excellent anode materials need to have good conductivity, large specific surface area, no biological toxicity, corrosion resistance, low price, good biocompatibility, and chemical stability. The anode electrocatalytic nanomaterials mainly include carbon nanomaterials and their composites, nanometals and their oxides, conductive polymers.

Carbon Nanomaterials

The anode of MFC is generally consist of carbon nanomaterials, including carbon paper, carbon cloth, carbon felt and so on. Carbon based materials have many advantages, such as high stability, high conductivity, good biocompatibility, low price and commercial applicability. According to the structure, carbon based materials can be divided into two-dimensional and three-dimensional electrodes. The researchers prepared graphite brush, which was used in MFC to generate electricity (Logan et al. 2007), as shown in Fig. 6.8. In this study, graphite brush was made of graphite fiber by brush making machine, and the center of the brush was made of anti-corrosion titanium wire. Because the diameter of graphite fiber was very small, the specific surface area of graphite brush was as high as 18,200 $m^2\ m^{-3}$ and the power density reached 2400 mW m^{-2}. Although this type of carbon materials had achieved good performance as anodes for MFC, it was found that the resistance and ability to attach microbes still limited the power generation of MFC. Therefore, it is necessary to develop carbon materials with better conductivity and biocompatibility.

As a new type of carbon material, graphene also has good mechanical and electrical properties. The scholars tested the electricity generation effect of graphene/graphite plate electrode prepared by electrolysis method and chemical reduction method. The results showed that the current of graphene/graphite plate electrode prepared by electrolytic stripping was about 40% higher than that of the

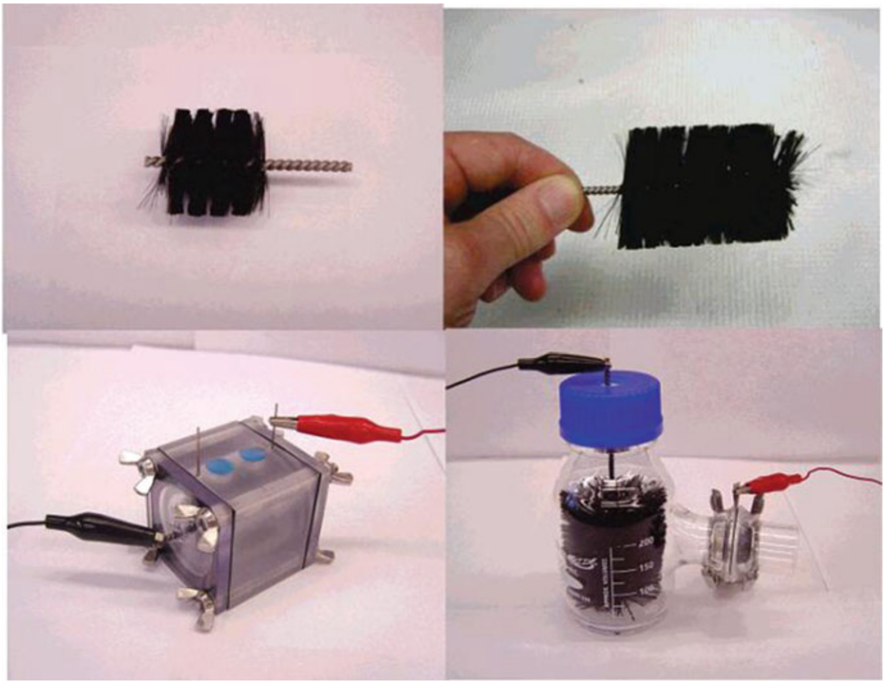


Fig. 6.8 Graphite fiber brush anode electrode used in (**a**) C-MFC and (**b**) B-MFCs, and photographs of the reactors containing the brush electrodes: (**c**) C-MFC shown with the brush anode, and (**d**) B-MFC with brush anode and side port cathode. (Figure adapted from Ref. Logan et al. (2007) with permission)

latter (Tang et al. 2015). Because graphene nanosheets are easy to stack, the real surface area for the attachment of the bacteria will be reduced to a great extent. Thus, in order to alleviate this phenomenon, many researchers use metal or metal oxide, conductive polymer, and carbon nanotubes to composite with graphene. As shown in Fig. 6.8, Graphene oxide/single wall carbon nanotubes hydrogel composite was prepared as MFC anode, *E. coli* bacteria as catalyst and the performance of MFC has been greatly improved. The reason was that CNTs were intercalated into the layers of GO, which reduced the stacking degree and increased the surface area of the materials. Moreover, CNTs in the composite could also promote the conductivity of the composites (Kumar et al. 2014).

Although carbon based materials have many advantages, such as good stability, high conductivity, and good biocompatibility, on the one hand, due to the high surface energy state of carbon based materials, it is easy for carbon materials to lose electrons, which makes the anode activation overpotential high and consumes more energy during the electron transfer process of electricity producing microbes; on the other hand, their inherent hydrophobicity property do harm to the adhesion of microbes, leading to the low efficiency of electron transfer. Besides, the traditional carbon based materials hardly have electrocatalytic activity. Therefore, using appropriate methods and materials to modify the surface of carbon based materials can effectively change the physical and chemical characteristics of the materials, which are beneficial to the adhesion of electricity producing microbes on the anode surface and reduce the anode activation overpotential, thus promoting the electron transfer rate between the microbe and the anode.

Metal Nanomaterials

As one kind of high conductivity materials, metals are easy to be corroded when used as MFC anode materials. So far, only stainless steel wire mesh and titanium nanosheets are widely used in MFC anode. The performance of MFC constructed by metal alone is generally not high, which may ascribe to the smooth surface of metal, because the smooth surface suppresses the growth of microorganisms. Noble metals with good biocompatibility can be used as anodes to alleviate these deficiencies. It was found that *Geobacter sulfurreducens* could grow on the surface of gold electrode, and the generated current was equivalent to that of graphite electrode under the same conditions (as shown in Fig. 6.9) (Richter et al. 2008). Previous studies have shown that metal combined with other materials can not only make full use of the

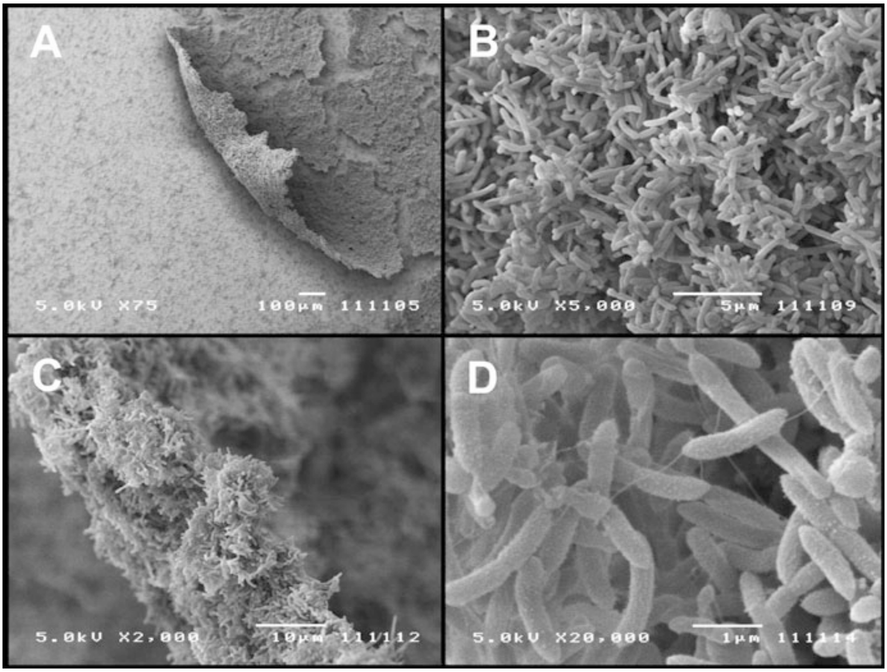


Fig. 6.9 SEM images of *G. sulfurreducens* growing on a gold electrode. (**a**) Biofilm attached to the surface, partially peeling off. (**b**) Closeup of Fig. 6.3a where the biofilm was attached to the electrode surface. (**c**, **d**) Closeups of Fig. 6.3a: the edge of the biofilm. (Figure adapted from Ref. Richter et al. (2008) with permission)

good conductivity of metal, but also improve the defects caused by the smooth metal surface (Sun et al. 2010).

Nanoscale metal oxides are widely used in the field of MFC anode, and Fe_3O_4 are the most common metal oxide. Since the most electrogenic bacteria commonly used in MFC reaction system were metal reducing bacteria, the number of electrogenic bacteria on the electrode surface could be increased through the interaction between Fe_3O_4 and the electrogenic bacteria. One of the major drawbacks of metal oxide used in MFC anode is its poor conductivity, which increases the internal resistance of MFC and reduces the battery performance. Therefore, in order to solve this problem, researchers utilize metal oxide nanomaterials to fabricate MFC anode (Mehdinia et al. 2014a, b; Park et al. 2014).

Conductive Polymers

The good conductivity and environmental durability of conductive polymers have made them widely used as the anode doping materials in MFC. They are mainly used to modify the anode and improve the adhesion ability of bacteria. So far, the conductive polymers used in MFC anode mainly include polyaniline (PANI), polypyrrole (PPy), polypropylene cyanogen (PAN). and poly (3,4-ethylenedioxythiophene) (PEDOT). PANI is the most widely used polymer owing to its low price, facile to synthesize and good biocompatibility. Qiao et al. studied the carbon nanotube/polyaniline (CNT/PANI) composite material as anode material of high power MFC as shown in Fig. 6.10. The results exhibited that 20 wt.% CNT composite anode possessed the highest electrochemical activity, and its maximum power density reached 42 mW cm^2 (Qiao et al. 2007).

In MFC system, the pH value of bacteria liquid is usually about 7. However, PANI has poor conductivity under neutral conditions. So some other conductive polymers with more stable conductivity are gradually used to construct MFC anodes. Song's group used PPy to increase the conductivity of artificial biofilm anode (Zhao et al. 2013). Chen et al. synthesized layered PAN fiber through electrospinning technology (Chen et al. 2011). The current generated by these electrodes was about

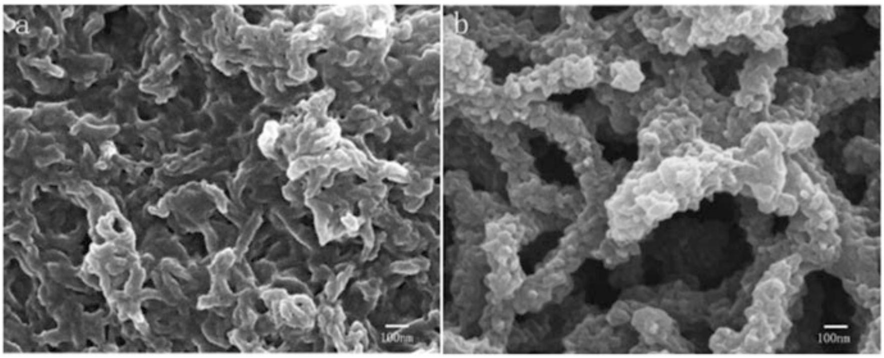


Fig. 6.10 SEM images of PANI and CNT/PANI composite films (**a**: plain PANI; **b**: 20 wt.% CNT/PANI composite). (Figure adapted from Ref. Qiao et al. (2007) with permission)

ten times larger than that of the control electrodes, which was mainly due to the increase of the specific surface area caused by the porosity of the materials. Besides, the conjugation of aromatic rings in polymer framework with riboflavin (the electron mediator secreted by the electric bacteria) improves the electron transfer rate.

6.4.2 Electrocatalytic Nanomaterials for MFC Cathode

As an important part of MFC, air cathode is one of the main factors affecting the performance of MFC. The oxygen reduction reaction (ORR) rate of cathode directly affects the power output of MFC. Oxygen is the most suitable electron acceptor for MFC because of its high oxidation potential, high practicability, low price, and no chemical pollution effluent (the only final product is water). The cathode ORR can be classified in two ways: $4e^-$ and $2e^-$ paths. The final product of $4e^-$ reaction path is water, and the relative potential of reduction reaction is 1.23 V, while the product of $2e^-$ reaction path is hydrogen peroxide, and the reaction potential is only 0.7 V. Therefore, the $4e^-$ reaction path can not only provide high cathode potential, but also can produce high voltage output, and the product is water, which can avoid the corrosion of electrode materials caused by hydrogen peroxide. In the actual cathodic ORR reaction, both $4e^-$ and $2e^-$ pathways affect the cathodic reaction, and efficient cathode catalysts can catalyze the reduction of oxygen towards $4e^-$ pathway. Therefore, the efficient cathode catalyst can not only reduce the reaction energy barrier and increase the reaction rate, but also promote the cathodic reaction to a higher potential. Thus, more and more electrocatalytic nanomaterials have been applied as cathode catalysts to improve the performance of MFC.

Noble Metal-Based Materials

Platinum (Pt) is widely used as catalyst in MFC air cathode to reduce ORR overpotential due to its excellent catalytic activity. It can reduce the activation energy of ORR, increase the reaction rate, and reduce the diffusion of oxygen to anode. Logan and co-workers (2004) used Pt coated carbon electrode as the cathode of double chamber MFC. After 120 h of operation, the maximum power obtained reached 0.097 mW. When Pt was removed from the electrode surface, its output power decreased by 78%. Although Pt exhibits excellent catalytic activity, its high price still makes the preparation cost of air cathode very high. Moreover, precious metals such as Pt are very sensitive to toxic ions in wastewater in practical application, resulting in rapid performance degradation of MFC.

Non-noble Metal-Based Materials

The metal oxide catalysts commonly used in MFC air cathode are manganese oxide, iron oxide, nickel oxide, cobalt oxide, and copper oxide. Manganese oxide is the most widely used metal oxide in the research of MFC air cathode. The oxygen reduction reactions catalyzed by manganese oxide are as follows:

$$Mn^{IV}O_2 + H_2O + e^- \rightarrow Mn^{III}OOH + OH^-;$$

$$2Mn^{III}OOH + O_2 \rightarrow Mn^{IV}OOH \cdot O^-;$$

$$Mn^{IV}OOH \cdot O^- + e^- \rightarrow Mn^{IV}O_2 + OH^-.$$

Mn^{III} is the catalytic intermediate of reducing oxygen, and its concentration determines the electrocatalytic ORR activity of MnO_2. It has been found that the crystal style of MnO_2 can affect its ORR catalytic activity. β-MnO_2 exhibits higher catalytic activity than α-MnO_2 and γ-MnO_2 because of its large specific surface area. As the cathode material, the maximum power density of MFC was much higher than that of bare electrode.

In addition to metal oxides, metal macrocyclic compounds are also widely used as air cathode catalysts for MFC, such as phthalocyanine metal complexes, porphyrin metal complexes, naphthalene cyanine metal complexes, and amino antipyrine metal compounds. However, under the acidic condition ($pH < 3$), the stability of the metal organic macrocyclic complexes is very poor, and the demetallization phenomenon will occur. For example, iron ions in the center of iron phthalocyanine (FePc) can be replaced by two hydrogen atoms and H_2Pc formed under acidic conditions.

Carbon nanomaterials have unique electrochemical and mechanical properties, so they have great potential applications in catalysis, supercapacitors, sensors, and hydrogen storage. Graphene, carbon nanotubes, carbon nanofibers, and other nanomaterials are widely used as air cathode catalysts for MFC due to their good conductivity, high specific surface area and easy to be doped (such as N, P). The researchers prepared carbon nanofibers by pyrolysis and electrospinning using polyacrylonitrile (PAN) as precursor (Ghasemi et al. 2011). After being chemically activated in KOH (8 mol L^{-1}) solution, the power density of MFC cathode was 2.65 times of that of Pt cathode, which was the highest ORR catalytic activity material in MFC field. In addition, CNTs/G composites have a higher initial reduction potential (0.89 V) for oxygen reduction catalysis. The Tafel slope indicates that the ORR catalyzed in four electron reduction pathway. Conductive polymer composites can be used as cathode materials for MFC, including PANI, PPy, and PTh. The catalytic mechanism of conducting polymer for ORR is to weaken the O–O molecular bond adsorbed on the surface of the conducting polymer, which makes it easier to break and participate in the reaction. The ORR catalytic activities of PANI, PPy, PTh, poly (3-methylthiophene), and PEDOT electrodes were measured. The results showed that PANI and PPy had higher catalytic activities than the other three conducting polymers (Khomenko et al. 2005).

6.5 Summary and Prospect

Microbes are ubiquitous in nature. Especially, owing to the energy crisis and environmental pollution becoming serious, the demand for green and new energy is more and more urgent. For microbial battery, it is greatly significant to improve its output power density and electric generation efficiency. The electrocatalytic nanomaterials applied in BFC field can greatly promote its productivity, extend the battery life, and reduce the electrode cost. At present, a series of electrocatalytic nanomaterials with excellent performance have been developed, such as nanowires and nanotubes, they can shorten the distance between the electrode surface and the active center of biocatalyst, thus improve the electron transport efficiency. Meanwhile, the good biocompatibility of electrocatalytic nanomaterials also increases the life of BFC from several hours to several months. Besides, the development and utilization of three-dimensional macroporous nanomaterials with large specific surface area and ordered array structure have provided more binding sites for biocatalysts in BFC and greatly improved the space utilization of electrodes. Finally, the cathode of BFC avoid using expensive platinum as catalyst, so the cost of the battery can be greatly reduced.

The development of BFC is no longer limited to energy storage and power generation devices, and its applications in new fields have become an important direction in the future. For example, BFC can be applied for water purification, waste degradation, self-powered sensors (Khomenko et al. 2005; Deng et al. 2010; Liu et al. 2012; Wen et al. 2011; Li et al. 2015; Wang et al. 2014), and EBFC based self-powered cell sensors for the detection of tumor cells (Gai et al. 2016).

Although there are still many challenges in applications of microbes in electric generation, such as low electric generation efficiency, poor stability, and so on, the development of biofuel cells in the future is promising and bright. Especially, with the rapid development of nanotechnologies and nanomaterials in recent years, they can provide profound and efficient technical support for biofuel cells.

References

Armstrong FA (2002) Insights from protein film voltammetry into mechanisms of complex biological electron-transfer reactions. J Chem Soc Dalton Trans 5:661–671

Armstrong FA, Hill HAO, Walton NJ (1988) Direct electrochemistry of redox proteins. Acc Chem Res 21(11):407–413

Babadi AA, Wan-Mohtar WAI, Chang JS, Ilham Z, Jamaludin AA, Zamiri G, Akbarzadeh O, Basirun WJ (2019) High-performance enzymatic biofuel cell based on three-dimensional graphene. Int J Hydrog Energy 44(57):30367–30374

Balandin AA, Ghosh S, Bao WZ, Calizo I, Teweldebrhan D, Miao F, Lau CN (2008) Superior thermal conductivity of single-layer graphene. Nano Lett 8(3):902–907

Banks CE, Crossley A, Salter C, Wilkins SJ, Compton RG (2006) Carbon nanotubes contain metal impurities which are responsible for the “electrocatalysis” seen at some nanotube-modified electrodes. Angew Chem Int Ed Engl 45(16):2533–2537

Canstein HV, Ogawa J, Shimizu S, Lloyd JR (2008) Secretion of flavins by Shewanella species and their role in extracellular electron transfer. Appl Environ Microbiol 74(3):615–623

Cao XX, Huang X, Liang P, Boon N, Fan MZ, Zhang L, Zhang XY (2009) A completely anoxic microbial fuel cell using a photo-biocathode for cathodic carbon dioxide reduction. Energy Environ Sci 2(5):498–501

Chakraborty I, Sathe SM, Dubey BK, Ghangrekar MM (2020) Waste-derived biochar: applications and future perspective in microbial fuel cells. Bioresour Technol 312

Chen T, Barton SC, Binyamin G, Gao ZQ, Zhang YC, Kim HH, Heller A (2001) A miniature biofuel cell. J Am Chem Soc 123(35):8630–8631

Chen SL, Hou HQ, Harnisch F, Patil SA, Carmona-Martinez AA, Agarwal S, Zhang YY, Sinha-Ray S, Yarin AL, Greiner A, Schroder U (2011) Electrospun and solution blown three-dimensional carbon fiber nonwovens for application as electrodes in microbial fuel cells. Energy Environ Sci 4(4):1417–1421

Chen Y, Gai PP, Zhang JR, Zhu JJ (2015) Design of an enzymatic biofuel cell with large power output. J Mater Chem A 3(21):11511–11516

Covington ED, Gelbmann CB, Kotloski NJ, Gralnick JA (2010) An essential role for UshA in processing of extracellular flavin electron shuttles by Shewanella oneidensis. Mol Microbiol 78 (2):519–532

Dai H (2003) Carbon nanotubes: synthesis, integration, and properties. ChemInform 34 (8):1035–1044

Davis F, Higson SPJ (2007) Biofuel cells – recent advances and applications. Biosens Bioelectron 22(7):1224–1235

Deng L, Chen CG, Zhou M, Guo SJ, Wang EK, Dong SJ (2010) Integrated self-powered microchip biosensor for endogenous biological cyanide. Anal Chem 82(10):4283–4287

Du ZW, Li HR, Gu TY (2007) A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Adv 25(5):464–482

Gai PP, Song RB, Zhu C, Ji YS, Chen Y, Zhang JR, Zhu JJ (2015) A ternary hybrid of carbon nanotubes/graphitic carbon nitride nanosheets/gold nanoparticles used as robust substrate electrodes in enzyme biofuel cells. Chem Commun 51(79):14735–14738

Gai PP, Ji YS, Wang WJ, Song RB, Zhu C, Chen Y, Zhang JR, Zhu JJ (2016) Ultrasensitive self-powered cytosensor. Nano Energy 19:541–549

Gao F, Viry L, Maugey M, Poulin P, Mano N (2010) Engineering hybrid nanotube wires for high-power biofuel cells. Nat Commun 1:7

Ghasemi M, Shahgaldi S, Ismail M, Kim BH, Yaakob Z, Daud WRW (2011) Activated carbon nanofibers as an alternative cathode catalyst to platinum in a two-chamber microbial fuel cell. Int J Hydrog Energy 36(21):13746–13752

Ghindilis AL, Atanasov P, Wilkins E (2010) Enzyme-catalyzed direct electron transfer: fundamentals and analytical applications. Electroanalysis 9(9):661–674

Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealson KH, Fredrickson JK (2009) Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms (vol 103, pg 11358, 2006). Proc Natl Acad Sci U S A 106(23):9535

Habermann W, Pommer EH (1991) Biological fuel cells with sulphide storage capacity. Appl Microbiol Biotechnol 35(1):128–133

He Z, Kan J, Mansfeld F, Angenent LT, Nealson KH (2009) Self-sustained phototrophic microbial fuel cells based on the synergistic cooperation between photosynthetic microorganisms and heterotrophic bacteria. Environ Sci Technol 43(5):1648–1654

Holzinger M, Le Goff A, Cosnier S (2012) Carbon nanotube/enzyme biofuel cells. Electrochim Acta 82:179–190

Huang YS, Wu DQ, Wang JZ, Han S, Lv L, Zhang F, Feng XL (2014) Amphiphilic polymer promoted assembly of macroporous graphene/SnO_2 frameworks with tunable porosity for high-performance Lithium storage. Small 10(11):2226–2232

Iijima S (1991) Helical microtubes of graphite carbon. Nature 354(6348):56–58

Jeon WY, Lee JH, Dasbnyam K, Choi YB, Kim TH, Lee HH, Kim HW, Kim HH (2019) Performance of a glucose-reactive enzyme-based biofuel cell system for biomedical applications. Sci Rep 9

Jiang Y, Yang XF, Liang P, Liu PP, Huang X (2018) Microbial fuel cell sensors for water quality early warning systems: fundamentals, signal resolution, optimization and future challenges. Renew Sustain Energy Rev 81:292–305

Jin XJ, Guo F, Ma WQ, Liu Y, Liu H (2019) Heterotrophic anodic denitrification improves carbon removal and electricity recovery efficiency in microbial fuel cells. Chem Eng J 370:527–535
Kadier A, Jain P, Lai B, Kalil MS, Kondaveeti S, Alabbosh KFS, Abu-Reesh IM, Mohanakrishna G (2020) Biorefinery perspectives of microbial electrolysis cells (MECs) for hydrogen and valuable chemicals production through wastewater treatment. Biofuel Res J 7(1):1128–1142
Kakarla R, Min B (2014) Photoautotrophic microalgae Scenedesmus obliquus attached on a cathode as oxygen producers for microbial fuel cell (MFC) operation. Int J Hydrog Energy 39 (19):10275–10283
Kang XH, Wang J, Wu H, Aksay IA, Liu J, Lin YH (2009) Glucose oxidase-graphene-chitosan modified electrode for direct electrochemistry and glucose sensing. Biosens Bioelectron 25 (4):901–905
Karaskiewicz M, Nazaruk E, Zelechowska K, Biernat JF, Rogalski J, Bilewicz R (2012) Fully enzymatic mediatorless fuel cell with efficient naphthylated carbon nanotube-laccase composite cathodes. Electrochem Commun 20:124–127
Khajeh RT, Aber S, Nofouzi K, Ebrahimi S (2020) Treatment of mixed dairy and dye wastewater in anode of microbial fuel cell with simultaneous electricity generation. Environ Sci Pollut Res 27 (35):43711–43723
Khomenko VG, Barsukov VZ, Katashinskii AS (2005) The catalytic activity of conducting polymers toward oxygen reduction. Electrochim Acta 50(7–8):1675–1683
Kim BH, Park DH, Shin PK, Chang IS, Kim HJ (1999a) Mediator-less biofuel cell. US Patent
Kim HJ, Hyun MS, Chang IS, Kim BH (1999b) A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, Shewanella putrefaciens. J Microbiol Biotechnol 9(3):365–367
Klüpfel L, Piepenbrock A, Kappler A, Sander M (2014) Humic substances as fully regenerable electron acceptors in recurrently anoxic environments. Nat Geosci 7(3):195–200
Kumar GG, Hashmi S, Karthikeyan C, GhavamiNejad A, Vatankhah-Varnoosfaderani M, Stadler FJ (2014) Graphene oxide/carbon nanotube composite hydrogels-versatile materials for microbial fuel cell applications. Macromol Rapid Commun 35(21):1861–1865
La Rottaz CE, Gonzalez ER (2013) Synthesis and characterization of chemical modified carbon-chitosan composites applied to glucose oxidase fuel cells. J Electrochem Soc 160(1):G37–G45
Lee C, Wei XD, Kysar JW, Hone J (2008) Measurement of the elastic properties and intrinsic strength of monolayer graphene. Science 321(5887):385–388
Li SA, Wang YH, Ge SG, Yu JH, Yan M (2015) Self-powered competitive immunosensor driven by biofuel cell based on hollow-channel paper analytical devices. Biosens Bioelectron 71:18–24
Li M, Zhou MH, Tian XY, Tan CL, McDaniel CT, Hassett DJ, Gu TY (2018) Microbial fuel cell (MFC) power performance improvement through enhanced microbial electrogenicity. Biotechnol Adv 36(4):1316–1327
Liu L, Choi S (2017) Self-sustaining, solar-driven bioelectricity generation in micro-sized microbial fuel cell using co-culture of heterotrophic and photosynthetic bacteria. J Power Sources 348:138–144
Liu ZH, Cho B, Ouyang TM, Feldman B (2012) Miniature amperometric self-powered continuous glucose sensor with linear response. Anal Chem 84(7):3403–3409
Logan BE, Regan JM (2006) Microbial fuel cells--challenges and applications. Environ Sci Technol 40(17):5172–5180
Logan B, Cheng S, Watson V, Estadt G (2007) Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environ Sci Technol 41(9):3341–3346
Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP, Woodward JC (1996) Humic substances as electron acceptors for microbial respiration. Nature 382(6590):445–448
Malig J, Englert JM, Hirsch A, Guldi DM (2011) Wet chemistry of graphene. Electrochem Soc Interface 20(1):53–56
Mano N, de Poulpiquet A (2018) O-2 reduction in enzymatic biofuel cells. Chem Rev 118 (5):2392–2468
Mano N, Heller A (2003) A miniature membraneless biofuel cell operating at 0.36 V under physiological conditions. J Electrochem Soc 150(8):A1136–A1138

Mehdinia A, Ziaei E, Jabbari A (2014a) Facile microwave-assisted synthesized reduced graphene oxide/tin oxide nanocomposite and using as anode material of microbial fuel cell to improve power generation. Int J Hydrog Energy 39(20):10724–10730

Mehdinia A, Ziaei E, Jabbari A (2014b) Multi-walled carbon nanotube/SnO_2 nanocomposite: a novel anode material for microbial fuel cells. Electrochim Acta 130:512–518

Min B, Logan BE (2004) Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environ Sci Technol 38(21):5809–5814

Minteer SD, Atanassov P, Luckarift HR, Johnson GR (2012) New materials for biological fuel cells. Mater Today 15(4):166–173

Mitra P, Hill GA (2012) Continuous microbial fuel cell using a photoautotrophic cathode and a fermentative anode. Can J Chem Eng 90(4):1006–1010

Neto SA, Hickey DP, Milton RD, De Andrade AR, Minteer SD (2015) High current density PQQ-dependent alcohol and aldehyde dehydrogenase bioanodes. Biosens Bioelectron 72:247–254

Niu ZQ, Liu LL, Zhang L, Shao Q, Zhou WY, Chen XD, Xie SS (2014) A universal strategy to prepare functional porous graphene hybrid architectures. Adv Mater 26(22):3681–3687

Noll TG (2011) Strategies for “wiring” redox-active proteins to electrodes and applications in biosensors, biofuel cells, and nanotechnology. Chem Soc Rev 40(7):3564–3576

Oh SE, Logan BE (2005) Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. Water Res 39(19):4673–4682

Oh S, Min B, Logan BE (2004) Cathode performance as a factor in electricity generation in microbial fuel cells. Environ Sci Technol 38(18):4900–4904

Palmore G, Bertschy H, Bergens S, Whitesides G (1998) A methanol/dioxygen biofuel cell that uses NAD+-dependent dehydrogenases as catalysts: application of an electro-enzymatic method to regenerate nicotinamide adenine dinucleotide at low overpotentials. J Electroanal Chem 443 (1):155–161

Park IH, Christy M, Kim P, Nahm KS (2014) Enhanced electrical contact of microbes using Fe_3O_4/CNT nanocomposite anode in mediator-less microbial fuel cell. Biosens Bioelectron 58 (1):75–80

Potter CM (1911) Electrical effects accompanying the decomposition of organic compounds. Proc R Soc Lond 84(571):260–276

Qiao Y, Li CM, Bao SJ, Bao QL (2007) Carbon nanotube/polyaniline composite as anode material for microbial fuel cells. J Power Sources 170(1):79–84

Qiu JD, Zhou WM, Guo J, Wang R, Liang RP (2009) Amperometric sensor based on ferrocene-modified multiwalled carbon nanotube nanocomposites as electron mediator for the determination of glucose. Anal Biochem 385(2):264–269

Raad NK, Farrokhi F, Mousavi SA, Darvishi P, Mahmoudi A (2020) Simultaneous power generation and sewage sludge stabilization using an air cathode-MFCs. Biomass Bioenergy 140

Rabaey K, Verstraete W (2005) Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol 23(6):291–298

Rabaey K, Boon N, Hofte M, Verstraete W (2005) Microbial phenazine production enhances electron transfer in biofuel cells. Environ Sci Technol 39(9):3401–3408

Rao CNR, Sood AK, Subrahmanyam KS, Govindaraj A (2009) Graphene: the new two-dimensional nanomaterial. Angew Chem Int Ed Engl 48(42):7752–7777

Reguera G, Mccarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. Nature 435(7045):1098–1101

Ren Z, Ward TE, Regan JM (2007) Electricity production from cellulose in a microbial fuel cell using a defined binary culture. Environ Sci Technol 41(13):4781

Reuillard B, Le Goff A, Agnes C, Holzinger M, Zebda A, Gondran C, Elouarzaki K, Cosnier S (2013) High power enzymatic biofuel cell based on naphthoquinone-mediated oxidation of glucose by glucose oxidase in a carbon nanotube 3D matrix. Phys Chem Chem Phys 15 (14):4892–4896

Rhoads A, Beyenal H, Lewandowski Z (2005) Microbial fuel cell using anaerobic respiration as an anodic reaction and biomineralized manganese as a cathodic reactant. Environ Sci Technol 39 (12):4666–4671

Richter H, McCarthy K, Nevin KP, Johnson JP, Rotello VM, Lovley DR (2008) Electricity generation by Geobacter sulfurreducens attached to gold electrodes. Langmuir 24 (8):4376–4379

Roden EE, Kappler A, Bauer I, Jiang J, Paul A, Stoesser R, Konishi H, Xu H (2010) Extracellular electron transfer through microbial reduction of solid-phase humic substances. Nat Geosci 3 (6):417–421

Sambavi SM, Vishali S, Varjani S, Mullai P (2020) Electricity generation in a microbial fuel cell using iron oxide nanoparticles. Indian J Exp Biol 58(8):571–577

Santoro C, Arbizzani C, Erable B, Ieropoulos I (2017) Microbial fuel cells: from fundamentals to applications. A review. J Power Sources 356:225–244

Smart SK, Cassady AI, Lu GQ, Martin DJ (2006) The biocompatibility of carbon nanotubes. Carbon 44(6):1034–1047

Stankovich S, Dikin DA, Dommett GHB, Kohlhaas KM, Zimney EJ, Stach EA, Piner RD, Nguyen ST, Ruoff RS (2006) Graphene-based composite materials. Nature 442(7100):282–286

Sun M, Zhang F, Tong ZH, Sheng GP, Chen YZ, Zhao Y, Chen YP, Zhou SY, Liu G, Tian YC, Yu HQ (2010) A gold-sputtered carbon paper as an anode for improved electricity generation from a microbial fuel cell inoculated with Shewanella oneidensis MR-1. Biosens Bioelectron 26 (2):338–343

Sun YQ, Wu QO, Shi GQ (2011) Graphene based new energy materials. Energy Environ Sci 4 (4):1113–1132

Sun HY, Xu Z, Gao C (2013) Multifunctional, ultra-flyweight, synergistically assembled carbon aerogels. Adv Mater 25(18):2554–2560

Tang JH, Chen SS, Yuan Y, Cai XX, Zhou SG (2015) In situ formation of graphene layers on graphite surfaces for efficient anodes of microbial fuel cells. Biosens Bioelectron 71:387–395

Tu X, Luo X, Luo S, Yan L, Zhang F, Xie Q (2010) Novel carboxylation treatment and characterization of multiwalled carbon nanotubes for simultaneous sensitive determination of adenine and guanine in DNA. Microchim Acta 169(1–2):33–40

Wan LL, Li XJ, Zang GL, Wang X, Zhang YY, Zhou QX (2015) A solar assisted microbial electrolysis cell for hydrogen production driven by a microbial fuel cell. RSC Adv 5 (100):82276–82281

Wang Y, Kern SE, Newman DK (2009) Endogenous phenazine antibiotics promote anaerobic survival of Pseudomonas aeruginosa via extracellular Electron transfer. J Bacteriol 192 (1):365–369

Wang YH, Ge L, Wang PP, Yan M, Yu JH, Ge SG (2014) A three-dimensional origami-based immuno-biofuel cell for self-powered, low-cost, and sensitive point-of-care testing. Chem Commun 50(16):1947–1949

Wei W, Li PP, Li Y, Cao XD, Liu SQ (2012) Nitrogen-doped carbon nanotubes enhanced laccase enzymatic reactivity towards oxygen reduction and its application in biofuel cell. Electrochem Commun 22:181–184

Wen D, Deng L, Guo S, Dong S (2011) Self-powered sensor for trace Hg(2+) detection. Anal Chem 83(10):3968–3972

Willner B, Katz E, Willner I (2006) Electrical contacting of redox proteins by nanotechnological means. Curr Opin Biotechnol 17(6):589–596

Woo S, Kim YR, Chung TD, Piao Y, Kim H (2012) Synthesis of a graphene-carbon nanotube composite and its electrochemical sensing of hydrogen peroxide. Electrochim Acta 59:509–514

Wu P, Shao QA, Hu YJ, Jin JA, Yin YJ, Zhang H, Cai CX (2010) Direct electrochemistry of glucose oxidase assembled on graphene and application to glucose detection. Electrochim Acta 55(28):8606–8614

Xin Y, Munge B, Patel V (2006) Carbon nanotube amplification strategies for highly sensitive immunodetection of cancer biomarkers. J Am Chem Soc 128(34):11199–11205

Xu F, Cao FQ, Kong Q, Zhou LL, Yuan Q, Zhu YJ, Wang Q, Du YD, Wang ZD (2018) Electricity production and evolution of microbial community in the constructed wetland-microbial fuel cell. Chem Eng J 339:479–486

Yahiro AT, Lee SM, Kimble DO (1964) Bioelectrochemistry. I. Enzyme utilizing bio-fuel cell studies. Biochim Biophys Acta 88(2):375–383

Yan Y, Zheng W, Su L, Mao L (2006) Carbon-nanotube-based glucose/O_2 biofuel cells. Adv Mater 18(19):2639–2643

Yang SY, Chang KH, Tien HW, Lee YF, Li SM, Wang YS, Wang JY, Ma CCM, Hu CC (2011) Design and tailoring of a hierarchical graphene-carbon nanotube architecture for supercapacitors. J Mater Chem 21(7):2374–2380

Yang X, Feng B, He XL, Li FP, Ding YL, Fei JJ (2013) Carbon nanomaterial based electrochemical sensors for biogenic amines. Microchim Acta 180(11–12):935–956

Yehezkeli O, Tel-Vered R, Reichlin S, Willner I (2011) Nano-engineered Flavin-dependent glucose dehydrogenase/gold nanoparticle-modified electrodes for glucose sensing and biofuel cell applications. ACS Nano 5(3):2385–2391

Zebda A, Gondran C, Le Goff A, Holzinger M, Cinquin P, Cosnier S (2011) Mediatorless high-power glucose biofuel cells based on compressed carbon nanotube-enzyme electrodes. Nat Commun 2:6

Zhao CE, Wang Y, Shi FJ, Zhang JR, Zhu JJ (2013) High biocurrent generation in Shewanella-inoculated microbial fuel cells using ionic liquid functionalized graphene nanosheets as an anode. Chem Commun 49(59):6668–6670

Zhou HL, Yu WJ, Liu LX, Cheng R, Chen Y, Huang XQ, Liu Y, Wang Y, Huang Y, Duan XF (2013) Chemical vapour deposition growth of large single crystals of monolayer and bilayer graphene. Nat Commun 4:8

Application of Microbes in Household Products

7

Farhana Nazira Idris and Masrina Mohd Nadzir

Abstract

Microbes (e.g., bacteria, fungi, yeast, and algae) are beneficial in our daily life. Utilization of microbes in the preparation of food products like cheese, yoghurt, and bread is well known. Still, in reality, there are many other uses of microbes that contribute massively in our life, such as the production of cleaning products, cosmetics, and textiles. This chapter summarized the utilization of microbes in household products either through enzyme production, secretion of substance, or directly used in the product. Furthermore, this chapter also highlighted the type of microbes involved and a variety of microbial-based household products that are available.

Keywords

Bacteria · Fungi · Cleaning products · Cosmetics · Household products · Microbes · Textiles

7.1 Introduction

Microbes are present in our surrounding either we are aware of it or not. These tiny living organisms exist in various sources such as air, soil, water, plant, and animal. Usually, people consider microbes as pathogens that cause disease and are not safe to be present in an indoor building, air, water, or food. Nevertheless, certain microbes are useful in our daily life, and these microbes can affect the quality, sensory properties, safety, acceptability, and consistency of the products involved

F. N. Idris (✉) · M. M. Nadzir
School of Chemical Engineering, Universiti Sains Malaysia, Nibong Tebal, Pulau Pinang, Malaysia

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(Ly et al. 2018). Moreover, many valuable products in the household are due to microbial activities. The microbes also have been involved in the food and feed preparation for centuries. Within the same species, the microbes can be either pathogenic or not based on selected strains. *Escherichia coli* from strain O157:H7, for example, is a common microbe that causes food-borne diseases. Conversely, strain K12 of *E. coli* is not harmful and used regularly in laboratory for scientific analysis (Spök et al. 2018).

In manufacturing microbial products, fermentation technology is the most applied method which utilized microbes to develop products that are beneficial to humans. In general, fermentation produces biomass, extracellular metabolites, or intracellular compounds based on the implementation (Behera et al. 2019). Protein biomass produced from microbes via pure or mixed cultures can be a good substitute ingredient in protein-enriched foods (Ritala et al. 2017). There is also the production of primary metabolites such as ethanol, organic acid, and polysaccharides and secondary metabolites such as penicillin, gibberellin, and lovastatin which are useful in cosmeceutical, textile, and cleaning products. Microbes are favorable as sources of metabolites since they are practicable and can be used in mass production with reasonable cost (Gupta et al. 2019). Microbial enzymes also have long been used as biocatalyst in many products to speed up the chemical reaction. It is used in cleaning products to break down chemical bonds upon addition of water (Sanchez and Demain 2017). Different microbial enzymes are used to process different substrates and raw materials. Advances in fermentation technology have the potential to change the way microbial products are produced. Figure 7.1 shows the application of microbes either through their production of enzymes or compounds in household products.

Due to concern on environment and consumer awareness, applications of green ingredients or formulations in household products keep increasing. Thus, usage of emulsifiers or surfactants derived from microbes is not only sustainable but also biodegradable (Gupta et al. 2019; Sałek and Euston 2019; Sanchez and Demain 2017). Emulsifiers and surfactants are commonly used in cosmetics, food, textiles, and cleaning products. For example, rhamnolipids are biosurfactant extracted from *Pseudomonas aeruginosa* and have been commercialized in personal care products, cosmetics, and cleaning (Sałek and Euston 2019). Conversely, there are also microbial exopolysaccharides (EPSs) such as xanthan, pullulan, emulsan, cellulose, galactan, dextran, hyaluronic acid, and alginate that can be synthesized by bacteria, fungi, yeast, algae, etc. for various applications like cosmetics, textiles, and therapeutics (Angelin and Kavitha 2020; Yildiz and Karatas 2018). The EPSs are biodegradable, non-toxic, biocompatible and can be produced by *Lactobacillus* sp. (e.g., *L. acidophilus*, *L. gasseri*, *L. plantarum*, and *L. rhamnosus*), *Lactococcus*, *Leuconostoc*, *Weissella*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, and *Pediococcus* (Angelin and Kavitha 2020). By using microbial ingredients, the products manufactured are having simplified process, improved and consistent quality beside their biocompatibility which is the main benefit (Gupta et al. 2019).

In this chapter, the diversity of microbes present in a variety of household products is reviewed which people may have unnoticed due to microbes commonly

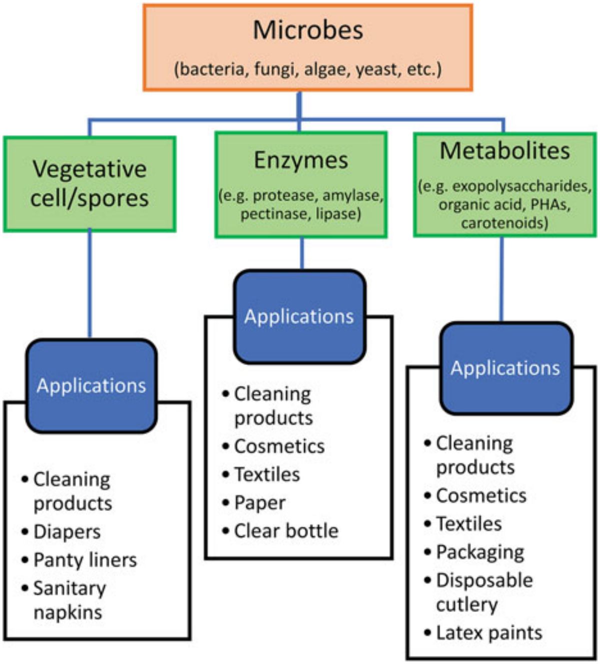

Fig. 7.1 Uses of microbes in household products

associated with harm or causing infection in the household. Hopefully, this information could open the eyes of consumers regarding the broad application of microbes.

7.2 Household Products

7.2.1 Cleaning Product

Recently, a range of cleaning products based on microbes or also known as microbial-based cleaning products (MBCPs) are highly marketed for domestic use aside from hospitals and daycare centers due to being non-toxic, biodegradable, and eco-friendly (OECD 2015). These MBCPs are also known as a probiotic cleaner, biological cleaner, or microbial cleaner (Spök et al. 2018). The living microorganisms are used as active ingredients to remove odors, dirt, food residues, and grease (OECD 2015; Spök et al. 2018). There exist various type of cleaning products based on microbes to clean the drains, remove the deposits in pipes, or to grease the machine parts. The MBCPs are also used to clean upholstery, carpets, and hard surface. Besides the chemical agents and/or enzymes, the MBCPs also contain the vegetative cells and spores (Arvanitakis et al. 2018).

In detergent, protease by microbes breaks down stains from protein. Amylases break down stains from starch, lipases are used for removing greases, and ureases degrade the organic high molecular weight substances in soil (Hettiarachchy et al.

2018; Sanchez and Demain 2017). The concept is the microbes enzymatically degrade the substance that causes dirt, odor, grease, or soil that attaches to the clothes. *Bacillus licheniformis* and *Bacillus amyloliquefaciens* have been used in detergents to produce enzymes for cleaning surface (Adisesh et al. 2011; OECD 2015). The proteases added to detergents are mostly derived from *Aspergillus* and selected strains of *B. amyloliquefaciens* (Park et al. 2017). There is also keratinase produced by *Bacillus* sp., *Streptomyces*, *Paenibacillus*, *Aspergillus sulphureus*, and *Paecilomyces woosongensis* used in bleaches, surfactants, and additives in the detergents to improve its washing performance and stains removal (Srivastava et al. 2020). Among global enzymes sales, 25% comes from their application in laundry detergents while the rest are from manufacturing foods, leather, pharmaceuticals, agrochemicals, and silk (Sanchez and Demain 2017). In addition, the microbial strains also have been engineered to produce recombinant enzymes in detergents to enhance their activity at the reduce temperature and prepare condition to be more alkaline (OECD 2015). The first recombinant lipase, named lipolase, is used in detergent made by replicating the lipase from *Humicola lanuginose* into *Aspergillus oryzae* (Sanchez and Demain 2017). Another application of microbes in cleaning products is by removing irritating and smelly odor. For example, the microbes metabolized NH_3 which is a substance that causes odor or the H_2S formation may be prevented by converting SO_4 into S_2 (Arvanitakis et al. 2018; Spök et al. 2018).

Microbes also provide competition between unwelcomed microorganisms in conquering area by utilizing all the nutrients from the polluted surfaces or soil or lowering the pH to restrict the competitor microbes' growth (Spök et al. 2018). Some of these MBCPs contain formulations of spore-forming bacteria (e.g., *Bacillus* spp.), thus hinder recolonization of unwelcomed microorganism since it will remain on the treated surface (Arvanitakis et al. 2018). Therefore, MBCPs provide more long-term effect compared to conventional cleaning products such as chlorine (Spök et al. 2018).

A variety of other bacteria such as *Achromobacter*, *Actinobacter*, *Alcaligenes*, *Arthrobacter*, and *Rhodopseudomonas* are found in MBCPs, but most of them are identified to the genus level only. *Achromobacter* is a marine bacterium which able to degrade xenobiotic compounds; meanwhile, *Alcaligenes*, *Rhodopseudomonas*, and *Arthrobacter* are used to break down azo dyes in textiles (Arvanitakis et al. 2018). Yeast, phytosynthetic and lactic acid bacteria (LAB) are commonly used in soap; meanwhile, phototrophic bacteria such as *Rhodobacter*, *Rhodospirillum*, *Chromatium*, and *Chlorobium* are used in deodorizer, degreaser, and mold inhibitor (OECD 2015). In terms of fungi, *Saccharomyces* and *Candida* are used as biodegradation agents for various harmful chemicals (Arvanitakis et al. 2018; OECD 2015). Itaconic acid, oxalic acid, and succinic acid are the primary metabolites produced by *Aspergillus* sp. used in detergents (Park et al. 2017). Details of the cleaning products and the microbes involved are presented in Table 7.1.

A number of cleaning products have been commercialized and patented to provide a selection for consumers in terms of natural active ingredients. Sophorolipids are biosurfactant derived from yeasts such as *Starmerella*, *Candida*,

Table 7.1 Examples of commercialized cleaning product

Cleaning product	Microbes involved in formulation	References
Soap	*Bacillus subtilis*, *Pseudomonas*, *Enterobacter*, *Citrobacter*, *Corynebacter*	Arvanitakis et al. (2018)
Detergents	*Aspergillus saccharolyticus*, *Aspergillus oryzae*, *Aspergillus niger*, *Streptomyces griseus*, *Aspergillus terreus*, *Saccharomyces cerevisiae*, *B. subtilis*, *Lactobacillus* sp., *Rhodobacter*, *Candida utilis*, *Streptomyces albus*, *Mucor hiemalis*	Park et al. (2017), Sanchez and Demain (2017), Srivastava et al. (2020)
Drain cleaners	*B. subtilis*, *Bacillus licheniformis*, *Bacillus polymyxa*, *A. oryzae*, *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, *Bacillus megaterium*, *Rhodopseudomonas palustris*	Arvanitakis et al. (2018), OECD (2015)
Odor control	*Lactobacillus delbrueckii Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, *B. licheniformis*, *Cryptococcus*, *Kluyveromyces*, *Candida*, *Metschnikowia*	Arvanitakis et al. (2018), OECD (2015), Srivastava et al. (2020)
Cleaning solution	*Achromobacter*, *Actinobacter*, *B. subtilis*, *Flavobacterium*, *Pseudomonas sp.*	Al-Marzooq et al. (2017), Arvanitakis et al. (2018)
Freshener, degreaser, deodorizer	*Lactobacillus*, *Rhodobacter*, *Rhodospirillum*, *Chromatium*, *Chlorobium*, *Rhodopseudomonas*, *Propionibacterium*, *Pediococcus*, *Streptococcus*, *Saccharomyces*, *Candida*	OECD (2015)
Surface cleaners	*Bacillus* sp. (*B. subtilis*, *B. circulans*, *B. megaterium*, *B. licheniformis*, *B. pumilus*, *B. sphaericus*)	Arvanitakis et al. (2018), OECD (2015)
Fish tank treatment	*Rhodopseudomonas palustris*, yeast, lactic acid bacteria	OECD (2015)

and *Pseudohyphozyma*. A Germany-based company utilized the biosurfactant under the name REWOFERM® for detergents and home care cleaning products (REWOFERM 2020; Sałek and Euston 2019). Various *Bacillus* strains have been used by Genesis Biosciences to produce Evogen microbial products for hard surface cleaning, odor control, bathroom cleaners, carpet, and fabric care (Evogen 2020). Another product, Probiotic eMC® by Multikraft can be used to clean the kitchen, windows, bathroom, floor, and furniture besides being gentle on the skin and materials (Multikraft 2020). A cellulase complex produced by *Humicola insolens* is used in detergent and marketed by Novozymes under the name Celluzyme which has effects of color brightening, softening, and removal of particulate soil (Celluzyme 2020; Sanchez and Demain 2017).

7.2.2 Cosmeceutical

Many consumers opted for natural product-based cosmetics formulations due to fear of possible harm effect from chemical ingredients. Bioactive compounds produced by microbes have the highest potential to be exploited for numerous commercial purposes, including cosmeceutical which the cosmetic products are intended for use on the skin, nail, hair, lips, or teeth. The compounds from microbes also are considered low-cost, sustainable, and rapid-producing substitute to other natural compounds in anti-aging, photo-protective, and skin-whitening products (Corinaldesi et al. 2017).

Cyanobacteria is a marine microbe that is useful for producing ultraviolet (UV)-absorbing compound, scytonemin, used in sunscreen. The cyanobacteria *Nostoc* sp., *Calothrix crustacean*, or *Chlorogloeopsis* sp. produce scytonemin which absorb UVA and UVB more efficiently than commercial formulation (Alves et al. 2020). Another formulation that can be used in sunscreen products is benzodiazepine alkaloids, which is isolated from marine fungi, *Exophiala* sp. (Corinaldesi et al. 2017; Zhang et al. 2008) and astaxanthin is obtained from *Haematococcus pluvialis* (Gupta et al. 2019).

Kojic acid, a compound secreted by fungi such as *Penicillium* sp., *A. flavus*, and *A. oryzae* has been used widely in skin-whitening products (Alves et al. 2020; Park et al. 2017). Kojic acid prevents the formation and accumulation of melanin in which the copper irons are chelated for the tyrosinase activity (Park et al. 2017). Chysophanol also is an active ingredient used in skin-whitening products and can be obtained from *Microsporum* sp. (MFS-YL) (Alves et al. 2020; Corinaldesi et al. 2017). Zeaxanthin also exhibits skin-whitening properties which can be obtained from microalgae, *Nannochloropsis oculata* (Gupta et al. 2019). Conversely, dihydroxyacetone (DHA) is an active tanning ingredients produced from species like *Schizochytrium*, *Aurantiochytrium*, and *Ulkenia* (Alves et al. 2020) in which function of DHA in skin care product is to even the skin tone (L'Oréal 2020).

Anti-aging products usually related to the ability to improve skin elasticity increase collagen and skin moisture content. In general, most anti-aging product contains moisturizing substances. *Streptococcus* species such as *Streptococcus zooepidemicus*, *Streptococcus equisimilis*, *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus equi*, and *Bacillus* are the bacteria that able to produce hyaluronic acid which is broadly utilized for anti-aging and moisturizing properties (Gupta et al. 2019; Yildiz and Karatas 2018). Another type of bacteria, *Vibrio diabolicus* is the producer of EPSs (HE800) which is comparable to hyaluronic acid in terms of encouraging the structuring of collagen and manufactured as anti-aging product (Corinaldesi et al. 2017). *Alteromonas macleodii* is one of the bacteria that able to secrete EPSs and has been applied in soothing products. The product is marketed under the name Abyssine® by Unipex (Abyssine 2020) for reducing and relieving pain of sensitive skin against mechanical, chemical, and UVB damage (Martins et al. 2014). Other anti-aging products contain EPSs that are extracted from *Pseudoalteromonas antarctica* and *Halomonas eurihalina* which both are marine microbes (Alves et al. 2020; Martins et al. 2014). Likewise, a mixture of EPSs secreted by *Pseudoalteromonas* sp. is implemented as an ingredient in anti-aging products, namely SeaCode® by Lipotec (SeaCode 2020). This mixture promotes the synthesis of collagen type I, thus improve the skin condition (Martins et al. 2014). Conversely, the Japanese dermatology company, KANEKA utilized surfactin produced by *B. subtilis* in its cosmetic formulation which has properties of anti-aging, anti-wrinkle, and enhancement of skin's collagen production (KANEKA 2020; Sałek and Euston 2019). Other sources of surfactin are *B. licheniformis*, *B. amyloliquefaciens*, and *B. pumilus* (Gupta et al. 2019). Carotenoids are among top active compounds for anti-aging properties which can be produced by many species of microalgae (Alves et al. 2020). There is also astaxanthin, one of the well-known carotenoids produced by *H. pluvialis*, *Rhodotorula*, *Phaffia*, and *Xanthophyllomyces*. This carotenoid is used to keep skin healthy and to protect skin from damage (Alves et al. 2020; Corinaldesi et al. 2017). Yeast such as *Saccharomyces* and *Candida* produce glutathione. It is a compound used widely in cosmetic not only for skin whitening and anti-aging but also for tooth gel and mouth rinse (Schmacht et al. 2017).

For moisturizing properties, squalene is a compound obtained from fungi-like protist, *Thraustochytrids* to keep skin moisturized (Zhang et al. 2017). *Spirulina* produces some proteins and hydrolytes used in hair products for keeping water retention besides very useful for dry skin treatment; meanwhile, *Chlorella* has smoothening and softening properties for hair and skin (Alves et al. 2020). Hair products such as shampoo, hair gel, hair sprays, hair colorant, hair tonics, and styling lotions also contained chitosan which is produced by *Pseudomonas*, *Acinetobacter*, *Halomonas*, *Arthrobacter*, *Myroides*, *Alteromonas*, *Bacillus*, and *Corynebacteria*, sp. (Corinaldesi et al. 2017). Chitosan is a well-known chitin-glucans obtained from cell wall of fungi and a good moisturizer (Gupta et al. 2019). There is also ectoine, which can be obtained from *Halomonas elongata*, *Corynebacterium glutamicum*, and *E. coli*, and it has properties to protect human tissues from dryness, thus been used as an ingredient in hair and skin care products like lotion, cream, and sprays (Becker and Wittmann 2020). Another common compound, lactic acid, which is produced by *Lactococcus lactic* has been used for centuries in food but also used as moisturizing agent and emulsifier in cosmetic (Pham et al. 2019). Yeasts such as *Pseudozyma*, *Ustilago*, and *Schizonella* can produce mannosylerythritol lipids (MELs) which are useful as biosurfactant in cosmetic for hydrating the skin. Various cosmetic products such as lipstick, nail care, eye shades, and body massage oils used MEL in their production (Gupta et al. 2019). The commercialized cosmetic products are marketed in the UA under the name SurfMellow® (Sałek and Euston 2019; TOYOBO 2020).

Carbohydrate fermentation by *Xanthomonas campestris* produces xanthan gum which has been applied widely in cosmetic products as surfactant-emulsifying agent, skin-conditioning agent, viscosity-improving agent, and emulsion stabilizer (Fiume et al. 2016; Sałek and Euston 2019). The compound is also used to make clear gel toothpaste (Verma et al. 2020). Emulsan is widely used in cleansing creams, soap, lotions, toothpaste, and shampoo in which these EPSs are produced by

Acinetobacter spp. during the stationary and late exponential phase of the growth cycle (Yildiz and Karatas 2018).

Other common ingredient found in cosmetic formulations is fatty acid esters which act as natural emollient and emulsifiers. This compound usually produced by higher plants, but there is also production of unique fatty acid esters by some bacteria. In many cosmetic products, ethyl oleate is commonly used as perfuming and emollient and can be produced by *Nocardiopsis dassonvillei*, which is an actinomycetes and a symbiont of *Dendrilla nigra* (Alves et al. 2020). Terpenoid, one of the ingredients in perfume can be synthesized by yeast, *Saccharomyces cerevisiae* (Zhang et al. 2017).

Microbial enzymes such as peroxidase and superoxide dismutase in the cosmetic products act as free radical scavengers to protect the skin against UV light. Protease has been utilized for skin treatments such as xerosis, psoriasis, and ichthyoses. Keratinase are used in creams and ointment for smoothness of heels, elbows, and knees and secreted by *B. licheniformis*, *Thermoanaerobacter*, *Thermosipho*, *Thermococcus*, *Lysobacter*, *Nesterenkonia*, *Kocuria*, *Vibrio*, *Xanthomonas*, *Stenotrophomonas*, and *Chryseobacterium* (Gupta et al. 2019). There is also esterase secreted by *A. niger* to hydrolase ester to make perfume (Park et al. 2017). Table 7.2 shows the range of cosmeceutical products in which the microbes are involved.

7.2.3 Textiles

Materials for clothes, bedding, curtains, diapers, aprons are made from natural and synthetic fibers. Cotton, linen, silk, and wool are some of the examples of natural fibers; meanwhile, synthetic fibers include those from polyester, polyamide, polyvinyl chloride, polyhydrazide, and polyprolines (Bajpai et al. 2011). Usually, uses of enzymes such as pectinase, amylase, cutinase, licasse, and cellulose produced by microbes in textile production are for treatment, desizing, scouring, mercerizing, dying, printing, finishing, and biopolishing (Hettiarachchy et al. 2018; Singh 2016). For an example, ramie fibers are an excellent natural textile for shirt, shorts, napkins, handkerchiefs, and tablecloths, in which process of degumming are done by *Bacillus* sp. producing alkaline pectinase to remove ramie gum (Kashyap et al. 2001). Laccase secreted by *A. niger* is used in denim finishing (Singh 2016). In manufacturing cotton, pectate lyase from actinomycete have a good degumming effect which shows a good separation of the bast fiber (Kashyap et al. 2001; Sanchez and Demain 2017) and cellulases have been utilized after weaving to eliminate starch-based sizes from fabrics (Singh 2016). Wool fibers that are used to make sportswear, sweaters, suits, dresses, and coats utilized keratinolytic protease from *Bacillus* sp., *Chryseobacterium*, *Pseudomonas*, and *Streptomyces* to degrade keratinous layers of the wool without damaging the other fiber part besides enhancing the quality of wool fabrics. This enzyme also improves the dyeing property of wool, tensile strength and prevent the wool from shrinking (Srivastava et al. 2020). Cellulase from *Aspergillus nidulans* used during biopolishing of jute fibers which later can be woven into curtain, carpets, rugs, cloth, and chair covers (Jabasingh and

Table 7.2 Cosmeceutical products containing compounds produced by microbes

Product	Compounds	Microbes	References
Sunscreen	Carotenoids	*Agrobacterium*, *Rhodotorula Phaffia*, *Xanthophyllomyces*	Corinaldesi et al. (2017)
	Benzodiazepine alkaloids	*Exophiala*	Zhang et al. (2008)
Anti-aging	EPS	*Agrobacterium* sp., *Xanthomonas campestris*, *Alcaligenes faecalis*, *Zymomonas mobilis*, *Bacillus* sp., *Pseudoalteromonas* sp., *Aureobasidium pullulan*, *Edwardsiella tarda*, *Alteromonas macleodii*	Alves et al. (2020); Corinaldesi et al. (2017); Martins et al. (2014)
	Carotenoids	Microalgae (*Dunaliella salina*), fungi (*Blakeslea trispora*), thraustochytrids, *Yarrowia lipolytica*, *Saccharomyces cerevisiae*	Corinaldesi et al., (2017); Moser and Pichler (2019)
	Hyaluronic acid	*Streptococcus equisimilis*, *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus equi*	Yildiz and Karatas (2018)
	Lipopeptides	*Bacillus subtilis*	Sałek and Euston (2019)
Skin-whitening	Pyrone	Marine fungi (*Aspergillus*, *Penicillium*, *Alternaria* sp., *Botrytis* sp.)	Alves et al. (2020)
	Chrysophanol	*Microsporum* sp.	Corinaldesi et al. (2017)
Moisturizer	Chitin, chitosan, protein polysaccharides	Zygomycetes, ascomycetes, basidiomycetes, chytridiomycetes, *Actinobacter*, *Pseudomonas*, *Azotobacter*, *Corynebacterium*, *Streptomyces*, *Myroides*	Alves et al. (2020)
	Squalene	*Schizochytrium*, *Aurantiochytrium*, *Ulkenia*	Alves et al. (2020)
	Lactic acid	*Lactococcus lactis*	Pham et al. (2019)
	Dextran	*Leuconostoc mesenteriodes*, *Streptococcus mutans*, *Weissella*, *Pediococcus, and Lactobacillus*	Yildiz and Karatas (2018); Gupta et al. (2019)
	Glycolipids	*Pseudozyma*, *Ustilago*, *Schizonella*	Sałek and Euston (2019)
	Keratinase	*Bacillus licheniformis*, *Thermoanaerobacter*, *Thermosipho*, *Thermococcus*, *Lysobacter*, *Nesterenkonia*, *Kocuria*, *Vibrio*, *Xanthomonas*, *Stenotrophomonas*, *Chryseobacterium*	Gupta et al. (2019)

(continued)

Table 7.2 (continued)

Product	Compounds	Microbes	References
Hair products	Protein, hydrolysates	*Spirulina* sp.	Alves et al. (2020)
	Oils	*Chlorella*	Gupta et al. (2019)
	Chitosan	*Acinetobacter*, *Arthrobacter*, *Pseudomonas*, *Halomonas*, *Myroides*, *Corynebacteria*, *Bacillus*, *Alteromonas* sp.	Corinaldesi et al. (2017)
Tooth gel/mouth rinse	Glutathione	Yeast	Schmacht et al. (2017)
	Emulsan	*Acinetobacter* sp.	Yildiz and Karatas (2018)
Perfume	Ethyl oleate	*Nocardiopsis dassonvillei*	Alves et al. (2020)
	Terpenoid	*Saccharomyces cerevisiae*	Zhang et al. (2017)

Nachiyar 2012; Singh 2016). For synthetic fibers, esterases have been used to improve their hydrophilicity and aid further finishing steps (Singh 2016).

The enzymatic activity of microbes can also be used in leather enhancement. Here, enzymes are used to remove unwanted parts such as hair from the animal skin, then the skin can be used as the raw materials. In leather production, different enzymes are used during different process which involved dehairing, soaking, bating, degreasing, dyeing, and solid waste treatment (De Souza and Gutterres 2012). Enzymes such as protease, keratinase, and lipase are the main enzymes of interest in production of leather because they remove globular protein, hydrolyze oils, greases, fats, and keratin of hair epidermis with the breakage of disulfide bonds (De Souza and Gutterres 2012; Srivastava et al. 2020). For example, alkaline protease from *B. subtilis* is used for dehairing leather, keratinase from *Aspergillus tamarii* during degreasing (Srivastava et al. 2020), and β-glucosidase, which is produced by *A. niger* used in dyeing textiles (Park et al. 2017).

Microbial polymers or known as EPS also have been utilized in textile production. The polymers are produced by chemical polymerization or fermentation of monomers (Verma et al. 2020). *Aureobasidium pullulans* is a black yeast-like-fungus that able to produce pullan, a water-soluble polymer (Pathak and Prasad 2014). For production of sports apparel, the soil bacterium, *Azotobacter vinelandii* produce linear polysaccharide alginate that possesses high moisture absorption that help to keep athletes body dry by absorbing sweat (Urtuvia et al. 2017). Algae is another type of microbes that able to produce alginate which is widely used in textiles. The alginate is commercially extracted from *Ascophyllum nodosum*, *Laminaria digitata*, *Laminaria japonica*, *Laminaria hyperborea*, and *Macrocystis pyrifera* (Lee and Mooney 2012). Another type of alginate, calcium alginate, exhibits flame-retardant properties. Fabric containing calcium alginate is effective in hindering the entry of heat and fire because of the firm burning residue char. Beside firefighter apparel, this fabric can be used in the production of upholstered furniture and furnishing decor textiles along with work clothing, military garments, carpet, and bedding (Kong et al. 2009; Pathak and Prasad 2014).

7.2.4 Others

Usage of biodegradable plastic is no longer foreign since people wants to decrease the impact of plastic in the environment. Polyhydroxyalkanoates (PHAs) is a natural biopolymer of biodegradable plastic produced by bacteria in the presence of surplus carbon, particularly when another essential nutrient (e.g., oxygen or phosphorus or nitrogen) is restricted in terms of amount or after a change of pH. When the cell is provided with a limiting nutrient, the compounds that stored energy deteriorate and are utilized as source of carbon for bacterial growth. The bacteria used for PHAs production are *Alcaligenes eutrophus*, *Alcaligenes latus*, *E. coli*, *Protomonas oleovorans*, *Protomonas extorquens*, and a mutant strain of *Azotobacter vinelandii*. Application of PHAs can be found in the form of a shampoo bottle, disposable razors, disposable cutlery, cosmetic containers, plastic beverage bottles, milk cartons, pens, combs, sanitary products, and latex paint (Anjum et al. 2016).

In paper production, microbial lipase secreted by *Candida rugosa* has been used by Nippon Paper Industries to remove 90% of hydrophobic compounds from wood, mainly triglycerides and waxes (Sanchez and Demain 2017). In another preparation of Japanese paper, alkaline pectinase produced by *Bacillus* spp. and *Erwinia carotovora* is used for retting Mitsumata bast which increases the strength of the pulp. Thus, paper sheets prepared from this pulps are very soft and uniform (Kashyap et al. 2001).

Sporulated *Lactobacilli*, *Lactococcus*, *Pediococcus*, and *Bacillus* coagulants are used in baby diapers, panty liners, and sanitary pads by mixing it with polyacrylic acid (superabsorbent), hard fat (hydrophobic carrier), and zeolite (odor absorbent). These applications in the sanitary pads avoid the occurrence of vaginal infections since these bacteria are the common vaginal microbes (Juturu and Wu 2016). There is also production of cyclodextrin by *B. subtilis*, *Brevibacterium* sp., and *Brevibacillus brevis* used in diapers, napkins, and menstrual pad as odor control (Gupta et al. 2019).

7.3 Benefits and Challenges

The products based on microbes kept increasing over the years. New technologies and insights continue to be discovered as natural products become more relevant. Spök et al. (2018) have identified more than 30 different species of microbes used in MBCPs in which most of them are yeast and bacteria with the most frequently used are *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Rhodopseudomonas*, and *Saccharomyces*. All these products are claimed to be safe, or qualified presumption of safety (QPS) and the microbes used belong in Group 1, which is harmless to humans and animals. This is in contrast to a typical household bleach that usually contains sodium hypochlorite, ammonium hydroxide which can be found in hard surface cleaners and sodium chlorite in detergents which tend to be reactive and corrosive (Arvanitakis et al. 2018; OECD 2015). The compounds in MBCPs are less hazardous and the products also usually presented in less amount of acids, surfactants, and organic solvents compared to chemical compounds (Spök et al. 2018). There was also no incident of health reported from consumer or professional regarding the use of MBCPs (Spök et al. 2018). In term of cosmetics, the 34 microbial polysaccharides gums used in formulations are found to be safe (Fiume et al. 2016). Moreover, the microbial-based household products are very suitable for those who are allergic to the chemical besides being environmentally friendly. As for PHAs, the products are more eco-friendly, biodegradable, and sustainable (Anjum et al. 2016).

Despite all the advantages, contrary to the application of microbes in the industry where the exposure of microbes might be controlled and monitored by the government and non-government body, general consumer might be less aware of such protocol or guidelines in handling the microbial products in the household. Besides, the active ingredients of the products are very often considered confidential business information, the consumers often clueless about what types of microbes are present in the products. Therefore, it is important for the manufacturers to have proper labeling on the products and specified the microbes used in the products at least to a species level to differ the pathogenic and non-pathogenic strains and to assess their risk towards human and environment (Arvanitakis et al. 2018).

There is also potential environmental issue due to the extensive use of such products and their emission into the environment especially regarding the microorganism itself and formulation/use of the product. For examples, *Acinetobacter baumannii* was found to cause healthcare-associated infection and *Candida* sp. is deemed as an opportunistic pathogen (OECD 2015). A cleaning product employs *A. oryzae* may possess allergic properties which cause lung inflammation (VKM et al. 2019). Application of MBCPs either by spray or powders can create aerosols resulting in inhalational exposure as well (Arvanitakis et al. 2018). There is also the possibility for oral ingestion mainly if these products are applied near the food. Spores-containing products also can last for a long time; thus, the consumers might be exposed to it for a long-term. The indoor setting of where the household products are placed and used also enhanced all of these exposures especially if there is poor ventilation. At present, the lack of information regarding the type and immensity of possible human exposures to microbes by utilizing these products causes any attempt of precise risk evaluation on human health from such products rather tricky. As a precaution, individual who is vulnerable such as infant, pregnant woman, and elderly might need to be careful when using certain microbial-based products.

7.4 Conclusion

Microbes appear to be widely used in household products and play a key role in producing modified enzymes with enhanced properties and applied as an active ingredient in a diverse application. In these past years, more microbial-based household products are being marketed and consumed due to the growing concern regarding the use of chemicals in products for daily use besides increasing awareness about the environment. Moreover, the microbes are presented as great sustainable resources of natural active compounds. Currently, various types of household products derived from microbes can be found commercially, thus proved microbes have prominent prospects for numerous sectors due to their distinct and special properties. With the development in biotechnology, it is expected more microbial-based products will be produced and well-established as metabolic engineering of the microbes, mode-of-action and formulation are improved besides more new microbes are discovered for their potential used in the industries.

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References

Abyssine (2020). http://www.lucasmeyercosmetics-us.com/en/products/product.php?id=1. Accessed 11 Aug 2020

Adisesh A, Murphy E, Barber CM, Ayres JG (2011) Occupational asthma and rhinitis due to detergent enzymes in healthcare. Occup Med (Lond) 61:364–369. https://doi.org/10.1093/occmed/kqr107

Al-Marzooq F, Al Bayat S, Sayyar F, Ishaq H, Nasralla H, Koutaich R, Al Kawas S (2017) Can probiotic cleaning solutions replace chemical disinfectants in dental clinics? Eur J Dent 11:192–195. https://doi.org/10.4103/ejd.ejd

Alves A, Sousa E, Sousa E, Kijjoa A, Kijjoa A, Pinto M, Pinto M (2020) Marine-derived compounds with potential use as cosmeceuticals and nutricosmetics. Molecules:25. https://doi.org/10.3390/molecules25112536

Angelin J, Kavitha M (2020) Exopolysaccharides from probiotic bacteria and their health potential. Int J Biol Macromol 162:853–865. https://doi.org/10.1016/j.ijbiomac.2020.06.190

Anjum A, Zuber M, Zia KM, Noreen A, Anjum MN, Tabasum S (2016) Microbial production of polyhydroxyalkanoates (PHAs) and its copolymers: a review of recent advancements. Int J Biol Macromol 89:161–174. https://doi.org/10.1016/j.ijbiomac.2016.04.069

Arvanitakis G, Temmerman R, Spök A (2018) Development and use of microbial-based cleaning products (MBCPs): current issues and knowledge gaps. Food Chem Toxicol 116:3–9. https://doi.org/10.1016/j.fct.2017.12.032

Bajpai V, Bajpai S, Jha MK, Dey A, Ghosh S (2011) Microbial adherence on textile materials: a review. J Environ Res Dev 5:666–672

Becker J, Wittmann C (2020) Microbial production of extremolytes — high-value active ingredients for nutrition, health care, and well-being. Curr Opin Biotechnol 65:118–128. https://doi.org/10.1016/j.copbio.2020.02.010

Behera SS, Ray RC, Das U, Panda SK, Saranraj P (2019) Microorganism in fermentation. In: Berenjian A (ed) Essentials in fermentation technology, learning materials in biosciences. Springer Nature, Switzerland, pp 1–39. https://doi.org/10.1007/978-3-030-16230-6

Celluzyme (2020). https://trademarks.justia.com/792/06/celluzyme-79206569.html. Accessed 5 Sept 2020

Corinaldesi C, Barone G, Marcellini F, Dell'Anno A, Danovaro R (2017) Marine microbial-derived molecules and their potential use in cosmeceutical and cosmetic products. Mar Drugs 15:1–21. https://doi.org/10.3390/md15040118

De Souza FR, Gutterres M (2012) Application of enzymes in leather processing: a comparison between chemical and coenzymatic processes. Brazilian J Chem Eng 29:473–481. https://doi.org/10.1590/S0104-66322012000300004

Evogen (2020). http://genesisbiosciences.co.uk/our-group/evogen-microbial-products/. Accessed 26 July 2020

Fiume MM, Heldreth B, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, Marks JG, Shank RC, Slaga TJ, Snyder PW, Andersen FA, Gill LJ (2016) Safety assessment of microbial polysaccharide gums as used in cosmetics. Int J Toxicol 35:5S–49S. https://doi.org/10.1177/1091581816651606

Gupta PL, Rajput M, Oza T, Trivedi U, Sanghvi G (2019) Eminence of microbial products in cosmetic industry. Nat Prod Bioprospect 9:267–278. https://doi.org/10.1007/s13659-019-0215-0

Hettiarachchy NS, Feliz DJ, Edwards JS, Horax R (2018) The use of immobilized enzymes to improve functionality. In: Proteins in food processing, 2nd edn. Elsevier Ltd, Amsterdam, pp 569–597. https://doi.org/10.1016/B978-0-08-100722-8.00022-X

Jabasingh SA, Nachiyar CV (2012) Process optimization for the biopolishing of jute fiberswith cellulases from Aspergillus nidulans AJ SU04. Int J Biosci Biochem Bioinforma 2:12–16. https://doi.org/10.7763/ijbbb.2012.v2.60

Juturu V, Wu JC (2016) Microbial production of lactic acid: the latest development. Crit Rev Biotechnol 36:967–977. https://doi.org/10.3109/07388551.2015.1066305

KANEKA (2020). https://www.kaneka.co.jp/en/business/qualityoflife/nbd_002.html. Accessed 25 July 2020

Kashyap DR, Vohra PK, Chopra S, Tewari R (2001) Applications of pectinases in the commercial sector: a review. Bioresour Technol 77:215–227. https://doi.org/10.1016/S0960-8524(00)00118-8

Kong QS, Wang BB, Ji Q, Xia YZ, Guo ZX, Yu J (2009) Thermal degradation and flame retardancy of calcium alginate fibers. Chinese J Polym Sci (English Ed.) 27:807–812. https://doi.org/10.1142/S0256767909004527

L'Oréal (2020). https://www.lorealparisusa.com/ingredient-library/dha.aspx. Accessed 2 Sept 2020

Lee KY, Mooney DJ (2012) Alginate: properties and biomedical applications. Prog Polym Sci 37:106–126. https://doi.org/10.1016/j.progpolymsci.2011.06.003

Ly D, Mayrhofer S, Domig KJ (2018) Significance of traditional fermented foods in the lower Mekong subregion: a focus on lactic acid bacteria. Food Biosci 26:113–125. https://doi.org/10.1016/j.fbio.2018.10.004

Martins A, Vieira H, Gaspar H, Santos S (2014) Marketed marine natural products in the pharmaceutical and cosmeceutical industries: tips for success. Mar Drugs 12:1066–1101. https://doi.org/10.3390/md12021066

Moser S, Pichler H (2019) Identifying and engineering the ideal microbial terpenoid production host. Appl Microbiol Biotechnol 103:5501–5516. https://doi.org/10.1007/s00253-019-09892-y

Multikraft (2020). https://www.multikraft.com/en/products-applications/cleaning-indoor-environment/. Accessed 29 July 2020

OECD (2015) Microbial-based cleaning products in use and the potential role of transgenic microorganisms. In: Biosafety and the environmental uses of micro-organism: conference proceedings, Paris, pp 129–141. https://doi.org/10.1787/9789264213562-13-en

Park HS, Jun SC, Han KH, Hong SB, Yu JH (2017) Diversity, application, and synthetic biology of industrially important Aspergillus fungi. In: Advances in applied microbiology. Elsevier Ltd, Amsterdam, pp 161–202. https://doi.org/10.1016/bs.aambs.2017.03.001

Pathak H, Prasad A (2014) Application and prospects of microbial polymers in textile industries. J Text Sci Eng 4:172. https://doi.org/10.4172/2165-8064.1000

Pham JV, Yilma MA, Feliz A, Majid MT, Maffetone N, Walker JR, Kim E, Cho HJ, Reynolds JM, Song MC, Park SR, Yoon YJ (2019) A review of the microbial production of bioactive natural products and biologics. Front Microbiol 10:1–27. https://doi.org/10.3389/fmicb.2019.01404

REWOFERM (2020). https://household-care.evonik.com/product/household-care/en/products/pages/Rewoferm-SL-ONE.aspx. Accessed 25 July 2020

Ritala A, Häkkinen ST, Toivari M, Wiebe MG (2017) Single cell protein-state-of-the-art, industrial landscape and patents 2001–2016. Front Microbiol 8. https://doi.org/10.3389/fmicb.2017.02009

Sałek K, Euston SR (2019) Sustainable microbial biosurfactants and bioemulsifiers for commercial exploitation. Process Biochem 85:143–155. https://doi.org/10.1016/j.procbio.2019.06.027

Sanchez S, Demain AL (2017) Useful microbial enzymes-an introduction. In: Biotechnology of microbial enzymes: production, biocatalysis and industrial applications. Elsevier Inc., Amsterdam, pp 1–11. https://doi.org/10.1016/B978-0-12-803725-6.00001-7

Schmacht M, Lorenz E, Senz M (2017) Microbial production of glutathione. World J Microbiol Biotechnol 33. https://doi.org/10.1007/s11274-017-2277-7

SeaCode (2020). https://www.lipotec.com/en/products/seacode-trade-marine-ingredient/. Accessed 11 Aug 2020

Singh S (2016) Aspergillus enzymes for textile industry. In: New and future developments in microbial biotechnology and bioengineering: Aspergillus system properties and applications. Elsevier B.V., Amsterdam, pp 191–198. https://doi.org/10.1016/B978-0-444-63505-1.00014-2

Spök A, Arvanitakis G, McClung G (2018) Status of microbial based cleaning products in statutory regulations and ecolabelling in Europe, the USA, and Canada. Food Chem Toxicol 116:1–24. https://doi.org/10.1016/j.fct.2017.12.057

Srivastava B, Khatri M, Singh G, Arya SK (2020) Microbial keratinases: an overview of biochemical characterization and its eco-friendly approach for industrial applications. J Clean Prod 252:1–26. https://doi.org/10.1016/j.jclepro.2019.119847

TOYOBO (2020). https://www.toyobo-global.com/seihin/cosme/surfmellow.htm. Accessed 25 July 2020

Urtuvia V, Maturana N, Acevedo F, Peña C, Díaz-Barrera A (2017) Bacterial alginate production: an overview of its biosynthesis and potential industrial production. World J Microbiol Biotechnol 33:1–10. https://doi.org/10.1007/s11274-017-2363-x

Verma ML, Kumar S, Jeslin J, Dubey NK (2020) Microbial production of biopolymers with potential biotechnological applications. In: Biopolymer-based formulations. Elsevier Inc., Amsterdam, pp 105–137. https://doi.org/10.1016/b978-0-12-816897-4.00005-9

VKM, Madslien EH, Asare N, Bergh Ø, Joner E, Trosvik P, Yazdankhah S, Eklo OM, Nielsen KM, Ytrehus B, Wasteson Y (2019). Current knowledge of the health and environmental risks of microbial-based cleaning products. Scientific opinion of the panel on microbial ecology of the Norwegian Scientific Committee for food and environment

Yildiz H, Karatas N (2018) Microbial exopolysaccharides: resources and bioactive properties. Process Biochem 72:41–46. https://doi.org/10.1016/j.procbio.2018.06.009

Zhang D, Yang X, Kang JS, Choi HD, Son BW (2008) Circumdatin I, a new ultraviolet—a protecting benzodiazepine alkaloid from a marine isolate of the fungus Exophiala. J Antibiot (Tokyo) 61:40–42. https://doi.org/10.1038/ja.2008.108

Zhang Y, Nielsen J, Liu Z (2017) Engineering yeast metabolism for production of terpenoids for use as perfume ingredients, pharmaceuticals and biofuels. FEMS Yeast Res 17:1–11. https://doi.org/10.1093/femsyr/fox080

8 Electricity Generation and Wastewater Treatment with Membrane-Less Microbial Fuel Cell

Chenar A. Tahir, Zoltán Pásztory, Charu Agarwal, and Levente Csóka

Abstract

Water pollution is a pressing issue due to growing levels of industrialization and increasing amounts of domestic wastewater. The possibility of harvesting energy from waste has captured the attention of the scientists across the globe. In this regard, the membrane-less microbial fuel cell (ML-MFC) has come across as a sustainable choice of technology for energy generation along with wastewater treatment. This unit behaves like a bioreactor relying on the bacteria that act as a biocatalyst oxidizing the organic matter to produce electricity. In this chapter, the focus is laid on the influence of chemical and physical parameters of the ML-MFC unit on the productivity of the process from electricity generation and wastewater treatment aspects.

Keywords

Anode · Cathode · Conductivity · Electricity generation · Membrane-less microbial fuel cell · Operating temperature · pH · Reactor design · Substrate pretreatment · Wastewater treatment

C. A. Tahir · Z. Pásztory · C. Agarwal
Innovation Center, University of Sopron, Sopron, Hungary

L. Csóka (✉)
Faculty of Informatics, ELTE University, Budapest, Hungary, Institute of Cellulose and Paper Technology, celltech-paper Ltd., Sopron, Hungary
e-mail: csoka.levente@celltech-paper.hu

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8.1 Introduction

Since the last few decades, immense thrust on the sustainability factor has driven the scientific community to seek ways of addressing the issues causing harm to the ecosystem (Nastro 2014). On the other hand, concerns regarding the depletion of crude oil and the consequential rush to find alternative energy sources have also gained momentum. In view of this, microbial fuel cells (MFCs) have become of global interest as a sustainable technology with immense potential to generate electricity from organic matter in wastewaters, concurrently treating the wastewaters and contributing to environmental remediation (Feng et al. 2008). The MFC technology holds promise to fulfill at least a part of the future energy needs (Logan 2010). Moreover, it is self-sustaining, affordable, and does not require huge capital investments.

The MFCs function by converting the chemical energy derived from the organic matter present in the wastewater directly into electrical energy, with the help of electrogenic bacteria working as a biocatalyst (Min and Logan 2004). An MFC primarily consists of three parts: an electrode system with a container, a microorganism culture (anaerobic or aerobic) in a growing medium, and a substrate solution to nourish the microbes (Cheng et al. 2006). The MFCs are designed with different configurations, in general, they are classified as single-chamber or dual-chamber MFCs (Khan et al. 2018). Most single-chamber MFCs usually contain a proton-exchange membrane (PEM) to separate the anode and the cathode compartments. However, in most cases, the membrane offers a considerable internal resistance that negatively influences the electrochemical performance of the MFCs. Besides, high costs of the membrane have rendered conventional MFCs commercially unviable (Logan 2010). Recent efforts have been directed to achieve high output without a membrane, thus giving rise to the concept of membrane-less microbial fuel cell (ML-MFC), as depicted in Fig. 8.1. The presence of an additional anode layer (in the middle of the cell) in Fig. 8.1b acts as a barrier preventing the movement of organic materials towards the cathode, which holds the key to high efficiency (Kim et al. 2016). Thus, the ML-MFCs overcome the drawback of conventional MFCs since the membrane limits the functioning of the MFC by decelerating the transfer of protons through it (Jang et al. 2004).

There are several major advantages offered by an ML-MFC with regard to the energy output, process economics, environmental impact, as well as the overall feasibility (Fig. 8.2). Firstly, ML-MFC does not require any energy input from an external source. Secondly, it can operate at ambient temperature and pressure conditions. Thirdly, it is eco-friendly as it does not produce any toxic by-products. It can help to curtain the environmental pollution levels, thus prevent damage to the planet (Yang et al. 2009). Fourthly, its operation without an ion-exchange membrane decreases the ohmic cell resistance, along with the manufacturing cost of the reactor (Clauwaert and Verstraete 2009). Finally, depending on the design, it can have a long operational lifespan with durability in keeping up the efficiency (Zhang and Ye 2015).

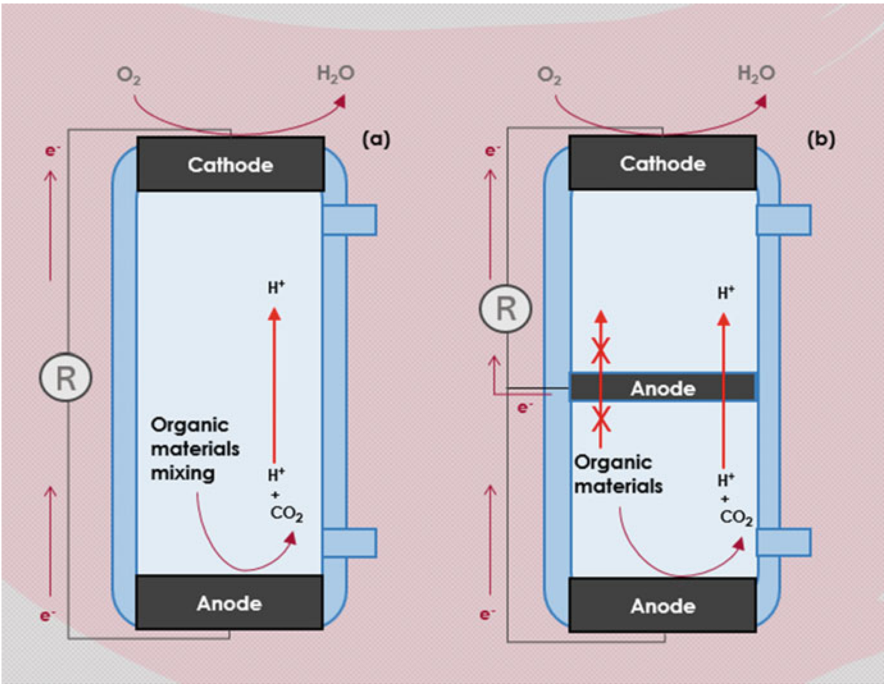

Fig. 8.1 Schematic representation of an ML-MFC (**a**) single-chamber unit, (**b**) dual-chamber unit. (Redrawn with modifications from Ref. Kim et al. (2016))

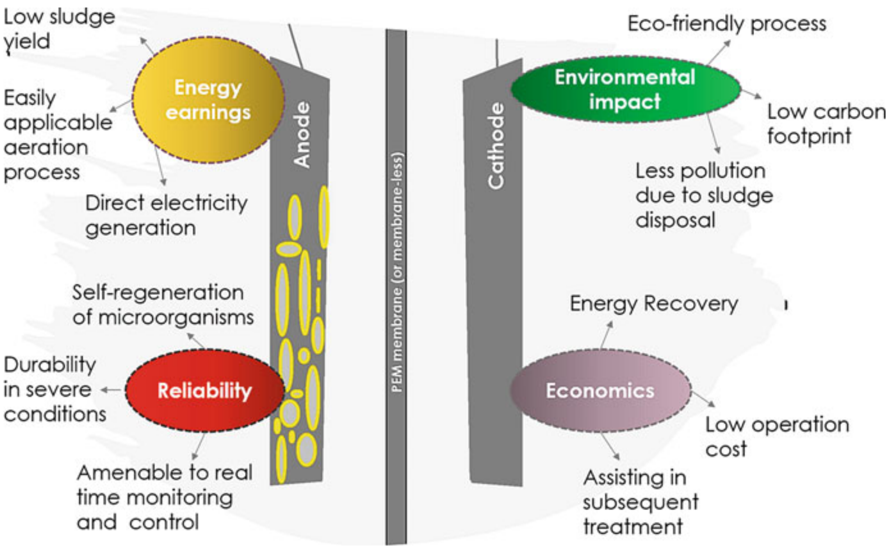

Fig. 8.2 Advantages of MFCs. (Redrawn with modifications from Ref. Li et al. (2014))

The process of bio-electricity generation in most kinds of MFCs is similar, where the substrate with microorganisms is contained in the anode compartment (if the anode is partitioned from the cathode using a separator) of the MFC chamber (Lin et al. 2013). The microorganisms play the role of a catalyst in the process of energy generation, producing electrons and protons along with carbon dioxide (CO_2) gas. With the passage of time, they create a biofilm on the anode surface thus catalyzing the anaerobic bacterial oxidation (Li et al. 2014; Waller and Trabold 2013). The protons or hydrogen ions (H^+) move from the anode towards the cathode, while the electrons transfer through an external electrical circuit connected to the cathode (Lin et al. 2013). Oxygen, which acts as the electron acceptor, is pumped from the air through the catholyte into the cathode compartment or, it is directly received if the cathode is in contact with the air (air-cathode) (Zhang and Ye 2015). In the cathode compartment, hydrogen ions and oxygen react with the help of electrons to form water or hydrogen peroxide (Luo et al. 2017). The cathode electrode is generally coated with a catalyst such as platinum to assist in the reaction of hydrogen ions with oxygen, or in other words, to facilitate the oxygen reduction reaction (Rahimnejad and Najafpour 2011). The basic chemical reactions taking place at the electrodes with the organic materials are depicted by Eqs. (8.1)–(8.3). The specific reactions may vary in anode compartment according to the type of substrate used; however, the products remain the same with altered stoichiometric coefficients (Khan et al. 2018). At the anode, the partition reaction in the case of glucose as substrate occurs as indicated by Eq. (8.1).

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \tag{8.1}$$

At the cathode, the partition reaction occurs as indicated by Eqs. (8.2) and (8.3).

$$4H^+ + 4e^- + O_2 \rightarrow 2H_2O \tag{8.2}$$

$$4H^+ + 4e^- + 2O_2 \rightarrow 2H_2O_2 \tag{8.3}$$

where, $\Delta G^o = -5792.2$ kJ/mol.

The substrate, which functions as a fuel for the MFC unit, contains organic compounds that are degraded by the microorganisms during the process of oxidation (Pauline and Boopathi 2018; Luo et al. 2007). Agri-food industries produce a lot of waste that is easily broken down by bacteria and can be a good substrate for MFCs. Other common substrates may include glucose, acetate, as well as wastewaters from the domestic household, dairy, slaughter-house, refinery, and dyeing industry (Pallavi and Udayashankara 2016; Jothinathan et al. 2018; Savizi et al. 2012). The functioning of MFCs is influenced by a number of chemical, physical, and biological factors that have a significant impact on the overall efficiency of the unit. The physical and chemical factors include the reactor design (Jang et al. 2004; Luo et al. 2007; Liu and Logan 2004; Ye et al. 2018); pH (Gil et al. 2003; Jadhav and Ghangrekar 2009); temperature (Feng et al. 2008; Jadhav and Ghangrekar 2009; Ahn and Logan 2010); electrode surface area with electrode spacing (Ghangrekar and Shinde 2007; Cheng and Logan 2011); and the pretreatment of the influent (Jadhav and Ghangrekar 2009; Ghangrekar and Shinde 2008; Yang et al. 2013). The biological factors include the type of microbial culture used, and the conditioning of the substrate for the microorganisms (Malvankar et al. 2012; Hassan et al. 2014). Many varieties of exogenous bacteria have been studied for their capability to produce electrons (Cheng and Logan 2007). A major issue in ML-MFCs is the mixing of the anolyte with the catholyte. This causes a decline in the efficiency of the cathode electrode due to biofouling. The ultimate aim is to reduce the internal resistance of the unit, enhance the maximum conversion rate of the organic materials inside the substrate, and increase the efficiency of the anode and cathode electrodes (Rabaey and Verstraete 2005).

The concept of an MFC was first postulated by Michael Cressé Potter from *Escherichia coli* and *Saccharomyces* with platinum electrodes way back in 1912, but its low power intensity led to little interest in it at the time (Yang et al. 2011). In the early 1990s, MFCs regained interest as a promising technology for bioenergy and wastewater treatment after substantial advancements lead to improved output efficiency (Rahimnejad et al. 2015). In 1999, Kim et al. reported the first mediator-less MFC (Logan 2008). Several developments have been made since then leading to increased power density of MFCs from less than 1 W/m^3 to over 4000 W/m^3. Many microorganisms have the ability to produce electrons from the metabolism of organic matter (Cheng et al. 2006; Liu et al. 2004). There are different methods by which the bacteria transfer electrons to the anode: transfer of electrons through mediator, direct transfer of electrons, and transfer of electrons through bacterial nanowires (Khan et al. 2018). The bacterial culture needs a favorable environment to thrive; the pH and temperature greatly affect the bacterial activity, thus influencing the performance of the reactor. Several reviews have been published on MFCs, each with a different flavor or emphasis in terms of the design features, substrates, microbial metabolism, their performance and challenges (Hindatu et al. 2017; Wei et al. 2011). In this chapter, the focus lies on the major chemical and physical factors impacting the performance and efficiency of ML-MFCs for power generation as well as water treatment. It brings up the recent advances in the ML-MFC technology and is expected to contribute to the future efforts in its development.

8.2 Electricity Generation

Bacteria are the crux of the MFC technology with the ability to produce and transfer electricity through their pili to their surroundings. Many factors play roles in the efficient harnessing of energy and accelerating the oxygen reduction reaction (ORR) such as the use of different substrates, pretreatment of the substrate, anode and cathode electrode performance, and the design of the reactor.

The design of MFC is the most significant factor influencing the electricity production and water treatment capability and overall process efficiency (Pant et al. 2010). One of the main design factors is the membrane, which has a huge impact on the productivity as well as the cost of MFC. Many studies have investigated and compared the performance of MFCs with and without membranes, under the same experimental conditions. For example, Sung and his team worked on improving the cathodic ORR without precious metals using a carbon cathode and replacing platinum as a catalyst due to its high cost. They compared a single-chamber MFC (SMFC) (air-cathode and without membrane) with a two-chamber MFC (TMFC) (consisting of PEM); and observed that the ML-MFC gave the highest power and ORR activity. This was due to the enormous internal resistance offered by the ion-exchange membrane, which is obvious from the resistance values of 45 and 80 Ω for SMFC and TMFC, respectively (Song et al. 2020). Another study found that an ML-MFC produced 140% higher power output of 520–570 μW with more stability compared to an MFC with ceramic membranes and salt-bridge connection (You et al. 2020). Similarly, MFC configurations having a metal anode with carbonaceous cathode showed that the ML-MFC dominated MFC with membrane giving superior performance in terms of maximum power density (MPD) (Yamashita et al. 2016). Thus, it is evident that the use of either salt-bridge or membrane contributes to a direct rise in the internal resistance of the reactor, which is a detrimental factor for achieving higher output power and overall efficiency (Min et al. 2005). It is not surprising that single-chamber ML-MFCs are preferable over the MFCs with membranes, and recent efforts have been directed towards the improvement of their performance to make them commercially successful (Logan 2010).

As stated, many factors influence the performance of MFCs and the final output of the reactor is a synergistic combination of each of the influencing factors, thus making the direct comparisons of the effect of individual factors among different studies difficult. Most studies on MFCs have probed the effects of pH variation, substrate type, design configuration, operating temperature, electrode materials, the oxygen amount in the anode, diffused oxygen sneaked into the system, etc. Therefore, the evaluations and comparison will be based on rational thoughts and research outcomes, and available theories and facts.

8.2.1 Anode and Cathode Electrodes

MFC has come across as a sustainable concept not only for electricity generation but also as a solution for the environmental concerns facing the globe (Palanisamy et al. 2019). The choice of electrodes is a crucial and challenging aspect in the design of MFCs for optimum performance. Efforts for the improvement of anode are directed towards the "optimal provision for bacterial attachment" and "better capability of collecting of more electrons from the bacteria and the medium" (Hindatu et al. 2017). For the cathode, its performance in terms of oxygen and hydrogen reaction (ORR) significantly affects the coulombic efficiency (CE) of the MFC and helps to boost the flow of electricity, durability, and long-term steadiness (Santoro et al. 2013; Jiménez González et al. 2020). Also, the placement of the cathode in the ML-MFC plays an important role. If the MFC is designed with an air-cathode, it faces some concerns like water loss, ORR activity, oxygen intrusion, cathode

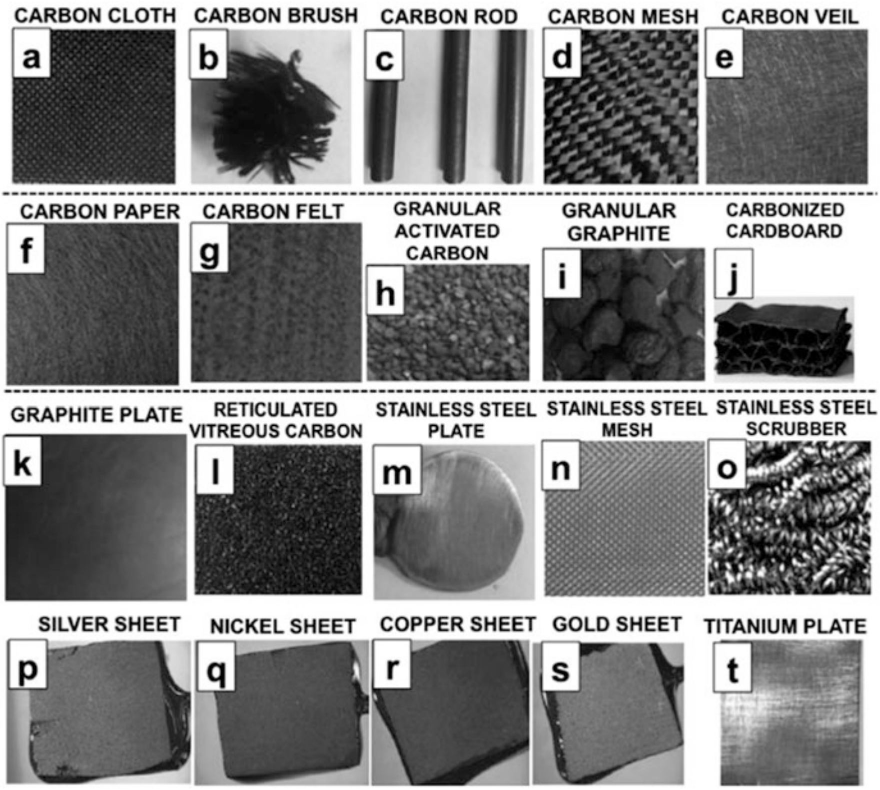

Fig. 8.3 Several types of carbonaceous and metallic materials used for anode and cathode electrode. (Published from Ref. Santoro et al. (2017) under CC BY 4.0 license)

deterioration, biofilm formation from the substrate-faced side, and anode performance and capability for fostering bacterial cultures. Many studies have been published in the last two decades dealing with these issues and their solutions. Although there has been a considerable progress in the electrodes to overcome their poor performance, some issues still remain unsolved that have made MFC technology unviable for large-scale operations (Zhou et al. 2012).

Till date, various materials have been explored for the development of electrode materials for the MFCs. Carbonaceous materials (like carbon cloth, carbon felt, graphite) and metallic materials (like copper, zinc, stainless steel) are widely used for making anode and cathode electrodes (Fig. 8.3) (Santoro et al. 2017). Carbon is abundantly available in nature, and possesses exceptional and unique characteristics like interaction with the electroactive-biofilm, conductivity, and the durability in toxic environments. For these reasons, carbonaceous materials find extensive applications as electrodes for MFCs (Santoro et al. 2017). Several analytical techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD) are commonly employed to characterize the structure of the electrode material. Most studies have evaluated the electrodes for their electrochemical performance (ORR) by cyclic voltammetry and electrochemical impedance spectroscopy, whereas some others have focused on oxygen diffusion through the cathode, MPD, CE, and chemical oxygen demand (COD).

Cathode Electrode

As mentioned, carbonaceous materials have unique properties such as electrical conductivity (Saba et al. 2017), corrosion resistivity (Slate et al. 2019), mechanical strength (Jia et al. 2018), high surface area (Yang et al. 2018), biocompatibility (Zhao et al. 2018), chemical stability (Cai et al. 2020a), environmental safety and low cost (Liu et al. 2020). In a study, a carbon cathode was prepared from mixed carbon powder (Vulcan XC-72) and 30 wt% polytetrafluoroethylene (PTFE) solution, coated with different numbers of diffusion layers (DLs) from the airside. The optimum performance was obtained with four DLs, which significantly improved the CE from 19.1% to 32%, while the MPD increased by 42% compared to an uncoated carbon cathode. Also, the open-circuit potential analyses revealed that the maximum potential difference between the cathode having four DLs and the uncoated cathode was 117 mV at 0.6 mA/cm^2. Obviously, the oxygen permeability and water loss from cathode decreased with increasing number of DLs (Cheng et al. 2006). Any attempt for sealing the cathode further lead to higher internal resistivity like in case of the MFC with PEM. For sealing the cathode to have lower resistivity, a spun-bonded olefin sheet was used in a study instead of a PTFE coating. In the beginning (on day 5), this cathode produced an MPD of 750 mW/m^2, current density of 2.0 A/m^2 (32 A/m^3), a CE of 55%, low resistance of about 4 Ω, and total internal resistance of 131 Ω, causing no water leakage in the cathode and a drop in resistance by 400%. After day 42, the overall internal resistance built up to 160 Ω due to the loss of platinum catalyst by 8.26% (w/w) and development of the bacterial biofilm on the catalyst, thus limiting the ORR ability. Finally, after 53 days, the cathode potential declined gradually to 280 mW/m^2 and 1.4 A/m^2 (Tugtas et al. 2011). In another work, different ratios of PTFE to carbon (200%, 100%, 80%, and 60%) were tested on the cathode by Guerrini et al. They found that a high PTFE ratio prevented the transfer of the protons and inhibited the ORR electrocatalysis. The optimum ratio was between 60% and 80%, which produced a current of 700 μA after 52 days. It was observed that more carbon contact enhanced the current production. Oxygen was not a limiting factor, instead, the biomass activity slightly influenced the cathode (Guerrini et al. 2015).

Despite all the attempts for improving the performance of the cathode, there is an increase in the resistance due to the coated layers and the biofilm formation over the cathode. Furthermore, the erosion of the cathode causes development of the cracks, which leads to wetting of the cathode with subsequent decrease in its ORR activity. Therefore, different materials with different properties have been explored for making cathode, producing a varying range of voltage and current. One of the reasons that make MFC non-commercial is the use of precious metals like platinum as a catalyst on cathode for increasing the ORR activity (Song et al. 2020). Many

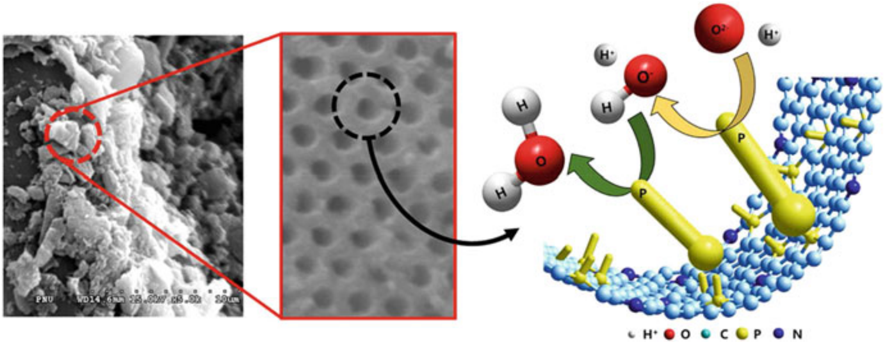

Fig. 8.4 Surface morphology of nitrogen- and phosphorus-doped ordered mesoporous carbon (NOPMC) illustrating the mechanism of oxygen reduction reaction. (Reproduced with permission from Ref. Song et al. (2020) © 2020 Elsevier publisher)

studies have been conducted on the cathode of an ML-MFC with efforts to achieve high ORR activity at a reasonable cost. Recently, in a research conducted by Song et al., nitrogen- and phosphorus-doped ordered mesoporous carbon (NPOMC) was used as a catalyst replacing platinum (Song et al. 2020). As elucidated in Fig. 8.4, the phosphate active sites on the mesoporous carbon surface are a doping center for the ORR, where the nanoporous structure of the catalyst helps to intensify the ORR activity and the nitrogen-doping sites cause a change in the charge distribution. The NPOMC in the ML-MFC gave only 30% of the ORR activity of Pt/C (154.0 mW/m^2) with 30–40% lower MPD. Moreover, gradual formation of biofilm on the surface of the catalyst (biofouling) blocked the nano-structured active sites of the NPOMC and drastically affected its performance and stability. The electrochemical performance showed results gave a current of 1.33 mA and an internal resistance of 286 Ω after 30 days of operation (Song et al. 2020).

In another work, canvas cloth was used for the fabrication of electrodes. The non-conductive material was made to conduct electricity by coating it with one of nickel or graphite along with manganese dioxide (MnO_2) as catalyst. The nickel-coated canvas produced a better result than the graphite-coated canvas with an MPD of 86.03 mW/m^2, COD reduction of 95%, and CE of 30.2% (Zhuang et al. 2009). In yet another study, bamboo charcoal coated with platinum was used as a cathode in an ML-MFC giving maximum power and voltage of 1.16 mW and 0.50 V, respectively (Yang et al. 2009). Feng et al. employed stainless steel coated with polypyrrole/anthraquinone-2-sulfonate film as a cathode. It produced an MPD of 575 mW/m^2, and the cathode showed a great ability to reduce oxygen and inhibit water leakage (Feng et al. 2011). Iron-based catalysts such as ricobendazole and niclosamide could be a possible alternative for platinum on the cathode electrode, as they have shown 20–25% higher efficiency than that of platinum (Santoro et al. 2016). Similarly, the ability pristine graphene to enhance extracellular electron transfer has been exploited for platinum-free electrodes, producing a volumetric power of 3.51 W/m^3 (Call et al. 2017). Thus, it can be concluded that sealing of the air-cathode electrode, choice of electrode material and cathode coating catalyst are crucial considerations to achieve a high ORR activity. Still, there is immense scope for improvement of the MFC with lots of undiscovered possibilities for the cathode—the main challenges being long lifetime, stable performance, and cost of the electrodes.

Anode Electrode

The anode material and its arrangement play a vital role in attachment of the bacteria, enrichment of the biofilm, oxidation of the substrate, as well as transfer of electrons between the bacteria and the electrode, which in turn affect the final output. Several methods of modification such as surface treatment (ammonia gas treatment (Cheng and Logan 2007), heat treatment (Feng et al. 2010), acid treatment, electrochemical oxidation), surface modification with nanomaterials, and surface coating with conductive polymers (Hindatu et al. 2017) have been used to improve the overall performance of an ML-MFC. All main parameters used for assessing the cathode electrode also hold true for the anode electrode; in addition, the anode should also be bio-compatible with the bacteria (Kumar et al. 2013).

A study used an anode made of carbon cloth treated with ammonia, while the cathode was carbon cloth with platinum catalyst. This combination led to a substantial improvement in CE by 20% compared to the untreated anode, and an MPD increment from 1640 to 1970 mW/m^2. The power attained was 7.5 times higher than that from the untreated electrode, and the start-up time was reduced by 50% (Cheng and Logan 2007). In another study, carbon fiber brush treated with acid and heat was used as the anode produced an MPD of 1370 mW/m^2, which was greater by 34% and 7% than that achieved by using untreated electrode and only heat treated electrode, respectively (Feng et al. 2010). Likewise, modification of the anode was done using nitric acid (CM-N) and ammonium nitrate (CM-A). CM-N performed better than CM-A giving an MPD of 792 mW/m^2 and CE of 24%, which were greater than that achieved using an untreated anode by 43% and 71%, respectively. Also, the modified anodes showed deep cracks and rough surface that aided in improved electron transferring property (Zhou et al. 2012). An MPD of 1788 mW/m^3 was achieved in a similar study by treating the anode surface with nitric acid and ammonia (Yang et al. 2014).

Lin et al. worked with different materials (stainless steel, copper, gold, and graphite carbon cloth) as electrodes. Copper anode showed erosion, while hindered electron transfer was observed in case of stainless steel. On the other hand, the gold electrode showed a very high performance. The best result in terms of open-circuit voltage (0.49 V) was achieved with the gold anode and carbon cloth cathode (Lin et al. 2013). Another study used an anode of graphite felt coated with iron oxide (Fe_2O_3) and ferric oxyhydroxide (FeOOH) giving an MPD of 18 W/m^3 (Wang et al. 2013). A voltage of 573 mV and an MPD of 884 mW/m^2 were obtained when polyaniline was used with a graphene-modified carbon cloth as an anode (Huang et al. 2016). A flame-oxidized stainless steel anode resulted in an MPD of 1063 mW/m^2 that was 24% higher compared to the untreated anode. Furthermore, it was also appreciably higher by 323% than the same MFC configuration with membrane (Yamashita et al. 2016).

Peng et al. added 5% of nickel-iron oxide ($NiFe_2O_4$) to the anode to achieve an MPD of 806.4 mW/m^2, reducing the internal resistance by 39% compared to the untreated anode (Peng et al. 2017). Using indium tin oxide coated glass as an anode produced the voltage and power output of 471 mV and 418.8 mW/m^2, respectively. However, its COD removal efficiency was lower than the carbon brush (Jiang et al. 2018). Another study found that the use of carbonized cotton textile modified with molybdenum carbide nanoparticles as an anode delivered an MPD of 1.12 W/m^2. This material offered a super performance in conductivity, high biocompatibility, strong electrochemical activity, and cost-effectiveness (Zeng et al. 2018). Graphene coated with iron sulfide (FeS_2) nanoparticles was employed as an anode on different substrates, achieving an MPD of 3220 mW/m^2 and an outstanding current density of 3.06 A/m^2 with COD removal of 1319 mg/l (Wang et al. 2018). Many studies have shown tremendous progress by improving the anode with different materials, nanoparticles and composites to give superior electrochemical performance. It is of prime significance to check the compatibility of the treated anode with bacteria, its conductivity and durability to ensure optimum efficiency.

8.2.2 Effect of Operating Temperature

Literature has proven that temperature has a great impact on the performance of ML-MFC by affecting the growth and survival of microbial communities, the conductivity of the substrate solution, internal resistance, and start-up time (Gadkari et al. 2020). A study on a single-chamber, air-cathode ML-MFC compared two operating temperatures (20 and 30 °C) and found a stable power density of 187 ± 8 mW/m^2 and CE of 10% at 30 °C. When the reactor was adjusted to 20 °C, MPD and CE decreased to 155 mW/m^2 and 8.9%, respectively. Moreover, as the temperature decreased from 30 to 20 °C, the cathode potential showed a massive drop by 315%; while the anode electrode potential lessened by 21% (Feng et al. 2008). Another work examined ML-MFC under different ranges of temperature, i.e. 20–35 and 8–22 °C. The higher working temperature range resulted in higher COD removal of 90%, lower current of 0.7 mA, and CE 1.5%. On the contrary, at lower temperature range, the COD removal reduced to 59%, current rose to 1.4 mA, and CE increased to 5%. Thus, the lower temperature range favored the current and coulombic efficiency, while higher temperature worked better for COD removal (Jadhav and Ghangrekar 2009). However, a different trend was observed in a continuous flow ML-MFC operating at 30 °C, which showed a power density of 422 mW/m^2 (12.8 W/m^3), COD removal of 26%, and CE of 1.7%. At ambient temperature (23 °C), the power density reduced to 345 mW/m^2 (10.5 W/m^3), COD removal reduced to 19%, while CE dropped to a mere 0.7% (Ahn and Logan 2010). Another study analyzed the effects of varying temperature in the range 15–35 °C, and found the best results at 35 °C showing MPD, current and CE of 74 mW/m^3, 2.51 mA, and 10%, respectively (Tee et al. 2018).

The start-up temperature must be taken into consideration for maintaining longer stability of power in batch and continuous mode ML-MFCs. Generally, the microbial community's sensitivity towards temperature decides on the maximum operating temperature, beyond which the bacterial activity degenerates. In most studies, the observed optimum temperature range from 20 to 32 °C favored the growth of methanogenic bacteria and demonstrated a linear rise in the power output. The highest limit of operating temperature is usually 35 °C, above which the reactor efficiency begins to decline.

8.2.3 Effect of pH

The bioactivity of the microbial community is considerably dependent on the pH of the substrate (Marashi and Kariminia 2015). In a study, an increase of the pH value above 8.5 led to the precipitation of carbonates on the cathode surface in batch mode of ML-MFC reactors. The deposition of the carbonate layer acted as a barrier and decreased the electrochemically-active area, similar to the PTFE effect on cathode activity (Guerrini et al. 2015). Another work tested the effect of pH in the range 5.5–7.5, and found that the internal resistance decreased as the pH difference between the anode and cathode solutions increased. The highest current was generated within an optimum pH range of 6.0–7.0 indicating the lower microbial activity at sub-optimal pH compared to the optimal pH (Jadhav and Ghangrekar 2009). Similar observations were found in yet another study that concluded the optimal pH of an ML-MFC as 7 (Gil et al. 2003). The effect of three different pH conditions (5.5, 7.0, and 8.5) was assessed on an ML-MFC. The highest power density was observed at pH 8.5 that was greater by 40% and 66% than the power densities observed at pH values of 7.0 and 5.4, respectively. This was evident as even though acidogenic and methanogenic bacteria are inactive in alkaline conditions, electrogenic bacteria are active in that environment (Marashi and Kariminia 2015). When the pH of the anodic solution increased from 5.4 to 9.0 due to the hydrolysis of the urea, it caused a decrease in the anodic performance, implying that the anode can take a limited pH working range (Santoro et al. 2013). The acidogenic bacteria have been shown to be active at pH 5.5, where the hydrogen production dominates overcoming the degradation of the pollutants and decreasing COD removal, as compared to neutral or alkaline conditions (Marashi and Kariminia 2015). In contrast, alkaline environment is favorable for the electrogenic bacteria leading to a higher power generation (Yuan et al. 2011).

8.2.4 Effect of Substrate Pretreatment

The quality of the substrate fed to the reactor is a crucial factor influencing the performance of the ML-MFC. The organic molecules need to be decomposed and dissolved in the substrate for the bacteria to be able to consume them. In a study, a sludge was kept in the anode chamber for 15 days, followed by heat treatment at 100 °C and cooling to room temperature. It was then re-inoculated, while no inoculation was done for the cathode. As a result, the COD removal efficiency reached 91.4% at an organic loading rate (OLR) of 2.65 kg COD/m^3.d, giving a maximum power density of 6.73 mW/m^2, and a current density of 70.74 mA/m^2 (Ghangrekar and Shinde 2008). In a similar way, a power density of 0.32 $\pm$ 0.01 W/m^2 was obtained with a sludge fermented for 9 days at 30 °C prior to dilution with the primary effluent, whereas the untreated primary effluent gave a power density of 0.24 $\pm$ 0.03 W/m^2. The fermentation caused a reduction in the total suspended solids from 26.1 to 16.5 g/l, and the pH from 5.7 to 4.5. Additionally, it increased the conductivity from 2.4 mS/cm to 4.7 mS/cm (Yang et al. 2013).

8.2.5 Effect of Reactor Design

The design of ML-MFC reactor is the cornerstone for deciding the type of cathode material and assembly suitable for the reactor. The cathode can be either submerged completely in the substrate or it can be placed as an air-cathode electrode, where one half has contact with the substrate and the other half is exposed to the air. For the air-cathode, the ORR reaction occurs with the oxygen in the air; while for the submerged cathode, air is supplied through a compressor. The anode electrode is placed inside the substrate.

In a study, an ML-MFC was designed with a cylindrical shape having a diameter of 10 cm and a height of 100 cm. Graphite felt was used as the anode (surface area of 465 cm^2) and placed at the bottom of the reactor; with glass wool (4 cm depth) and glass bead (4 cm depth) placed above the anode, as shown in Fig. 8.5. The cathode, made of graphite felt (surface area of 89 cm^2), was placed at the top of the reactor and the compartment was aerated. The electrode spacing was varied between 10 and 30 cm. The inlet of wastewater was from the bottom and after passing through the layers, it was discharged from the top. The set-up yielded a power density of 1.3 mW/m^2 at a current density of 6–9 mA/m^2 (Jang et al. 2004). A similar design by Ghangrekar and Shinde gave the maximum voltage of 358 mV, power density of 10.9 mW/m^2, COD removal of 88%, and BOD removal of 87% (Ghangrekar and Shinde 2007). A two-chambered cylindrical ML-MFC of Plexiglas was designed with a diameter of 75 mm and a height of 100 mm. The two chambers were separated by a carbon paper, and the electrodes too were made of carbon paper. The cathode electrode was coated with platinum and dipped into the cathode chamber. After 400 h long run, the maximum voltage output of 551 mV and power density of 121 mW/m^2 were attained (Luo et al. 2007). In another work, the cathode and anode compartments were placed at different levels, the cathode chamber was located above from the anode chamber so that the outlet of anode was connected to the inlet of cathode through a valve. The substrate was driven by gravity into the anode from the storage container that was placed at a higher level than the cathode. The air was pumped into cathode chamber, the influent entered from the anode and went through the connection to the cathode. The reactor assembly resulted in a maximum voltage of 160.7 mV, an MPD of 24.33 mW/m^3, COD removal efficiency of 90.45% (Du et al. 2011).

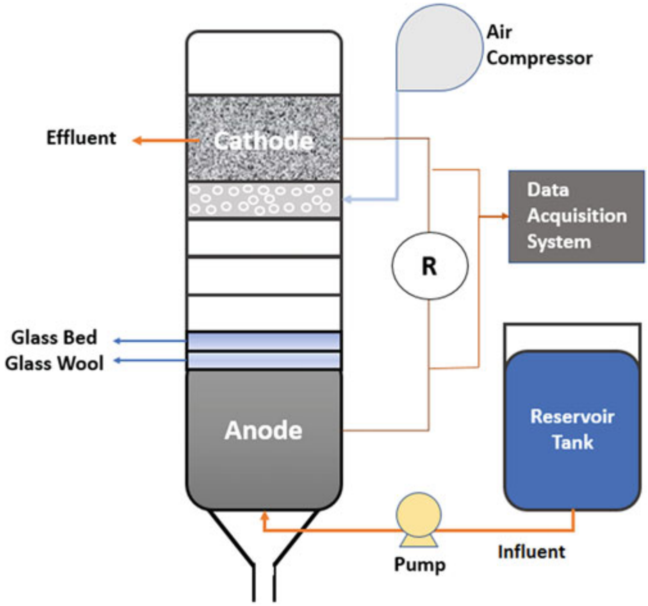

Fig. 8.5 Cylindrical ML-MFC with anode and cathode placed inside the reactor. (Redrawn with modifications from Ref. Jang et al. (2004))

The cylindrical reactors have the advantage of capturing maximum number of protons escaping from the anode, where the influent enters in from the bottom of the reactor and leaves from the top. The influent comes in contact with the cathode as it emerges out of the reactor, which accelerates the movement of hydrogen from anode to cathode. Moreover, the speed of the fluid flow affects the electricity production and the treatment of influent. Despite many advantages of the cylindrical reactor, there are some drawbacks associated with these types of designs. Firstly, the spacing between the two electrodes affects the reactor output and needs to be optimized. Secondly, there is a requirement for external air pumping into the cathode compartment. Thirdly, when the cathode is completely immersed in the substrate, it leads to the rapid formation of the biofilm resulting in biofouling, thus lowering the performance of the cathode. Finally, the cathode area may not be enough for the reactor; the literature shows that the optimal ratio of the cathode size to the anode size must be 2:1 or higher (Cheng and Logan 2011).

Another variation in the reactor design could be wherein the cathode is placed outside the reactor. In a study, a cylinder-shaped reactor made of Plexiglas was designed having 3 cm diameter, 13 cm height, and evenly drilled holes on the wall, as shown in Fig. 8.6. The cathode was a cylinder wrapped with a flexible carbon cloth coated with C/Pt, as an air-cathode. Carbon granules, with a surface area of 31 cm^2, were used as the anode. The inlet was from the bottom, while the effluent exited from the top. The reactor displayed a voltage of 0.384 V with a maximum volumetric power of 50.2 W/m^3 at a current density of 216 A/m^3 (with an internal

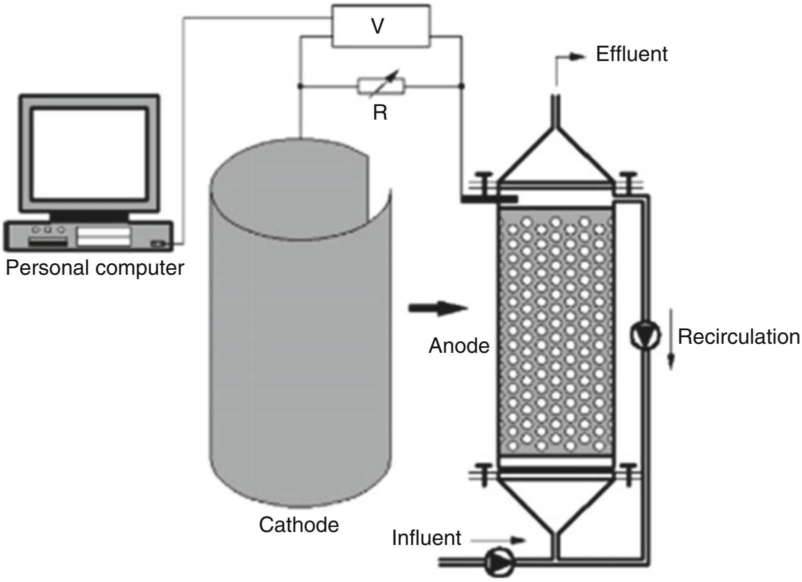

Fig. 8.6 Cylindrical ML-MFC with anode inside and cathode placed outside the reactor. (Reproduced with permission from Ref. You et al. (2007) © 2007 Elsevier publisher)

resistance of 27 Ω) (You et al. 2007). Furthermore, this design helped to have a longer lifespan and higher performance of the cathode, simultaneously decreasing the overall internal resistance by minimizing the distance between the cathode and the anode.

A modified ML-MFC configuration was developed to solve the issue of distance between cathode and anode. The design consisted of a twin cylindrical compartment with a volume of 1.85 l, an air-cathode made of carbon cloth coated with platinum from the air-facing side, and a brush-type anode made of carbon cloth connected with titanium wires. The anode and cathode were placed on each end of the compartment, with the cathode being at a distance of 1 cm from the anode. The design resulted in an MPD of 39–53 mW/m^2 from the cattle manure solid waste (Lee and Nirmalakhandan 2011). Liu and Logan used a Plexiglas cylindrical container open from both the sides with 4 cm length and 3 cm diameter, as shown in Fig. 8.7. The anode and cathode electrodes were placed on opposite sides of the cylinder. The anode was made of carbon paper and the cathode was made of carbon cloth coated with platinum on the air-facing side. The inner side of the cathode was examined with and without PEM. The MFC in the absence of PEM could achieve an MPD of 146 mW/m^2 with 20% CE using domestic wastewater as the substrate; while in the presence of PEM, it produced an MPD of 28 mW/m^2 at 28% CE (Liu and Logan 2004). A similar reactor was built with a glass tube placed on top of the reactor (4 cm long and 1.4 cm inner diameter) with a perforated cap to help the aerobic bacteria access air. The anode was made of carbon fiber brush, and the cathode was made of carbon cloth coated with platinum. The results showed an MPD of 268.5 mW/m^2,

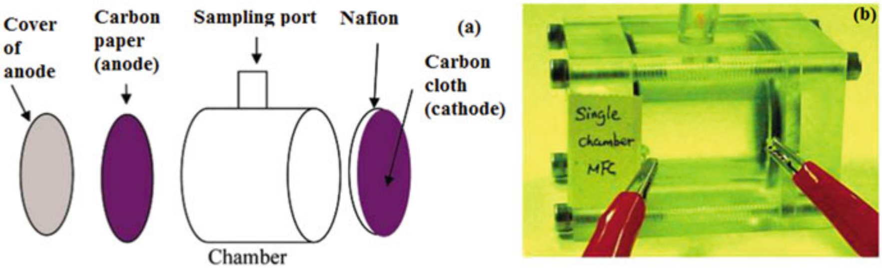

Fig. 8.7 Design of a lab-scale single-chamber ML-MFC. (Reproduced with permission from Ref. Liu and Logan (2004) © 2004 ACS publisher)

COD removal of 67%, phosphorus removal of 97%, and ammonia removal of 99% (Jiang 2017). It implies that the productivity of small-sized reactors is generally higher than that of big-sized reactors due to the lower internal resistance of the former. However, a disadvantage of these designs is that the mixing of cathodic and anodic compartments is inevitable, thus creating biofouling on the inner side of cathode.

In a different work, a micro-sized ML-MFC was designed with dimensions of 15 × 5 mm, thickness of 0.37 mm with a volume of 83 μl, with the anode and cathode made of carbon cloth (Fig. 8.8). Glass fiber was placed between the electrodes to alleviate the mixing of the fluids from the two compartments and for assisting in hydrogen transfer. The electrons were transferred through the titanium foils connected to each electrode. The electrodes were held with the help of acrylic cover plates. The cell was sealed with a silicone pad stacked vertically, resulting in an MPD of 3.2 mW/cm^3 (Ye et al. 2018).

Many efforts have been made for using biochar as electrode to develop economical and environmentally-friendly MFCs without compromising on their performance. A study fabricated a cathode using a bamboo tube, by carbonizing it at 900 °C in nitrogen atmosphere followed by heat treatment at 350 °C to increase porosity (Fig. 8.9). The cathode was also brushed with polytetrafluoroethylene solution on the external side to make it water proof. A carbon fiber brush was used as the anode. The cell produced an MPD of 40.4 W/m^3 and CE of 55% (Yang et al. 2017). The biomass materials can be a great replacement for expensive electrode materials for ML-MFCs due to their renewability, wide availability, and low cost. One of the approaches to better design with low cost is preventing mixing of anolyte with catholyte, minimizing oxygen intrusion, avoiding biofilm formation, and alleviating cathode deterioration. This can be achieved by putting a separator or an additional anode in the container to create a two-chamber ML-MFC (Kim et al. 2016). Recently, Nawaz and his team assembled a conical dual-chambered ML-MFC fabricated from graphite-based materials, as shown in Fig. 8.10. The cathode chamber was concentrically placed inside the anode chamber with 4 mm space in between them. The anode was sealed from the top with an acrylic lid, and air was pumped into the cathode. This design accomplished a treatment efficiency of 84.4% and MPD of 15.03 W/m^3 (Nawaz et al. 2020).

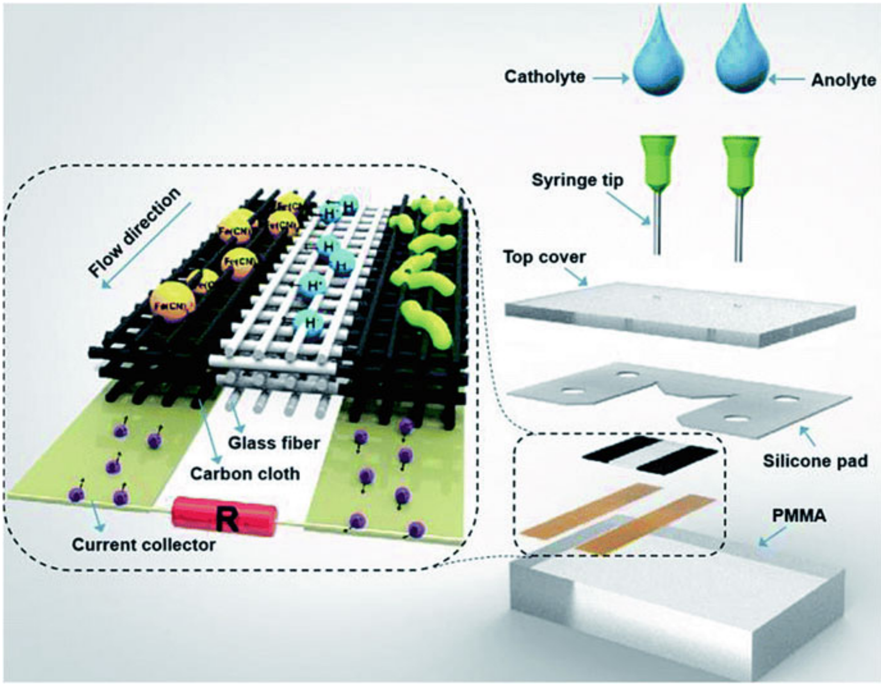

Fig. 8.8 Design of a millimeter scale ML-MFC. (Published from Ref. Ye et al. (2018) under CC BY-NC 3.0 license)

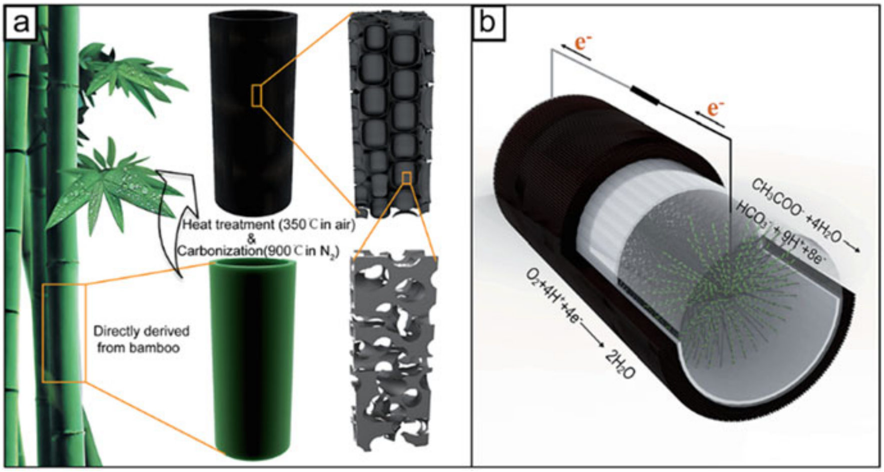

Fig. 8.9 (**a**) Fabrication process of cathode from bamboo tube; (**b**) MFC with cathode made from bamboo tube. (Published from Ref. Yang et al. (2017) under CC BY 3.0 license)

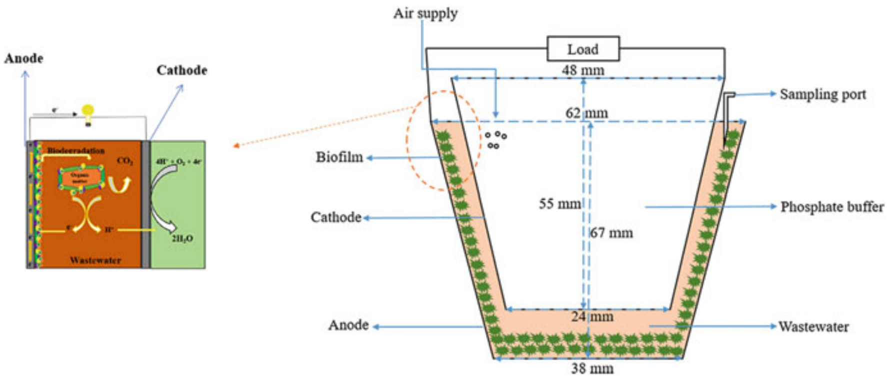

Fig. 8.10 Schematic representation of an ML-MFC with two containers concentrically placed inside one another. (Reproduced with permission from Ref. Nawaz et al. (2020) © 2020 Elsevier publisher)

8.2.6 Effect of Electrode Surface Area and Electrode Spacing

The surface area of electrodes and the spacing between them are crucial factors to be taken into account for optimum performance of the ML-MFCs. A study investigated the effects of varying the electrode spacing (20, 24, and 28 cm) and anode area. It was found that more MPD could be achieved when the electrodes were placed close to each other. The maximum voltage of 358 mV was obtained at a distance of 20 cm. The power densities of 4.66, 6.45, and 10.13 mW/m^2 were achieved at anode surface area of 210.64, 140.43, and 70.21 cm^2 respectively. It shows that the power density decreases with increasing surface area of the anode (Ghangrekar and Shinde 2007). The trials for scale-up of ML-MFC showed that doubling the cathode surface area increased the power output by 62% and doubling the anode surface area increased the power output by 12% (Cheng and Logan 2011). In an experiment with a three-column MFC connected in series, an enhancement in the anode surface area from 360 to 1080 cm^2 in each column increased the maximum power output by 264% for column 1, 118% for column 2, and 151% for column 3. Also, the COD and BOD removal efficiencies were increased by 137% for column 1, 279% for column 2, 182% for column 3, and 63% for column 1, 161% for column 2 and 159% for column 3, respectively (Gálvez et al. 2009).

8.2.7 Effect of Substrate Conductivity

The conductivity of the substrate is another factor having a profound influence on the output performance of the ML-MFCs. One of the ways of enhancing the conductivity is by adding metal ions to the substrate. However, the addition of metal ions creates a toxic environment for the microorganisms. So MFCs are limited by the requirement to compromise between the toxicity issue and conductivity of the substrate (Dong et al. 2015). It has been found that when the ions are added, the MPD initially increased linearly with rising electrolyte conductivity, the MPD escalated from 0.11 W/m^2 at a conductivity of 1e^{-4} Sm^{-1} to 1.02 W/m^2 at a conductivity of 1e^{-2} Sm^{-1}. Further increase in conductivity beyond 2e^{-1} Sm^{-1} showed no more improvement in MPD (Gadkari et al. 2020).

In a trial, phosphate buffer was added to the substrate solution to increase its conductivity, which increased the power density from 1330 to 1640 mW/m^2 (Cheng and Logan 2007). In a similar test, increasing the concentration of phosphate buffer from 50 to 200 mM caused the MPD to rise from 438 to 528 mW/m^2 (Feng et al. 2008). In another study, addition of sodium acetate solution to the substrate was found to enhance the overall power density to 400 mW/m^2 (Jiang and Li 2009). Likewise, putting as little as 0.5% of NaCl and Na_2SO_4 (1:1 ratio) changed the power density from 34 to 43 mW/m^2 and augmented the phenol removal rate by 4%. Increasing the amount of salt to 1% raised the MFC power density to 45 mW/m^2, whereas 2% salt resulted in an inhibitory effect on power generation (Du et al. 2015; Mousavi et al. 2016). Thus, inorganic salts can greatly improve the conductivity and decrease the resistance in the solution, thus boosting the efficiency of ML-MFCs.

Most studies are in agreement with each other, where the output power increases with increasing ionic strength of the substrate. Higher conductivity implies better ionic conduction and ohmic loss reduction, which results in the improvement of power output from the ML-MFCs. Having said that, there is a limit to the degree of salinity that the substrate solution can take. It is important to ensure that the bacterial cultures do not get negatively impacted by the high salinity of the solution, since high content of salt may be intolerable for bacteria (Mousavi et al. 2016; Aaron et al. 2010). Thus, the bacterial response to external stimuli as regards the substances added to improve the ionic strength of the substrate should be carefully evaluated.

8.3 Water Treatment (Substrate)

The substrate is of paramount importance in any biological process, serving as the nutrient and energy source, and has a tremendous impact on the energy production in ML-MFCs (Yang et al. 2009). A countless variety of substrates comprised of organic matter in pure forms or diverse mixtures from wastewaters or lignocellulosic biomass are widely used in MFCs (Pant et al. 2010). Several ways are available for treating the wastewaters, the most common method being the aerobic activated sludge process, which requires pumping large amounts of air or oxygen into the reserve tanks (Capodaglio and Olsson 2020). The oxygen supplying expenses are estimated to be between 50% and 70% of the entire energy demand of a conventional facility (Capodaglio and Olsson 2020). Here, we focus on the ability of ML-MFC in wastewater treatment in respect of its organic, inorganic, and heavy metal content. Different researchers have connoted the ML-MFC performance with different measurable parameters such as current (or current density in mA/cm^2 or mA/m^3), voltage (open circuit or close circuit), COD/BOD reduction, MPD, CE, inorganic or heavy metal reduction to evaluate the efficiency of various substrate.

Many studies have attempted to maximize the efficiency of ML-MFCs for treating wastewater substrates containing organic materials as well as producing power. According to a study, glucose and wastewater produced an MPD of 494 and 146 mW/m^2 with CE of 9–12% and 20%, respectively. The glucose-fed unit could achieve glucose removal efficiency of 98%. Using a PEM, the same study gave an MPD of 262 and 28 mW/m^2 with CE of 40–55% and 28%, respectively (Liu and Logan 2004). It indicated that in the absence of PEM, the MPD increased and CE decreased due to considerable oxygen diffusion into the anode. Moreover, the concentration of glucose also affected the power output, the MPD improved with increasing glucose concentration. Similar observations were made in another research using acetate and butyrate as substrates, resulting in an MPD of 506 and 305 mW/m^2, respectively (Liu et al. 2005). The power density was found to be 54% higher for acetate, and 57% higher for butyrate compared to that obtained using a PEM (Liu et al. 2005). Feng et al. reported MPD values for beer brewery wastewater, glucose (0.6 g/l), and domestic wastewater as 205, 494, and 146 mW/m^2, respectively (Feng et al. 2008). The COD removal efficiency increased from 54% to 98% when the strength of brewery wastewater rose from 84 to 1600 mg/l.

Coal-tar refinery wastewater as substrate was reported to produce an MPD of 4.5 mW/m^2 at a voltage of 543 mV, along with a COD reduction of 88%, 57% of sulfate elimination, and 41% of sulfur removal. Furthermore, the ML-MFC could remove over 90% of phenol and 2-methyl phenol (Park et al. 2012). Passage of human urine through an ML-MFC achieved a current of 0.18–0.23 mA (Santoro et al. 2013). The initial COD of 10.9 g/l was reduced to 3.6 g/l after 4-day retention in the batch mode MFC unit. A study analyzed the effect of varying substrate concentration using phenol in the range of 25–200 mg/l. As the concentration of phenol increased from 25 to 100 mg/l, an increase in the removal of phenol from 80% to 97% was observed. It could attain an MPD of 49.8 mW/m^2 and a current density of 292.8 mA/m^2 (Buitrón and Moreno-Andrade 2014). An ML-MFC unit used rice straw pretreated with acid (to degrade cellulose) to generate an MPD of 137.6 mW/m^2, and COD removal efficiency of 79% at an initial COD of 400 mg/l (Wang et al. 2014). Similarly, purified terephthalic acid wastewater produced an MPD of 65.6 mW/m^2 at COD of 8000 mg/l (Marashi and Kariminia 2015).

In another study, dairy, leather, and sewage wastewaters generated maximum power levels of 1.98, 1.95, and 1.28 mW, along with COD reduction levels of 85.4%, 80%, and 65%, respectively (Aswin et al. 2017). Similarly, other lignocellulose materials used as substrate have resulted in an MPD of 29 mW/m^3 (Adekunle et al. 2016). Dye processing wastewater tested at several organic loadings resulted in an MPD of 515 mW/m^2, CE of 56%, and COD reduction of 85% at an organic loading of 1.0 g/l COD (Karuppiah et al. 2018). In a different work, petroleum refinery wastewater demonstrated treatment ratio and power density of 45.06% and 28.27 W/m^3, while the corresponding values for whey wastewater were found to be 72.76% and 23.23 W/m^3, respectively (Mohanakrishna et al. 2018). Wood, being rich in organic materials, also has potential as fuel for MFCs; for example, an MPD of 8555 mW/m^2 was reported using poplar wood (Erensoy and Çek 2018). Yet another investigation used tomato waste as the substrate producing an MPD of

60.041 mW/m^2, current density of 99.174 mA/m^2, and voltage of 0.701 V (Kamau et al. 2018).

Lately, many studies have attempted the heavy metal (sulfur, copper, mercury) removal using ML-MFCs due to their extreme toxicity and carcinogenicity. Various heavy metals are released in the effluents from tanning, cement, electroplating, and dye industries. In a study, a hydrolyzed heavy metal-containing wheat grain (HMWG) used as a substrate produced an MPD of 381 mW/m^2, a CE of 15.7%, and COD reduction of 83.4% (Yuan et al. 2018). Increasing the concentration of the HMWG hydrolysate slowed down the electricity production in the reactor. In another work, Cu (II) was used as an electron acceptor to explore the mechanism of metal treatment in an ML-MFC. A low ratio of Cu (II) resulted in heavy metal reduction efficiency of 87.56%, whereas the heavy metal reduction efficiency dropped to 36.98% with increasing ratio of Cu (II) in the substrate. Furthermore, it also caused the voltage to drop from 71 to 11.1 mV (Chan et al. 2020).

In a similar way, the treatment of inorganics has been achieved in ML-MFCs. In a study on domestic wastewater treatment, the results showed maximum power generation of 200 mW, COD reduction of 75%, 68% removal of ammonical nitrogen, and 90% reduction in suspended solids (Ge and He 2016). Likewise, yogurt wastewater tested as substrate produced an MPD of 1043 mW/m^2, with COD and NH_4-N reduction efficiencies of 87% and 74%, respectively (Luo et al. 2017). A supercapacitor-MFC showed high pollutant removal rates; 59.4% of COD, 78.2% of NH_4-N, 77.8% of nitrogen, and achieved an MPD of 298 mW/m^2 (Cai et al. 2020b).

8.4 Conclusion

The MFC technology has a huge potential for clean, safe, and sustainable production of bioenergy using industrial or domestic wastewater. The ML-MFCs have outperformed the conventional MFCs with a membrane with far better efficiencies for electricity generation as well as water treatment. The presence of a membrane restricts the speed of hydrogen ion movement, thus negatively influencing the power output. Despite the several advantages of ML-MFCs and progress achieved, many challenges still need to be addressed. One of the challenges is the decline in electricity generation over time due to the biofouling of the cathode, which adds to the operational cost for replacing the cathode once it loses efficiency. Also, the oxygen intrusion from the cathode to the anode compartment leads to a decline in the performance of the anode. Furthermore, cathode catalyst degradation is still a major issue affecting the efficiency of the cathode and the CE of the MFC.

The design of ML-MFC is a crucial aspect as it can help save energy losses by decreasing the internal resistance and enhance cathode efficiency. Having a well-sealed air-cathode in a single-chamber ML-MFC could provide high output, and stable performance in the long run with easy maintenance. As for the substrate, the concentration of the metals and organics have a significant impact on the power generation and pollutant reduction. High toxicity of the substrate may result in decline of the bacteria activity. Also, excessive salinity of the substrate may prove detrimental for the bacteria, thus it is important to achieve a balanced solution conductivity for optimal bacterial activity.

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References

Aaron D, Tsouris C, Hamilton CY, Borole AP (2010) Assessment of the effects of flow rate and ionic strength on the performance of an air-cathode microbial fuel cell using electrochemical impedance spectroscopy. Energies 3:592–606. https://doi.org/10.3390/en3040592

Adekunle A, Gariepy Y, Lyew D, Raghavan V (2016) Energy recovery from cassava peels in a single-chamber microbial fuel cell. Energy Sources Part A Recover Util Environ Eff 38:2495–2502. https://doi.org/10.1080/15567036.2015.1086909

Ahn Y, Logan BE (2010) Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures. Bioresour Technol 101:469–475. https://doi.org/10.1016/j.biortech.2009.07.039

Aswin T, Sabarunishabegum S, Sikkandar MY (2017) Optimization of microbial fuel cell for treating industrial wastewater and simultaneous power generation. Int J Chem Sci 15:132–143

Buitrón G, Moreno-Andrade I (2014) Performance of a single-chamber microbial fuel cell degrading phenol: effect of phenol concentration and external resistance. Appl Biochem Biotechnol 174:2471–2481. https://doi.org/10.1007/s12010-014-1195-5

Cai T, Meng L, Chen G et al (2020a) Application of advanced anodes in microbial fuel cells for power generation: a review. Chemosphere 248:125985. https://doi.org/10.1016/j.chemosphere.2020.125985

Cai T, Jiang N, Zhen G et al (2020b) Simultaneous energy harvest and nitrogen removal using a supercapacitor microbial fuel cell. Environ Pollut 266:115154. https://doi.org/10.1016/j.envpol.2020.115154

Call TP, Carey T, Bombelli P et al (2017) Platinum-free, graphene based anodes and air cathodes for single chamber microbial fuel cells. J Mater Chem A 5:23872–23886. https://doi.org/10.1039/c7ta06895f

Capodaglio AG, Olsson G (2020) Energy issues in sustainable urban wastewater management: use, demand reduction and recovery in the urban water cycle. Sustainability 12:226. https://doi.org/10.3390/su12010266

Chan KK, Thung WE, Ong SA et al (2020) Simultaneous heavy metal reduction and voltage generation with synergy membrane-less microbial fuel cell. IOP Conf Ser Earth Environ Sci 463. https://doi.org/10.1088/1755-1315/463/1/012067

Cheng S, Logan BE (2007) Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. Electrochem Commun 9:492–496. https://doi.org/10.1016/j.elecom.2006.10.023

Cheng S, Logan BE (2011) Increasing power generation for scaling up single-chamber air cathode microbial fuel cells. Bioresour Technol 102:4468–4473. https://doi.org/10.1016/j.biortech.2010.12.104

Cheng S, Liu H, Logan BE (2006) Increased performance of single-chamber microbial fuel cells using an improved cathode structure. Electrochem Commun 8:489–494. https://doi.org/10.1016/j.elecom.2006.01.010

Clauwaert P, Verstraete W (2009) Methanogenesis in membraneless microbial electrolysis cells. Appl Microbiol Biotechnol 82:829–836. https://doi.org/10.1007/s00253-008-1796-4
Dong Y, Qu Y, He W et al (2015) A 90-liter stackable baffled microbial fuel cell for brewery wastewater treatment based on energy self-sufficient mode. Bioresour Technol 195:66–72. https://doi.org/10.1016/j.biortech.2015.06.026
Du F, Xie B, Dong W et al (2011) Continuous flowing membraneless microbial fuel cells with separated electrode chambers. Bioresour Technol 102:8914–8920. https://doi.org/10.1016/j.biortech.2011.07.056
Du Y, Feng Y, Teng Q, Li H (2015) Effect of inorganic salt in the culture on microbial fuel cells performance. Int J Electrochem Sci 10:1316–1325
Erensoy A, Çek N (2018) Alternative biofuel materials for microbial fuel cells from poplar wood. ChemistrySelect 3:11251–11257. https://doi.org/10.1002/slct.201802171
Feng Y, Wang X, Logan BE, Lee H (2008) Brewery wastewater treatment using air-cathode microbial fuel cells. Appl Microbiol Biotechnol 78:873–880. https://doi.org/10.1007/s00253-008-1360-2
Feng Y, Yang Q, Wang X, Logan BE (2010) Treatment of carbon fiber brush anodes for improving power generation in air-cathode microbial fuel cells. J Power Sources 195:1841–1844. https://doi.org/10.1016/j.jpowsour.2009.10.030
Feng C, Wan Q, Lv Z et al (2011) One-step fabrication of membraneless microbial fuel cell cathode by electropolymerization of polypyrrole onto stainless steel mesh. Biosens Bioelectron 26:3953–3957. https://doi.org/10.1016/j.bios.2011.02.046
Gadkari S, Fontmorin JM, Yu E, Sadhukhan J (2020) Influence of temperature and other system parameters on microbial fuel cell performance: numerical and experimental investigation. Chem Eng J 388:124176. https://doi.org/10.1016/j.cej.2020.124176
Gálvez A, Greenman J, Ieropoulos I (2009) Landfill leachate treatment with microbial fuel cells; scale-up through plurality. Bioresour Technol 100:5085–5091. https://doi.org/10.1016/j.biortech.2009.05.061
Ge Z, He Z (2016) Long-term performance of a 200 liter modularized microbial fuel cell system treating municipal wastewater: treatment, energy, and cost. Environ Sci Water Res Technol 2:274–281. https://doi.org/10.1039/c6ew00020g
Ghangrekar MM, Shinde VB (2007) Performance of membrane-less microbial fuel cell treating wastewater and effect of electrode distance and area on electricity production. Bioresour Technol 98:2879–2885. https://doi.org/10.1016/j.biortech.2006.09.050
Ghangrekar MM, Shinde VB (2008) Simultaneous sewage treatment and electricity generation in membrane-less microbial fuel cell. Water Sci Technol 58:37–43. https://doi.org/10.2166/wst.2008.339
Gil GC, Chang IS, Kim BH et al (2003) Operational parameters affecting the performance of a mediator-less microbial fuel cell. Biosens Bioelectron 18:327–334. https://doi.org/10.1016/S0956-5663(02)00110-0
Guerrini E, Grattieri M, Faggianelli A et al (2015) PTFE effect on the electrocatalysis of the oxygen reduction reaction in membraneless microbial fuel cells. Bioelectrochemistry 106:240–247. https://doi.org/10.1016/j.bioelechem.2015.05.008
Hassan SHA, Gad El-Rab SMF, Rahimnejad M et al (2014) Electricity generation from rice straw using a microbial fuel cell. Int J Hydrog Energy 39:9490–9496. https://doi.org/10.1016/j.ijhydene.2014.03.259
Hindatu Y, Annuar MSM, Gumel AM (2017) Mini-review: anode modification for improved performance of microbial fuel cell. Renew Sust Energ Rev 73:236–248. https://doi.org/10.1016/j.rser.2017.01.138
Huang L, Li X, Ren Y, Wang X (2016) In-situ modified carbon cloth with polyaniline/graphene as anode to enhance performance of microbial fuel cell. Int J Hydrog Energy 41:11369–11379. https://doi.org/10.1016/j.ijhydene.2016.05.048

Jadhav GS, Ghangrekar MM (2009) Performance of microbial fuel cell subjected to variation in pH, temperature, external load and substrate concentration. Bioresour Technol 100:717–723. https://doi.org/10.1016/j.biortech.2008.07.041

Jang JK, Pham TH, Chang IS et al (2004) Construction and operation of a novel mediator- and membrane-less microbial fuel cell. Process Biochem 39:1007–1012. https://doi.org/10.1016/S0032-9592(03)00203-6

Jia Y, Feng H, Shen D et al (2018) High-performance microbial fuel cell anodes obtained from sewage sludge mixed with fly ash. J Hazard Mater 354:27–32. https://doi.org/10.1016/j.jhazmat.2018.04.008

Jiang HM (2017) Combination of microbial fuel cells with microalgae cultivation for bioelectricity generation and domestic wastewater treatment. Environ Eng Sci 34:489–495. https://doi.org/10.1089/ees.2016.0279

Jiang D, Li B (2009) Novel electrode materials to enhance the bacterial adhesion and increase the power generation in microbial fuel cells (MFCs). Water Sci Technol 59:557–563. https://doi.org/10.2166/wst.2009.007

Jiang Q, Xing D, Zhang L et al (2018) Interaction of bacteria and archaea in a microbial fuel cell with ITO anode. RSC Adv 8:28487–28495. https://doi.org/10.1039/c8ra01207e

Jiménez González ML, Benítez CH, Juarez ZA et al (2020) Study of the effect of activated carbon cathode configuration on the performance of a membrane-less microbial fuel cell. Catalysts 10:619. https://doi.org/10.3390/catal10060619

Jothinathan D, Nasrin Fathima AH, Mylsamy P et al (2018) Microbial fuel cell research using animal waste: a feebly-explored area to others. In: Microbial fuel cell technology for bioelectricity. Springer, Cham, pp 151–168

Kamau JM, Mbui DN, Mwaniki JM, Mwaura FB (2018) Utilization of rumen fluid in production of bio–energy from market waste using microbial fuel cells technology. J Appl Biotechnol Bioeng 5:227–231. https://doi.org/10.15406/jabb.2018.05.00142

Karuppiah T, Pugazhendi A, Subramanian S et al (2018) Deriving electricity from dye processing wastewater using single chamber microbial fuel cell with carbon brush anode and platinum nano coated air cathode. 3 Biotech 8. https://doi.org/10.1007/s13205-018-1462-1

Khan MD, Khan N, Sultana S et al (2018) Microbial fuel cell: waste minimization and energy generation. In: Khan OM, Mohammad Z, Iqbal I (eds) Modern age environmental problems and their remediation. Springer, Cham, pp 129–146

Kim J, Kim B, An J et al (2016) Development of anode zone using dual-anode system to reduce organic matter crossover in membraneless microbial fuel cells. Bioresour Technol 213:140–145. https://doi.org/10.1016/j.biortech.2016.03.012

Kumar GG, Sarathi VGS, Nahm KS (2013) Recent advances and challenges in the anode architecture and their modifications for the applications of microbial fuel cells. Biosens Bioelectron 43:461–475. https://doi.org/10.1016/j.bios.2012.12.048

Lee Y, Nirmalakhandan N (2011) Electricity production in membrane-less microbial fuel cell fed with livestock organic solid waste. Bioresour Technol 102:5831–5835. https://doi.org/10.1016/j.biortech.2011.02.090

Li WW, Yu HQ, He Z (2014) Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies. Energy Environ Sci 7:911–924. https://doi.org/10.1039/c3ee43106a

Lin CC, Wei CH, Chen CI et al (2013) Characteristics of the photosynthesis microbial fuel cell with a Spirulina platensis biofilm. Bioresour Technol 135:640–643. https://doi.org/10.1016/j.biortech.2012.09.138

Liu H, Logan BE (2004) Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ Sci Technol 38:4040–4046. https://doi.org/10.1021/es0499344

Liu H, Ramnarayanan R, Logan BE (2004) Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ Sci Technol 38:2281–2285. https://doi.org/10.1021/es034923g

Liu H, Cheng S, Logan BE (2005) Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ Sci Technol 39:658–662. https://doi.org/10.1021/es048927c

Liu SH, Lai CY, Chang PH et al (2020) Enhancing copper recovery and electricity generation from wastewater using low-cost membrane-less microbial fuel cell with a carbonized clay cup as cathode. J Clean Prod 247:119118. https://doi.org/10.1016/j.jclepro.2019.119118

Logan BE (2008) Microbial fuel cells. John Wiley & Sons, Hoboken, NJ

Logan BE (2010) Scaling up microbial fuel cells and other bioelectrochemical systems. Appl Microbiol Biotechnol 85:1665–1671. https://doi.org/10.1007/s00253-009-2378-9

Luo H, Liu G, Zhang R, Jin S (2007) Characteristics of generating electricity with microbial fuel cell by different organics as fuel. In: Proceedings of ISES world congress 2007, vol I–V. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 2449–2452

Luo H, Xu G, Lu Y et al (2017) Electricity generation in a microbial fuel cell using yogurt wastewater under alkaline conditions. RSC Adv 7:32826–32832. https://doi.org/10.1039/c7ra06131e

Malvankar NS, Tuominen MT, Lovley DR (2012) Biofilm conductivity is a decisive variable for high-current-density Geobacter sulfurreducens microbial fuel cells. Energy Environ Sci 5:5790–5797. https://doi.org/10.1039/c2ee03388g

Marashi SKF, Kariminia HR (2015) Performance of a single chamber microbial fuel cell at different organic loads and pH values using purified terephthalic acid wastewater. J Environ Heal Sci Eng 13:27. https://doi.org/10.1186/s40201-015-0179-x

Min B, Logan B (2004) Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environ Sci Technol 38:5809–5814

Min B, Cheng S, Logan BE (2005) Electricity generation using membrane and salt bridge microbial fuel cells. Water Res 39:1675–1686. https://doi.org/10.1016/j.watres.2005.02.002

Mohanakrishna G, Abu-Reesh IM, Al-Raoush RI, He Z (2018) Cylindrical graphite based microbial fuel cell for the treatment of industrial wastewaters and bioenergy generation. Bioresour Technol 247:753–758. https://doi.org/10.1016/j.biortech.2017.09.174

Mousavi SMS, Ayati B, Ganjidoust H (2016) Phenol removal and bio-electricity generation using a single-chamber microbial fuel cell in saline and increased-temperature condition. Energy Sources Part A Recover Util Environ Eff 38:3300–3307. https://doi.org/10.1080/15567036.2016.1156196

Nastro RA (2014) Microbial fuel cells in waste treatment: recent advances. Int J Performability Eng 10:367–376

Nawaz A, Raza W, Gul H et al (2020) Upscaling feasibility of a graphite-based truncated conical microbial fuel cell for bioelectrogenesis through organic wastewater treatment. J Colloid Interface Sci 570:99–108. https://doi.org/10.1016/j.jcis.2020.02.099

Palanisamy G, Jung HY, Sadhasivam T et al (2019) A comprehensive review on microbial fuel cell technologies: processes, utilization, and advanced developments in electrodes and membranes. J Clean Prod 221:598–621. https://doi.org/10.1016/j.jclepro.2019.02.172

Pallavi CK, Udayashankara TH (2016) A review on microbial fuel cells employing wastewaters as substrates for sustainable energy recovery and wastewater treatment. IOSR J Environ Sci Toxicol Food Technol 10:31–36. https://doi.org/10.9790/2402-1012023136

Pant D, Van Bogaert G, Diels L, Vanbroekhoven K (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 101:1533–1543. https://doi.org/10.1016/j.biortech.2009.10.017

Park HI, Wu C, Lin LS (2012) Coal tar wastewater treatment and electricity production using a membrane-less tubular microbial fuel cell. Biotechnol Bioprocess Eng 17:654–660. https://doi.org/10.1007/s12257-011-0374-2

Pauline S, Boopathi A (2018) Treatment of slaughterhouse wastewater using microbial fuel cell. Int J Adv Res Ideas Innov Technol 4:228–230

Peng X, Chu X, Wang S et al (2017) Bio-power performance enhancement in microbial fuel cell using Ni-ferrite decorated anode. RSC Adv 7:16027–16032. https://doi.org/10.1039/C7RA01253E

Rabaey K, Verstraete W (2005) Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol 23:291–298. https://doi.org/10.1016/j.tibtech.2005.04.008

Rahimnejad M, Najafpour GD (2011) Microbial fuel cells: a new source of power. In: Biochemical engineering and biotechnology, 2nd edn. Elsevier B.V., Amsterdam, pp 1–31

Rahimnejad M, Adhami A, Darvari S et al (2015) Microbial fuel cell as new technology for bioelectricity generation: a review. Alexandria Eng J 54:745–756. https://doi.org/10.1016/j.aej.2015.03.031

Saba B, Christy AD, Yu Z, Co AC (2017) Sustainable power generation from bacterio-algal microbial fuel cells (MFCs): an overview. Renew Sust Energ Rev 73:75–84. https://doi.org/10.1016/j.rser.2017.01.115

Santoro C, Ieropoulos I, Greenman J et al (2013) Current generation in membraneless single chamber microbial fuel cells (MFCs) treating urine. J Power Sources 238:190–196. https://doi.org/10.1016/j.jpowsour.2013.03.095

Santoro C, Serov A, Stariha L et al (2016) Iron based catalysts from novel low-cost organic precursors for enhanced oxygen reduction reaction in neutral media microbial fuel cells. Energy Environ Sci 9:2346–2353. https://doi.org/10.1039/c6ee01145d

Santoro C, Arbizzani C, Erable B, Ieropoulos I (2017) Microbial fuel cells: from fundamentals to applications. A review. J Power Sources 356:225–244. https://doi.org/10.1016/j.jpowsour.2017.03.109

Savizi ISP, Kariminia HR, Bakhshian S (2012) Simultaneous decolorization and bioelectricity generation in a dual chamber microbial fuel cell using electropolymerized-enzymatic cathode. Environ Sci Technol 46:6584–6593. https://doi.org/10.1021/es300367h

Slate AJ, Whitehead KA, Brownson DAC, Banks CE (2019) Microbial fuel cells: an overview of current technology. Renew Sust Energ Rev 101:60–81. https://doi.org/10.1016/j.rser.2018.09.044

Song YE, Lee S, Kim M et al (2020) Metal-free cathodic catalyst with nitrogen- and phosphorus-doped ordered mesoporous carbon (NPOMC) for microbial fuel cells. J Power Sources 451:227816. https://doi.org/10.1016/j.jpowsour.2020.227816

Tee PF, Abdullah MO, Tan IAW et al (2018) Bio-energy generation in an affordable, single-chamber microbial fuel cell integrated with adsorption hybrid system: effects of temperature and comparison study. Environ Technol (UK) 39:1081–1088. https://doi.org/10.1080/09593330.2017.1320433

Tugtas AE, Cavdar P, Calli B (2011) Continuous flow membrane-less air cathode microbial fuel cell with spunbonded olefin diffusion layer. Bioresour Technol 102:10425–10430. https://doi.org/10.1016/j.biortech.2011.08.082

Waller MG, Trabold TA (2013) Review of microbial fuel cells for wastewater treatment: large-scale applications, future needs and current research gaps. In: International conference on fuel cell science, engineering and technology. American Society of Mechanical Engineers, New York, p V001T01A011

Wang P, Li H, Du Z (2013) Deposition of iron on graphite felts by thermal decomposition of Fe $(CO)_5$ for anodic modification of microbial fuel cells. Int J Electrochem Sci 8:4712–4722

Wang Z, Lee T, Lim B et al (2014) Microbial community structures differentiated in a single-chamber air-cathode microbial fuel cell fueled with rice straw hydrolysate. Biotechnol Biofuels 7:1–10. https://doi.org/10.1186/1754-6834-7-9

Wang R, Yan M, Li H et al (2018) Fe_S2 nanoparticles decorated graphene as microbial-fuel-cell anode achieving high power density. Adv Mater 30:1800618. https://doi.org/10.1002/adma.201800618

Wei J, Liang P, Huang X (2011) Recent progress in electrodes for microbial fuel cells. Bioresour Technol 102:9335–9344. https://doi.org/10.1016/j.biortech.2011.07.019

Yamashita T, Ishida M, Asakawa S et al (2016) Enhanced electrical power generation using flame-oxidized stainless steel anode in microbial fuel cells and the anodic community structure. Biotechnol Biofuels 9:1–10. https://doi.org/10.1186/s13068-016-0480-7
Yang S, Jia B, Liu H (2009) Effects of the Pt loading side and cathode-biofilm on the performance of a membrane-less and single-chamber microbial fuel cell. Bioresour Technol 100:1197–1202. https://doi.org/10.1016/j.biortech.2008.08.005
Yang Y, Sun G, Xu M (2011) Microbial fuel cells come of age. J Chem Technol Biotechnol 86:625–632. https://doi.org/10.1002/jctb.2570
Yang F, Ren L, Pu Y, Logan BE (2013) Electricity generation from fermented primary sludge using single-chamber air-cathode microbial fuel cells. Bioresour Technol 128:784–787. https://doi.org/10.1016/j.biortech.2012.10.021
Yang G, Sun Y, Yuan Z et al (2014) Application of surface-modified carbon powder in microbial fuel cells. Cuihua Xuebao/Chinese J Catal 35:770–775. https://doi.org/10.1016/s1872-2067(14)60023-1
Yang W, Li J, Zhang L et al (2017) A monolithic air cathode derived from bamboo for microbial fuel cells. RSC Adv 7:28469–28475. https://doi.org/10.1039/c7ra04571a
Yang Y, Yan L, Song J, Xu M (2018) Optimizing the electrode surface area of sediment microbial fuel cells. RSC Adv 8:25319–25324. https://doi.org/10.1039/c8ra05069d
Ye D, Zhang P, Zhu X et al (2018) Electricity generation of a laminar-flow microbial fuel cell without any additional power supply. RSC Adv 8:33637–33641. https://doi.org/10.1039/C8RA07340F
You S, Zhao Q, Zhang J et al (2007) A graphite-granule membrane-less tubular air-cathode microbial fuel cell for power generation under continuously operational conditions. J Power Sources 173:172–177. https://doi.org/10.1016/j.jpowsour.2007.07.063
You J, Fan H, Winfiel J, Ieropoulos IA (2020) Complete microbial fuel cell fabrication using additive layer manufacturing. Molecules 25:3051
Yuan Y, Zhao B, Zhou S et al (2011) Electrocatalytic activity of anodic biofilm responses to pH changes in microbial fuel cells. Bioresour Technol 102:6887–6891. https://doi.org/10.1016/j.biortech.2011.04.008
Yuan GE, Deng H, Ru X, Zhang X (2018) Electricity generation from heavy metal-containing wheat grain hydrolysate using single-chamber microbial fuel cells: performance and long-term stability. Int J Electrochem Sci 13:8589–8601. https://doi.org/10.20964/2018.09.05
Zeng L, Zhao S, Zhang L, He M (2018) A facile synthesis of molybdenum carbide nanoparticles-modified carbonized cotton textile as an anode material for high-performance microbial fuel cells. RSC Adv 8:40490–40497. https://doi.org/10.1039/C8RA07502F
Zhang Y, Ye JS (2015) Graphene-based microbial fuel cells. In: Yusoff AR b M (ed) Graphene-based energy devices. Wiley-VCH Verlag GmbH & Co KGaA, Weinheim, Germany, pp 339–354
Zhao Y, Ma Y, Li T et al (2018) Modification of carbon felt anodes using double-oxidant HNO_3/H_2O_2 for application in microbial fuel cells. RSC Adv 8:2059–2064. https://doi.org/10.1039/c7ra12923h
Zhou M, Chi M, Wang H, Jin T (2012) Anode modification by electrochemical oxidation: a new practical method to improve the performance of microbial fuel cells. Biochem Eng J 60:151–155. https://doi.org/10.1016/j.bej.2011.10.014
Zhuang L, Zhou S, Wang Y et al (2009) Membrane-less cloth cathode assembly (CCA) for scalable microbial fuel cells. Biosens Bioelectron 24:3652–3656. https://doi.org/10.1016/j.bios.2009.05.032

Microbes: Applications for Power Generation

9

Zahra Pezeshki, Mashallah Rezakazemi, and Atiye Pezeshki

Abstract

Energy is one of the critical needs of human being that plays an imperative role for countries in the world. Today high prices of conventional energy like oil, natural gas, and coal are a serious problem around the world, and in order to overcome this problem, many technologies have been introduced. One of them is electricity generation from microbes, which has many applications as clean energy, reduction of environmental and air pollution, energy efficiency, availability, and sustainability. It can supply the demands of the current generation without decreasing the potential of the future generation. This chapter investigates the research papers in this regard to show the applications of microbes in electric generation.

Keywords

Application · Microbe · Microorganism · Bacteria · Yeast · Electricity · Mediator · Membrane · MFC · Renewable energy · Electricity generation · Power generation

Z. Pezeshki (✉)
Faculty of Electrical and Robotics Engineering, Shahrood University of Technology, Shahrood, Semnan, Iran

M. Rezakazemi
Faculty of Chemical and Materials Engineering, Shahrood University of Technology, Shahrood, Semnan, Iran

A. Pezeshki
Institut National de la Recherche Scientifique, Energy, Materials and Telecommunications Research Centre, Montreal, QC, Canada

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9.1 Introduction

Electrochemically, the electric generation from microbes requires active and effectual microbes which can be improved from different environments such as ocean sediments (Reimers et al. 2001), domestic wastewater (Liu and Logan 2004; Liu et al. 2004), anaerobic sewage sludge (Kim et al. 2004, 2005, 2006, 2007), etc. The microbes obtained from these environments can act as microorganisms such as bacteria/yeast or make them and then transfer electron activity to anode surfaces. In this process, kinetics equations are used to show the relationship between the maximum voltage output and the substrate concentration (Pezeshki et al. 2021). Fig. 9.1 shows supply chains and typical process providing end use of this technology.

In this chapter, we want to answer this question: what is the application of electricity generation from microbes for us? So, we follow our answer step by step from Sects. 9.2–9.6. Then in Sect. 9.7, we conclude our answer, and at the end, Section 9.8 shows the future approaches which must be performed in this regard.

9.2 Reduction of the Environmental and Air Pollution

However, the environmental and air pollution are seen as a local problem, but they are global problems. In fact, they affect both human and environment of the countries, and the consequences of such problems are spreading all over the world. These problems are the main factors of climate change and interaction between ecosystems; nitrogen, N; and ozone. Todays, renewable reserves can play a key role especially using microbes for energy generation in this phenomenon. In this section, the impact of microbes on reduction of the environment and air pollution will be discussed.

9.2.1 Natural Aerosols from Vegetation

One of the sources for electrical generation is the blue haze above heavily forested regions. Blue haze is the continuous atmospheric haze which can produce powerful electrical fields at growing covered plant surfaces such as pine needles. Such organic gases play an important role in air pollution by production of submicrometer-sized liquid or solid particles that is produced by photochemical impacts of sunlight on airborne hydrocarbons resulting in toxic blue natural aerosols. The blue haze is considered as a conduction path become molten during the brush discharge occurs due to atmospheric phenomenon (Fish 1972).

In order to use this source, it is considered such a closed system mounted on pine needles as a pair of electrodes subjected to different electrical gradients in the laboratory. Collecting the particles is done by attaching carbon-coated disks to the opposite electrodes. Figure 9.2 depicts the collected particles at 20 kV potential in

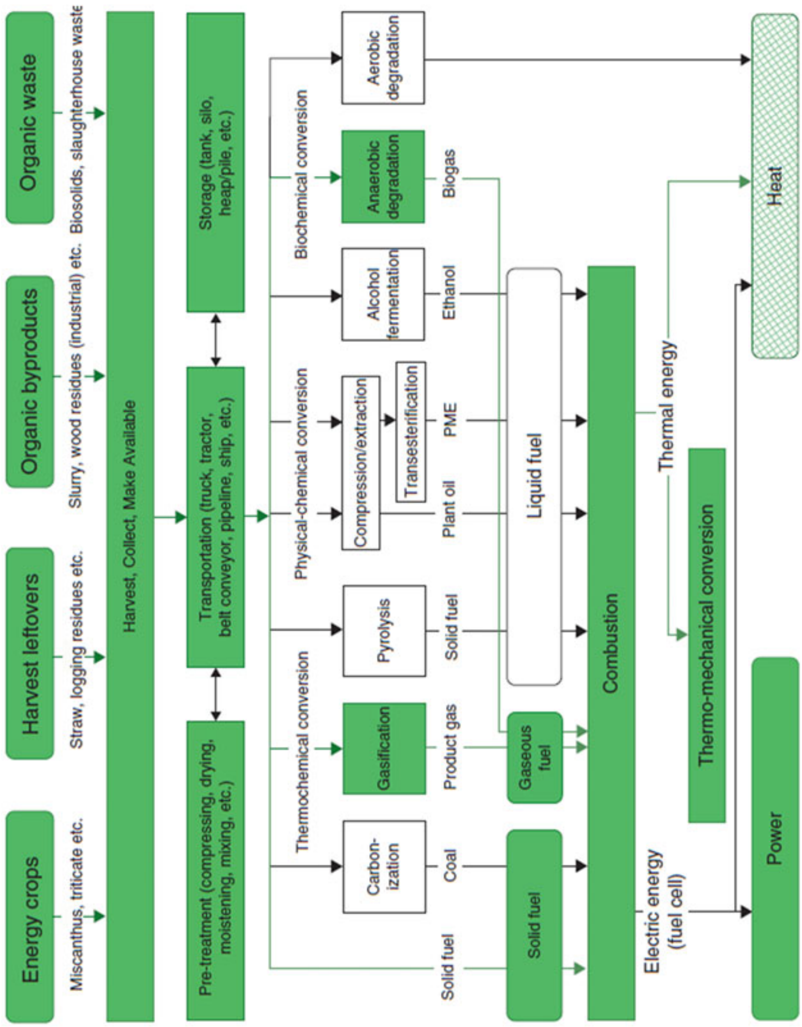

Fig. 9.1 The supply chains and typical process of providing end use of electric generation from microbes (Wiese 2017)

Fig. 9.2 Electron micrograph of particles produced by a pine needle at 20 kV (Fish 1972)

electron micrograph. At less potentials, the particles are less concentrated, and at higher, e.g., 30 kV, the particles start to shatter (Fish 1972).

The major factor in blue haze production is the particles are the sized range less than 0.6μm (Fish 1972). The electric generation from this source can help us to reduce air pollution.

9.2.2 Landfill Gas

Using landfill gas (LFG) for electricity generation is an expensive approach, but it has benefit for markets because of the decrease of pollution especially greenhouse gas (GHG) emissions and speed of power transportation. In fact, it generates green energy, and researchers have shown that there is not any correlation between the produced LFG and ambient temperature (Morgan and Yang 2001; Salihoglu 2018). Other uses of LFG consist of local direct use and pipeline injection. Among these, local direct use is the easiest and cheapest option if a customer is around and willing to buy this gas. But still for many areas, electricity generation is the best option (Morgan and Yang 2001).

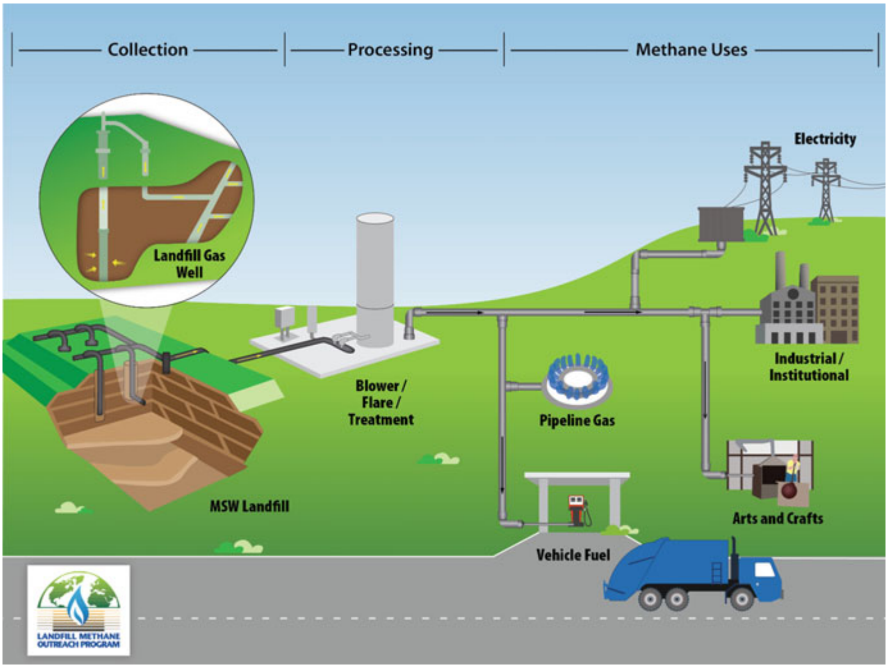

Fig. 9.3 The process of LFG use (United States Environmental Protection Agency: https://www.epa.gov/)

Figure 9.3 depicts the process of LFG use. In this process, perforated pipes are located between the gravel-filled trenches which are opened on the waste-filled parts of the valley. Then the trenches are affixed to a main collection pipe connected with a booster to extract the gas from the landfill and transfer it to the utilization system used for energy generation, for example, electricity. In this process, temperature is a key factor and has an essential role in the microbial process (Salihoglu 2018), because it must double the methanogensis rate to convert waste quicker (Christensen et al. 1996).

Today, due to increasing rate of production of wastes involving industrial waste, packaging waste, and municipal solid waste (MSW), such as household, commercial, streets, industries, and markets wastes, known as nonhazardous wastes (Zuberi and Ali 2015), caused by the urbanization growth, maybe using LFG for electricity generation and other uses the best option to decrease these wastes. Because, revenues resulted in this energy generated leads to energy recovery, i.e., gas extraction, which is an essential parameter that affects the revenues and environment by economical and environmental effects (Aghdam et al. 2018; Calabrò 2009; Calabrò et al. 2011).

Certainly, it is very important to have the field measurement before using LFG for landfill operating principles, waste specifications, MSW management policy, and

Table 9.1 The essential financial parameters for the LFG to energy (Salihoglu 2018)

Parameter	Unit	Value
Total investment cost	$	17,432,000
Annual operational expenses	$	1,764,000
Annual electricity generation	GWh/year	68.6
Installed capacity	MW	9.8
Share of the municipality	%	41

climatic impact on geographical region (Salihoglu 2018). Table 9.1 shows the main financial characteristic for LFG to energy.

Today LFG plants can generate 200 and 2000 tons of waste to energy, e.g., in Turkey (Salihoglu 2018).

9.2.3 Biogas

Biogas is a kind of fuel gas involving of methane, i.e., CH_4, CO_2, and other gases, produced through microbial processes under anaerobic condition from biodegradable materials (Shah and Nagarseth 2015). As a renewable energy, biogas has the many applications for power generation and energy efficiency increase. It is one of the natural resources which can decrease the problem of oil prices in the world. Here some applications of it for decrease of environmental and air pollution are defined (Mitan and Badarulzaman 2020).

Using Leachate of the Waste

The leachate of the waste is an environmental problem which severely endangers the environment due to contaminants such as manures and so on. One of the solutions for this issue is construction of a plant reactor for the leachate treatment. This plant can generate a big amount of biogas in anaerobic digestion (AD) (Bacenetti et al. 2016) phase to be utilized to run a power plant. For example, 33,504 m^3/d biogas is enough for running a power plant with capacity of 3.4 MW, i.e., two gas turbines units (Rashidi et al. 2012).

In this plant, in AD phase, CH_4 is consumed in turbines, and then the outlet gases from the gas turbines heat water and regulate the temperature of reactors due to having high temperature. The carbon dioxide, CO_2, is also generated which reduces the GHG effect of these gases and particulate matter (PM). The reactors with 75% chemical oxygen demand (COD) removal efficiency are the hybrid anaerobic baffled reactors. In the process, leachate enters into the reactors after cleaning manually and mechanically by bar screens (Rashidi et al. 2012).

For estimation of abovementioned unit, the parameters of the leachate are measured according to the standard methods (American Public Health Association, American Water Works Association, Water Pollution Control Federation,, and Water Environment Federation 1915), and the amount of biogas is estimated by mass balance conversion of COD to methane gas (Metcalf et al. 1979). The leachate chemical formula is as follows:

$$C_3H_{43.37}O_{6.8}NS_{029}$$

Then, the leachate and water chemical reaction occurs which is as follows:

$$C_cH_hO_oN_nS_s + mH_2O \rightarrow xCH_4 + yCO_2 + sH_2S + nNH_3$$

9.2.4 Biodiesel

Transportation is one of the sections using fossil fuels such as oil which increases the emissions of GHG and global warming. These effects call us to utilize alternative fuels.

In the transportation industry, biodiesel can be an ideal alternative for diesel engines especially heavy-duty vehicles such as bus, taxi, and other passenger cars.

In fact, it results in decrease of dependency upon imports of the fossil fuel imports and increase of employment in the industrial and agricultural sectors (Mittelbach 2013).

Biodiesel is made of fats and oils. Chemically, it is a fatty acid methyl matter of ethyl esters which is made of animal fats such as recycled frying oil, waste animal fat, etc. or vegetable oils, e.g., rapeseed oil, sunflower oil, soybean oil, palm oil, and suchlike (Mittelbach 2013).

Biodiesel can be also mixed with hydrocarbons obtained from hydrogenation of vegetable oils by removing the oxygen. These kinds of biodiesels are known as hydrogenated vegetable oils (HVOs) or NExBtl fuels. They can also be obtained of transesterification of vegetable oils with less alcohols yielding the fatty acid esters and free glycerol (Di Pascoli et al. 2001).

The feedstock price for biodiesel production is very important. Between the vegetable oils, rapeseed oil is the most expensive oil and palm oil the cheapest. Today, there are standards for biodiesel markets (Mittelbach 2013). Table 9.2 depicts the limits and parameters between two standards, i.e., CEN and ASTM, for these markets.

After increasing the biodiesel markets in transportation industry, researchers were perused to utilize it for electricity generation. It is not long since biodiesel is also used for power generators. These kinds of generators, called biodiesel generator, use biodiesel as a renewable source to burn clean and have a great reduction in GHG emissions and pollutants (Mittelbach 2013).

They are used as a prime power supply in remote regions where connecting to a power grid is not easily available to run essential standby power/electricity for facilities such as hospitals, datacenters, residential neighborhoods, etc.

Table 9.2 The limits and parameters between the CEN and ASTM standards (Mittelbach 2013)

Parameter	Unit	CEN standard	ASTM standard
Density	15 °C [kg/m^3]	860–900	–
Flash point	[°C]	≥101	≥93
Viscosity	40°C [mm^2/s]	3.5–5	1.9–6
Sulfated ash	[%m/m]	≤0.02	≤0.02
Conradson carbon residue	[%m/m]	≤0.30 (10% distillation residue)	≤0.05
Water	[mg/kg]	≤500	0.05 vol%
Oxidation stability	[h]	≥6	≥3
Copper corrosion	–	Class 1	–
Iodine number	[g Iodine/ 100g]	≤120	–
Acid number	[mg KOH/g]	≤0.50	≤0.50
Cetane number	–	≥51	≥47
Sulfur (S)	[mg/kg]	≤10	≤15
Total contamination	[mg/kg]	≤24	–
Distillation, T90	[°C]	–	≤360
Linolenic acid	[% m/m]	≤12	–
Methanol	[% m/m]	≤0.20	≤0.20
Diglycerides	[% m/m]	≤0.20	–
Polyunsaturated fatty acids (>4 double bonds)	[% m/m]	≤1	–
Monoglycerides	[% m/m]	≤0.80	–
Free glycerol	[% m/m]	≤0.25	≤0.250
Triglycerides	[% m/m]	≤0.20	–
Sum of ca, mg	[mg/kg]	≤5	–
Phosphorus (P)	[mg/kg]	≤4	≤10
Sum of Na, K	[mg/kg]	≤5	≤5

9.2.5 Bioethanol

The ethanol used as a fuel is negligible due to the petroleum industry development. Now because of the increasing prices of oil, ethanol is considered as a fuel-based renewable material obtained from celluloses, starch, and sugar, called bioethanol which reduces environmental and air pollution. This matter has been shown significant advantages in comparison to fossil fuels. In this section we want to speak about the bioethanol use especially for electricity generation.

Using Celluloses

In transportation industry, bioethanol is a well-established fuel for gasoline vehicles, but it can be utilized for power production especially electricity generation too.

Making this fuel from cellulose is the same as from starch. In the process, firstly, celluloses, hemicelluloses, and carbohydrates are hydrolyzed to monomer sugars. Then fermentation is performed by microorganisms on them caused the lignocellulosic material to be created. The lignocellulosic material is composed of 20–35% lignin, 50–60% carbohydrates in the form of hemicellulose and cellulose, as well as other components such as ash, extractives, and fatty acids. These materials protect the carbohydrates from degradation and enzymatic hydrolysis and make the heating process of bioethanol production much warmer than the biomass process. Thus, bioethanol is considered as a valuable chemically products in heat and power generation (Galbe and Zacchi 2012).

The process of bioethanol production from lignocellulosic material is including four parts (see Fig. 9.4) (Galbe and Zacchi 2012):

1. The carbohydrates hydrolysis (i.e., celluloses and hemicelluloses) as follows:

$$[C_6H_{10}O_5]_n + nH_2O \rightarrow nC_6H_{12}O_6$$

2. Using fermentation to convert all sugars to ethanol by using microorganisms like bacteria or yeast, separating and improving the ethanol and production of water-free ethanol as follows:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

3. The treatment of wastewater.
4. The combustion of solid residues for generating steam and electricity required.

Using Starch

Bioethanol can be obtained from starch too. Starch is a polysaccharide involving D-glucose molecules joined by 1,4-α glycosidic bonds (amylose) and 1,6-α glycosidic bonds (amylopectin). The D-glucose is produced by green plants from CO_2 released in air and water through photosynthesis and sunlight, necessary for growing human and animals. So, starch is a substrate based on animal feeding and human nutrition.

Today, a big amount of the yielded grain crops is utilized for animal feeding. So, bioethanol production can be combined with animal feeding and also human nutrition too which results in a growth of bioethanol production (Friedl 2012).

The process of the bioethanol produced from starch has been shown in Fig. 9.5. In this process, two enzymatic processes after milling the starch, including saccharification and liquefaction, are required to get the fermentable mixture. Then the fermentation is done usually using bacteria or yeast. The next processes are a rectification and distillation of liquid increasing the concentration of the bioethanol, and for its quality, adsorption process is used. Then in the separation process, a thin stillage steam and wet cake stream are separated and can partly be concentrated or recycled in the evaporation process. Afterward, wet cake and thin stillage can be mixed together to generate animal feeding, known as dried distiller grains with soluble (DDGS) (Friedl 2012).This kind of bioethanol is less used as a fuel for

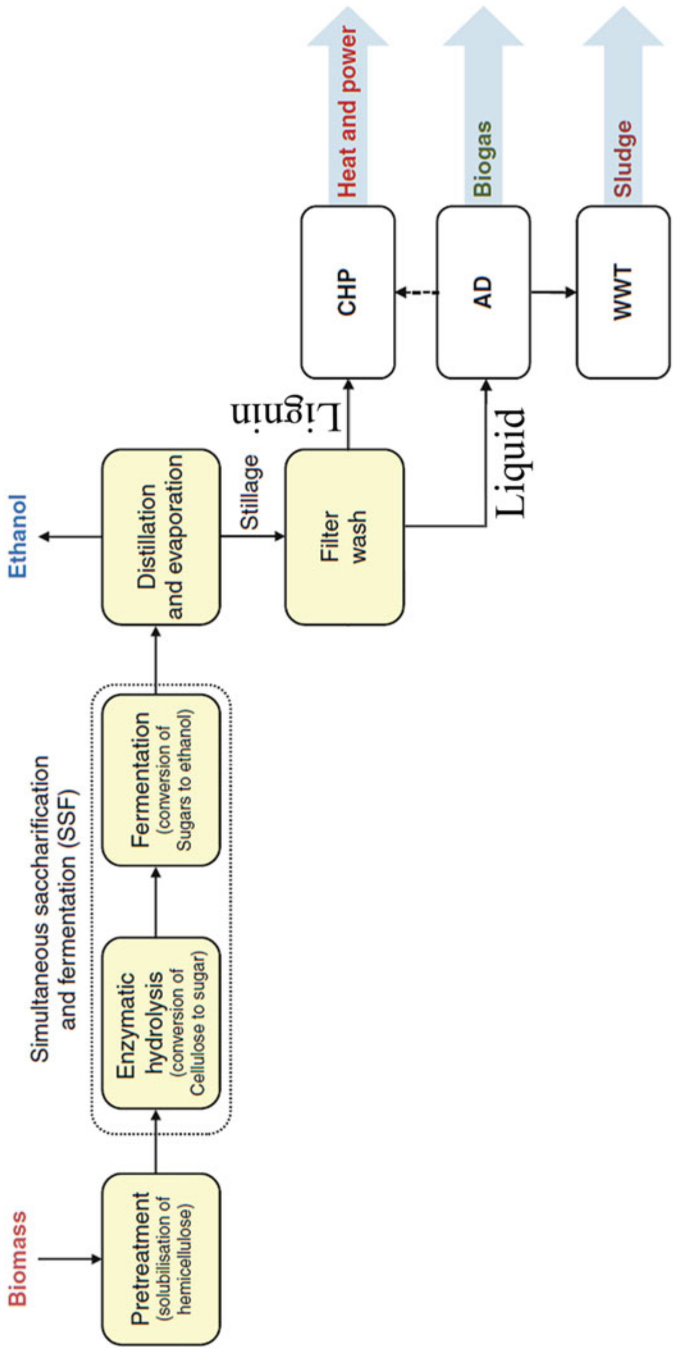

Fig. 9.4 Bioethanol production process from celluloses (WWT is the wastewater treatment and CHP the combined heat and power)

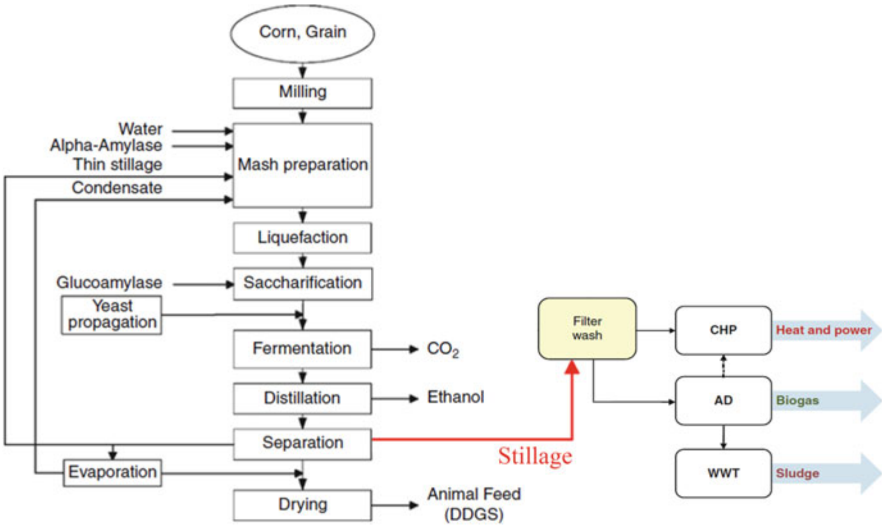

Fig. 9.5 Bioethanol production process from starch

power generation and transportation and mostly used for beer-producing and chemical industries as a lamp fuel which is called pure ethanol due to GHG reduction till 60%. For production of steam and electricity, combustion of the solid residue is required (Friedl 2012).

Using Sugar

Since the sugar price in the international markets is low, manufacturers try to find another way for solving this problem to compensate that. Since the sugarcane can be transformed into sugar or ethanol, ethanol is one of these solutions in this section. The bioethanol obtained from sugar, called pure ethanol, can be used in cars which have been begun since 1978 with taking part the companies such as Volkswagen, General Motors (Opel), Fiat, Ford. For using bioethanol for engines, after harvesting, sugarcane must be processed during 72 h preventing quality losses because of the bacterial activity.

In particular, sugarcane is considered as a semi-perennial crop, because after planting it can harvest 6–7 times without any replanting. So, 20% of this crop are exchanged with crops such as corn, beans, or peanuts helping the soil to recover (Coelho et al. 2013).

Filter cake, bagasse, tops, leaves (trash), and vinasse (stillage) are the other products obtained from sugarcane utilized in modern actions. The bagasse can be utilized for electricity generation as well. It includes 50% moisture and 30% cane. It has more energy than ethanol due to having cellulose. The electricity and heat of this by-product are generated by boilers (Coelho et al. 2013).

The Vinasse is produced by distillation. It consists of nutrients and organic materials, e.g., calcium, Ca, and potassium, K, so it can be pollutant if not well monitored. It is utilized for ethanol production (Coelho et al. 2013).

The filter cake is obtained from juice filtration, including rests of bagasse and sludge. Tops and trash consist of 30% sugarcane utilized as a cogeneration fuel. Cogeneration process is a process for mechanical/electrical as well as thermal energy production. The surplus of this generated energy is sent into the facilities in short-term contracts (Coelho et al. 2013).

9.2.6 Sewer

Sewer is the wastewater and refuse from animal or human that is usually driven to underground in cities. They both help generate energy and reduce environmental and air pollution. Today, the modern technologies can help us to generate electricity; with them we can name sewer electrical generation apparatus (Fig. 9.6). The sewer electrical generation apparatus consists of a generator affixed to a sewer pipe communicated with a turbine (Gotay 2013).

The turbine works with the help of sewer flow and runs the generator to produce electricity. This turbine is self-cleaning because of its reversible blades. It can be made of non-corroding materials such as stainless steel (Gotay 2013).

The generator can be disposed outside or inside of the sewer pipe. The design of the external generator is more cost-effective than the internal due to its installation which provides access and maintenance more easily. The generator is embedded into a chamber which has a lift hook on the top. This hook makes easier the generator removal (Gotay 2013).

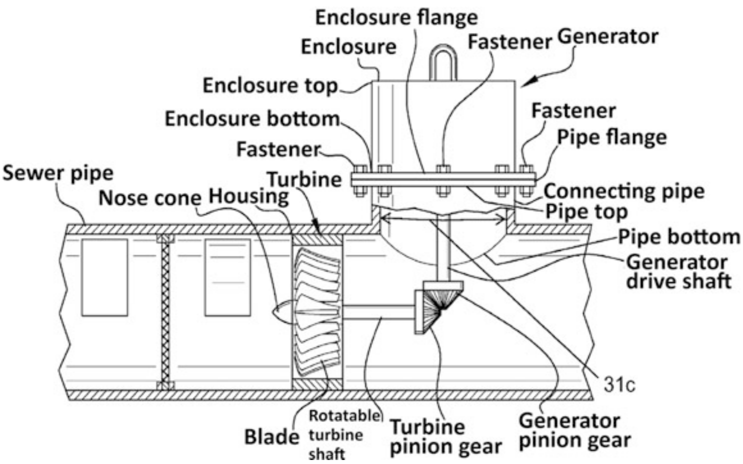

Fig. 9.6 Schema of the sewer electrical generation apparatus (Gotay 2013)

9.3 Energy Efficiency

Efficient and renewable energies obtained from microbes can play important roles in the future supply of electricity generation according to the new environmental requirements and standards. This section describes this application of microbes in the electricity generation.

9.3.1 Microorganisms

Microorganisms are divided into three groups: (1) exoelectrogens (Logan 2009; Logan and Regan 2006), (2) electrogens (Lovley 2006), and (3) anode-respiring bacteria (Torres et al. 2008). They are living in cells which are the active resource for microbial electricity generation. They are the alive creatures metabolize food to produce and release energy-rich substances from carbohydrates by oxidation which their reaction, called enzyme-catalyzed reaction shown, is as follows (Bennetto 1990):

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

In the enzyme-catalyzed reaction, the carbohydrates are initially oxidized without oxygen participation, and the enzymatic reactions cause their electrons to release. These electrons are utilized to provide the microorganisms for growth and maintenance of bio-synthetic reactions. The reaction is as follows (Bennetto 1990):

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

These electrons are called reduced intermediates. They enter the external cell membrane and become decreased and leave again. Then, the reduced ones are moved through mediators like humic acid, thionine, methyl viologen, etc. (Lithgow et al. 1986; Vega and Fernández 1987; Kreysa and Krämer 1989; Kim et al. 1999a, b, c, 2000, 2002; Yamazaki et al. 2002; Jang et al. 2004) to an electrode which is an electro-generic negatively charged electrode to provide an electric current through an outer circuit which is joined with a second electrode as a positive. In this chain, the oxidizing material is oxygen gas, but it can be a soluble solid oxidizing reagent, for example, potassium ferrocyanide, i.e., potassium hexacyanoferrate III (Bennetto 1990). Figure 9.7 shows the schematic diagram of this process.

The generated current is detected by a micrometer/multimeter and transforms energy and power from the microbial oxidation to mechanical energy in a small motor. This current can also provide light and heat from a small lamp or a light-emitting diode (LED) (Bennetto 1990).

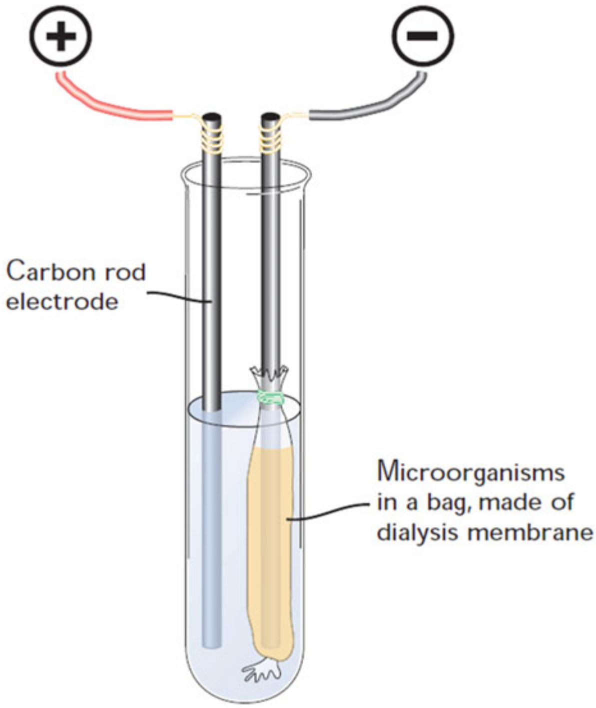

Fig. 9.7 The main features for electricity generation by microorganisms (Bennetto 1990)

9.3.2 Microbial Fuel Cells

Microbes exist everywhere in environment like canal, lakes, rivers, etc. They can oxidize various materials and transform their chemical energy to electrical energy. The microbial fuel cells (MFCs) help this transformation to be done (Sam and Mercy 2013; Yaqoob et al. 2020). MFCs are the biofuel cells or bio-electrochemical systems (BESs) (Li and Yu 2014) such as microbial electrochemical technologies (METs) (Zhao et al. 2019) which can produce electricity through biological processes by conversion of organic and inorganic materials (Asiri 2019). They were invented in 1911. In an MFC, two approaches are employed for electricity generation using most of the non-photosynthetic and photosynthetic microorganisms for fermentation of different substrates such as glucose, molasses, lactose, wastewater, sodium acetate, etc. with various bacteria or yeast including (1) without using any mediators and (2) using mediators as a membrane to spread the electron transfer rate over the anode. Two kinds of MFC exist including single-chambered microbial fuel cell (SMFC) and dual-chambered microbial fuel cell (DMFC) (Yaqoob et al. 2020; Khan 2009; Fu and Wu 2010; Jin et al. 2020). The SMFCs having mediators are called three-dimensional electrode microbial fuel cell (3DEMFC) (Dong et al. 2020).

In the first approach, biochemical reaction in microorganisms called bacteria and yeast (Mizil 2016) such as *Enterobacter aerogenes*, *Escherichia coli* (*E. coli*), *Clostridium perfringens*, *Clostridium butyricum*, *Clostridium acetobutylicum*

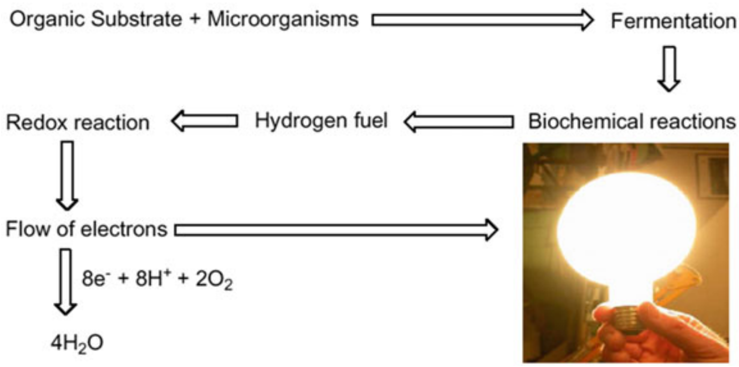

Fig. 9.8 Electricity generation by fermentation (Khan 2009)

(Lewis 1966; Raeburn and Rabinowitz 1971; Akiba et al. 1987; Ardeleanu et al. 1983), *Geobacter sulfurreducens*, *Shewanella putrefaciens* (Sam and Mercy 2013; Ilieva et al. 2018; Yang et al. 2020), *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera* (Rahayuningwulan et al. 2014), etc. causes fermentation in organic substrates which is converted into hydrogen fuel. Then this fuel can convert into water and electrical energy via redox reaction (see Fig. 9.8) or other reactions. In these kinds of MFCs, the anode can be as a terminal electron acceptor, or they can accept electron from cathode directly (Sam and Mercy 2013). Usually for sulfur compounds, the cathode is the electron acceptor (Sulonen et al. 2015). Anode and cathode can be in the form of plate or rod.

Several microorganisms such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Ferroplasma acidarmanus*, and/or *Ferroplasma acidiphilum* can oxidize sulfur compounds, such as tetrathionate called acidophilic (Sulonen et al. 2015).

In MFCs, electrode substrates, membranes, cathode, and anode play a key role in their work. If the more porous electrodes are used, they diffuse oxygen to anode and decrease the cell efficiency. The SMFCs have one chamber, and cathode and anode materials mostly made of carbon or graphite (Yaqoob et al. 2020) can also cause the polarization activity to lose. In these kinds of MFCs, the electrode surface area is important (Sam and Mercy 2013).

In a DMFC, the cathode and anode chambers are used and the space between cathode and anode chambers is filled by cation exchange membrane (CEM)/proton exchange membrane (PEM) fixed on the cathode surface. In the anode chamber, it is usually used the plates made of stainless steel (Mohamed et al. 2017), titanium (Ti) rod (Liu et al. 2020), etc. utilized as a current collector or blade agitator (Mohamed et al. 2017; Liu et al. 2020). After entering the wastewater into anode chamber, the microorganisms start to operate till to stabilize the open circuit voltage (OCV). Then the reactions of the electrodes occur and then the circuit is closed (Mohamed et al. 2017). In these reactions, the CO_2 can be produced by the anodic chamber. So the cathode chamber needs electron acceptor, e.g., O_2, ferricyanide, etc., for adsorbing the electrons and protons from anodic chamber and external

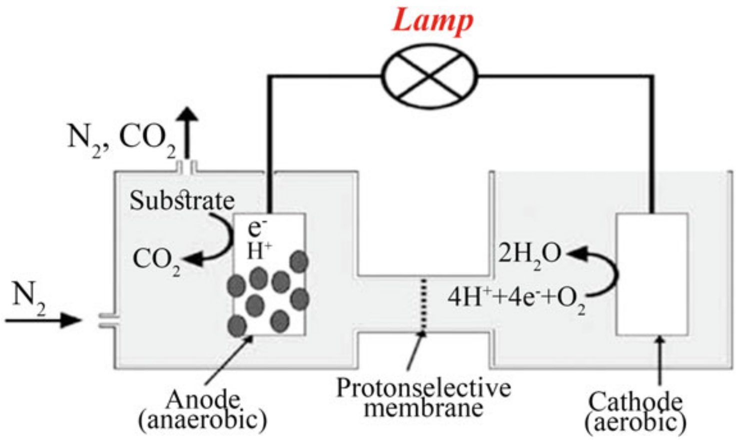

Fig. 9.9 The schematic diagram of MFC (Ma'arof et al. 2020)

circuit, respectively (Lee et al. 2015). These reactions at anode and cathode are as follows:

$$\text{Organics} \rightarrow CO_2 + H^+(\text{to cathode}) + e^-(\text{external circuit})$$

$$O_2 + H^+(\text{from cathode}) + e^-(\text{external circuit}) \rightarrow H_2O$$

The overall reaction created by above reactions is as follows:

$$\text{Organics} + O_2 \rightarrow CO_2 + H_2O + \text{external power}$$

This process changes chemical energy into electrical energy (Lee et al. 2015). Figure 9.9 illustrates the schematic diagram of MFC. The membrane usually is salt bridge, e.g., agar-agar salt bridge (Njoku et al. 2020), but it can be used other substrates, e.g., neutral red (NR), biochar, etc. instead of it (Dong et al. 2020). A longer membrane usually creates higher voltage than shorter membrane.

It can be used in a closed circuit by using an external circuit for measuring the current-voltage (I–V) relationship that is obtained from the maximum OCV.

The current density is obtained at a constant cell voltage. So the power, P, is calculated as the Eq. (9.1) (Mohamed et al. 2017):

$$P = IV \tag{9.1}$$

where V is the cell voltage in volt (V) and I the current in ampere (A) between anode and cathode. If the rod is used in anodic chamber, the power can be calculated as Eq. (9.2) (Liu et al. 2020):

$$P = N_p \rho n^3 d^5 \tag{9.2}$$

where N_p is the power number, ρ the solution density, d the diameter, and n the rotating rate of the anodic chamber rod.

The power density, D, is obtained by the Eq. (9.3) as follows:

$$D = JV/A_{an} \tag{9.3}$$

where J is the current density and A_{an} is the electrode surface area. The J is computed as follows according to the ohm law (Eq. 9.4) (Wang et al. 2019):

$$J = V/(R \cdot A_{an}) \tag{9.4}$$

where R represents the external resistance.

The maximum charge occurs when the microorganisms can digest all the materials. It can be computed by Eq. (9.5) as follows:

$$\text{The toral charge} = \left(C_p/C_T\right) \times 100 \tag{9.5}$$

where C_T is the theoretical amount of coulombs generated by the wastewater material and C_p is the total coulombs obtained from the current over time. The coulombic efficiency (CE) is introduced by the overall charge percentage moved to the anode through the material and can be computed as Eq. (9.6) (Mohamed et al. 2017; Liu et al. 2020):

$$\text{CE} = \frac{M \int_0^t I dt}{F b V_{an} \Delta \text{COD}} \tag{9.6}$$

where F is the Faraday's constant, M the molecular weight of oxygen, V_{an} the liquid volume in the anode, b the number of electrons changed per mole of oxygen, and t is time.

Using Natural Fermentation

The upflow microbial fuel cells (UMFCs) are the newer and large-scale types of MFCs which are utilized for electricity generation aided bioelectrically assisted microbial reactor (BEAMR). This technology can overcome the energy management problems as a current global issue.

The UMFC cells using the artificial systems called benthic unattended generators (BUGs) can collect the energy and utilize to power and manage devices. Today this system is utilized for remote areas such as bottom of the oceans more. In the oceans, there are many organic matters which can bear the fermentation process by the microorganisms that exist in the bottom. So because of this natural fermentation, chemical energy is changed into the electrical energy (Khan 2009). Figure 9.10 shows the electricity generation by UMFCs.

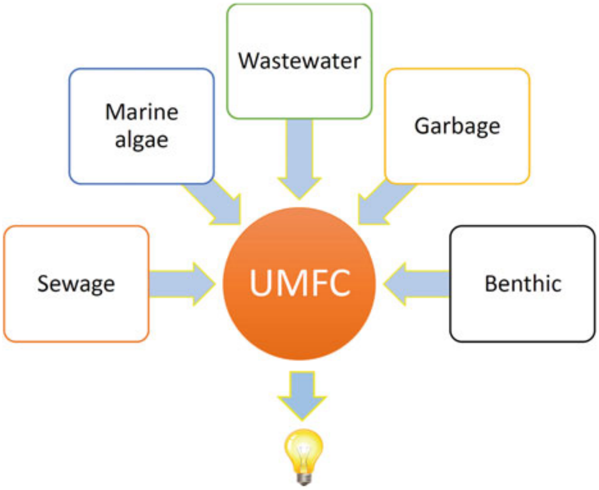

Fig. 9.10 Electricity generation by UMFC

Using Biomass

When it is used, the photosynthetic microorganism such as *Spirulina platensis* (*S. platensis*) for electricity generation in MFCs, using biomass, maintains the chemical process and conducts the generated electrons to the anode straightaway instead of moving them through an exchange membrane, mediator, or reactant gradient. In these kinds of MFCs known as photosynthetic/plant microbial fuel cell (PMFC), the biomass weight is appended to the anode surface. The electricity voltage of the MFC can increase by connecting the external resistances, but current density decreases (Fig. 9.11) (Fu and Wu 2010; Liu 2010).

The used biomass in this process can be biomass-derived sugars (Liu 2010).

Using Domestic Wastewater

Domestic wastewater has many applications in power generation. It consists of animal wastewater and human wastewater. Among them, animal such as abattoir wastewater (Njoku et al. 2020), swine wastewater, etc. can be collected to utilize in an aerobic and anaerobic fuel cells. As the same as other MFCs, these kinds of MFCs use microorganisms such as *Bacillus*, *Citrobacter*, *Pseudomonas*, *Lactobacillus*, *E. coli*, *Aspergillus*, *Rhizopus*, etc. to degrade organic substrates in wastewater and convert the chemical energy to electricity. These microbes act as a utilizer due to having metabolic activities in wastewater degradation. The percentage of the biochemical oxygen demand (BOD) and COD degradation of these MFCs is calculated as Eq. (9.7):

$$\%\text{degradation} = \frac{I - F}{I} \times 100 \tag{9.7}$$

where I is the initial value of the obtained organic materials and F the final value of them.

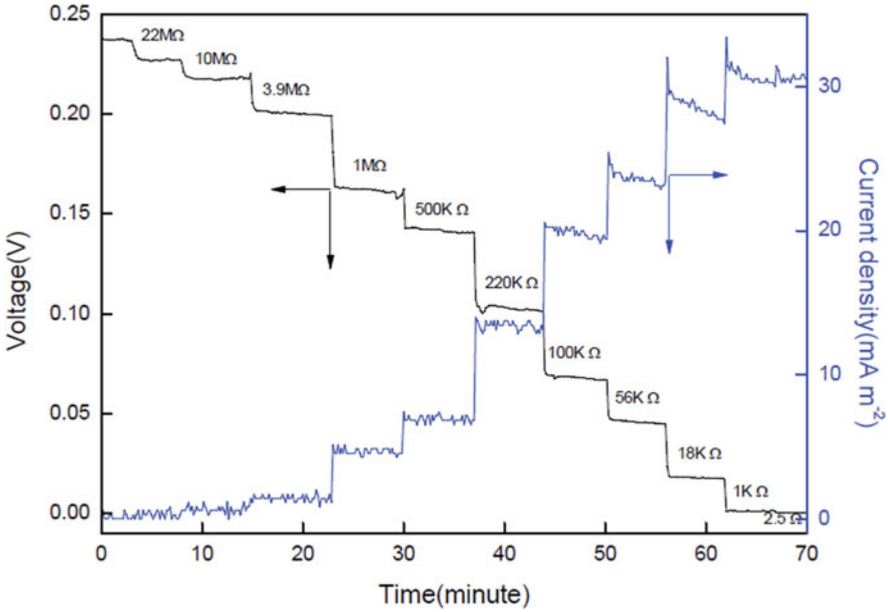

Fig. 9.11 Depending voltage and current density to the external resistance (Fu and Wu 2010)

In this MFC, the total power output depends on the distance between the cathode and anode, types of mediators and electrode materials, and oxygen reaction of the cathode. Figure 9.12 depicts the schematic diagram of this MFC. This MFC has four sections: (1) the cathode for holding the conductive saltwater solution as a salt bridge which can be agar-agar salt bridge (Njoku et al. 2020); (2) the anode for holding the organic materials and bacteria in an aerobic and anaerobic environment, e.g., granular sludge (Zhao et al. 2019); (3) the PEM, means that salt bridge, for separating the anode and cathode; and (4) the external circuit.

It is designed such a DMFC with two plastic bottles with a pipe filled with polyvinyl chloride (PVC) with the length of 5–10 cm. The PEM usually is a salt bridge with 4 cm diameter as well as two rubber loops. In this MFC, one electrode through the wires is passed via the lid to be connected to the external circuit and another electrode.

In the treatment process which is done by microbes on the organic materials, the bacteria create protons and electrons. Then the solution adsorbs the electrons into itself for conduct of an external circuit. Then the electrons move to cathode trough the circuit, and protons also move via PEM to join with electrons at the cathode. Then they merge with oxygen creating water. During this biochemical process, the chemical energy converts to electricity with high efficiency which is not harmful for environment because of GHG decrease (Zhao et al. 2019; Ogugbue et al. 2015).

The MFCs using domestic wastewater such as yogurt wastewater (Luo et al. 2017), etc. can be designed as a tricking microbial fuel cell (TMFC) depicted in

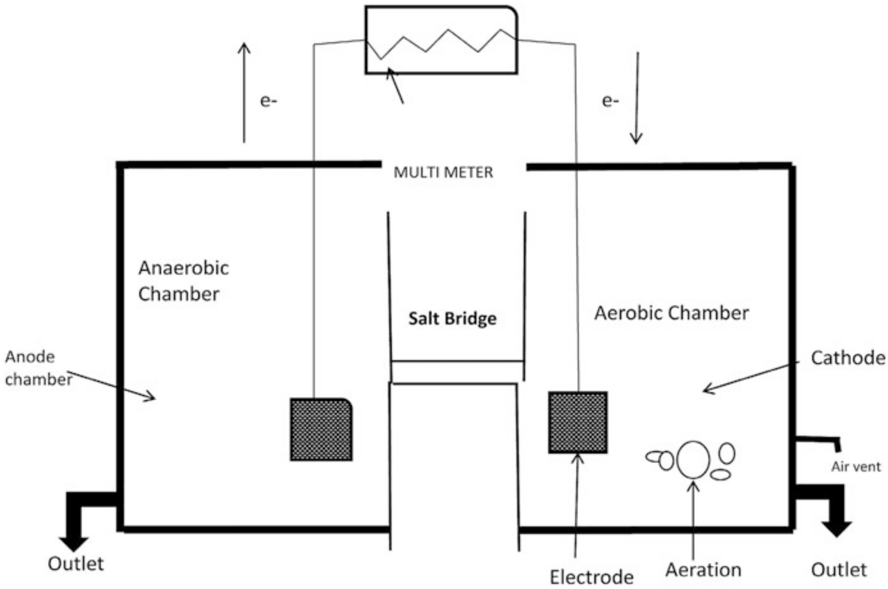

Fig. 9.12 The schematic diagram of the MFC using domestic wastewater (Ogugbue et al. 2015)

Fig. 9.13. It is a reactor which works in trickling mode continuously. The wastewater is added to TMFC 1 from the PVC tube. Then it is trickled from TMFC 1 to 4 (Fig. 9.13a). The vessel of this reactor has been made of acrylic plates with 1cm thick. There are nine small holes on the side of PVC tube to drip water. This PVC tube is connected to the pump from one side. The wastewater treatment is performed by the anode surface which has the 620 cm^2 surface area and is sewed by Ti wires (Fig. 9.13b) and cut into wavy strips (Fig. 9.13c). The anode is usually placed under alkaline condition to do better wastewater treatment and enhance electricity generation (Luo et al. 2017). The cathode is stainless steel mesh (SSM) outside the reactor with 427cm^2 surface area (Fig. 9.13b). The cathode and anode are connected to each other via an outer resistance to decrease the inner resistance created by the solution. Also, there is a two-layer separator material between the electrodes to clear short circuit (Gao et al. 2020).

Using Industrial Wastewater

Industrial wastewater treatment is the more expensive process than the treatment of domestic wastewater. So today new MFCs have been introduced using activated carbon (AC) as an efficient anode which they do not require any mediator. These are cheaper, because carbon materials are replaced with AC. It has a large surface area and porous structure which make easier the electron transfer. The other features of AC are its adhesion and mechanical strength. This kind of MFC has the high efficiency of 60–71% converting the chemical energy to electricity (Mohamed et al. 2017).

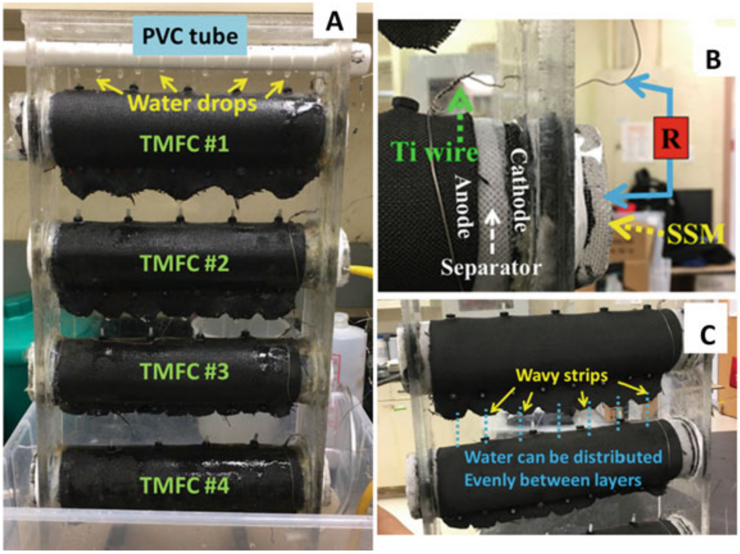

Fig. 9.13 (**a**) The schema of the TMFC using domestic wastewater; (**b**) cathode and anode; (c) schema of the anode for wastewater treatment (Gao et al. 2020)

In some locations, the industrial wastewater usually is mixed with domestic wastewater. So the amount of power generation produced by this wastewater will be more than that of only industrial wastewater (Ma'arof et al. 2020).

Using Sewage

Sewage is a domestic/municipal wastes, e.g., sewer, animal waste/manure/dung, etc. It is a type of waste that is generated by the people community. It is specified by rate of flow or volume, chemical and toxic constituents, physical condition, and its bacteriologic status. Sewage can be used for electric generation. It can be collected from different farms, then sieved, and mixed with other things such as sea sands. Afterward, they are soaked with distilled water. Before transferring them to the MFC, they are heated and afterward cooled down. Then they are digested by adding H_2SO_4. The MFC cover is plastic to utilize sun for microorganisms' growth (El-Nahhal et al. 2020).

The MFCs for using these kinds of wastes usually are SMFCs connected to each other in series (see Fig. 9.14) to produce high voltage and current for generating electricity. The cathode and anode electrodes of the SMFC are made of copper, Cu, and stainless steel, respectively.

After measurement, researchers have shown that the electricity generation from the horse manure is the most and that from the sludge is the least (El-Nahhal et al. 2020).

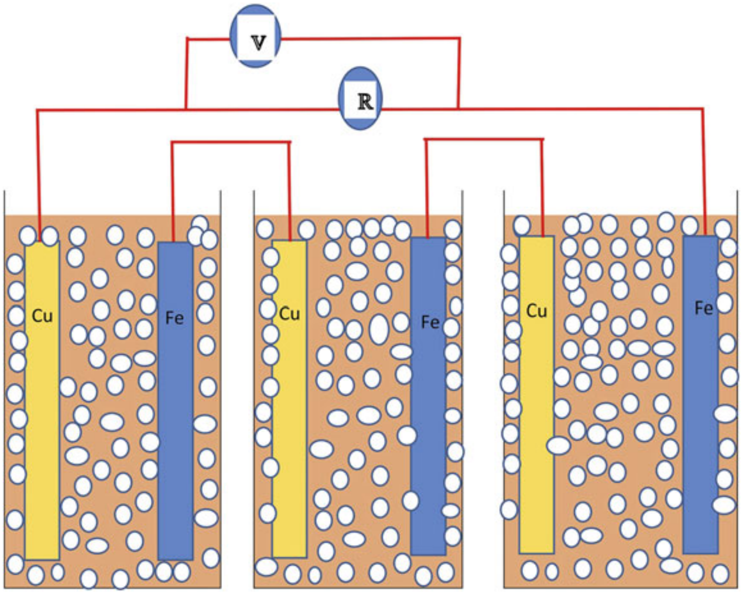

Fig. 9.14 The SMFC using agricultural waste for electricity generation (El-Nahhal et al. 2020)

Table 9.3 The chemical properties of the animal manure before and after electric generation (El-Nahhal et al. 2020)

	mg/kg		COD (mg/g)		
Manure type	SO_4	NO_3	Before	After	%*R*
Cow	81.01±4.6	9.19±0.67	91.48±0.42	1.94±0.94	98
Chicken	84.66±5.76	22.70±2.13	487.1±98	1.1±0.52	99
Horse	23.15±0.86	8.29±0.45	85.43±5.1	1.37±0.59	98
Sludge	188.2±21.5	2.43±0.5	51.5±6.5	0.62±0.12	98

$$\text{hourse manure} > \text{chicken manure} > \text{cow manure} > \text{sludge}$$

These SMFCs are cheap and fermentation is done under aerobic conditions to biodegrade organic materials in that. The manure sterilization reduces the current and voltage, because of killing the microorganisms (El-Nahhal et al. 2020). Table 9.3 shows the manure properties after and prior to electric generation.

The cathode and anode reactions are as follows (El-Nahhal et al. 2020):

$$\left\{\begin{array}{l} Fe^0 \rightarrow Fe^{2+} + 2e^- \\ H_2O \rightarrow 2H^+ + O^{2-} \\ 2H^+ + 2e^- \rightarrow H_2 \\ Fe^{2+} + O^{2-} \rightarrow FeO \end{array}\right. \quad \rightarrow \text{anode recations}$$

$$\left\{\begin{array}{l} Cu^0 \rightarrow Cu^{2+} + 2e^- \\ Cu^{2+} + SO_4^{2-} \rightarrow CuSO_4 \end{array}\right. \quad \rightarrow \text{cathode reactions}$$

The overall reaction is as follows (El-Nahhal et al. 2020):

$$2H_2S + 4O_2 \rightarrow 2SO_4^{2-} + 4H^+$$

$$3NH_3 + H_2O + O_2 \rightarrow 3NO_3^- + 11H^+ + 8e^-$$

The photosynthetic reaction also happened due to existing light and produces carbohydrate as follows (El-Nahhal et al. 2020):

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

$$6CO_2 + 6H_2O + NO_3^- + 30H^+ + 29e^- \rightarrow C_6H_{12}O_6 + 0.5N_2 + 15H_2O$$

$$6CO_2 + 6H_2O + SO_4^{2-} + 34H^+ + 32e^- \rightarrow C_6H_{12}O_6 + H_2S + 16H_2O$$

As the H_2S and ammonia create air and environmental problems, so it is better to pretreatment is done before using this kind of waste (El-Nahhal et al. 2020).

Using Crop Residue

Today using crop residue including by-product such as rice bran, etc. and defective products such as defective tomatoes, onions, potatoes, etc. for generating electricity has been started in some countries such as Japan and the US (Takahashi et al. 2016; Shrestha et al. 2016). It is considered as a fuel for MFCs. This kind of waste is the potent organic waste. Usually it is utilized by SMFCs (Takahashi et al. 2016) due to reducing cost, but DMFCs (Shrestha et al. 2016) are also used for this work.

The chamber of SMFCs can contain water or mineral solution as an inorganic nutrient solution. The reason for using inorganic matter is that it does not need oxygen for the degradation process (Logan and Rabaey 2012). Both of them can generate electricity with maximum of power density. The anode electrode mostly is made of graphite/carbon, and cathode commonly is multi-layer with a platinum catalyst layer on one side as well as four polytetrafluoroethylene layers on the another side, known as air cathode (Takahashi et al. 2016).

After entering the waste into the chamber, it is heated, and the operation is done by occurring anode and cathode reactions. After oxidizing the active waste microorganisms created such as *Allium cepa* from onions, *Solanum lycopersicum* from tomatoes, *Solanum tuberosum* from potatoes (Rojas Flores et al. 2020), etc.,

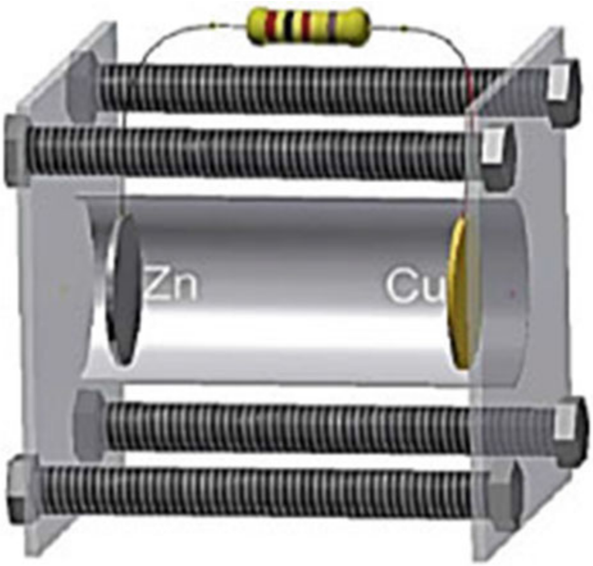

Fig. 9.15 Schema of SMFC using crop residue for electricity generation (Rojas Flores et al. 2020)

electrons are released and moved to the anode to produce electricity (Takahashi et al. 2016).

The anode and cathode of these SMFCs can be made of zinc, Zn, and Cu too, respectively (Rojas Flores et al. 2020). Figure 9.15 shows these kinds of SMDCs.

The chamber is usually made of PVC to absorb light for producing carbohydrates, amino acids, etc. (Takahashi et al. 2016; Rojas Flores et al. 2020).

If Zn and Cu are used for anode and cathode, the reactions are as follows (Rojas Flores et al. 2020):

$$Zn \rightarrow Zn^{2+} + 2e^- \qquad \text{anodic reaction}$$

$$2H^+ + 2e^- \rightarrow H_2 \qquad \text{cathodic reaction}$$

$$Zn + 2H^+ \rightarrow Zn^{2+} + H_2 \qquad \text{overal reaction}$$

Like rice bran, defective agricultural waste/crop residue such as tomatoes can generate electricity with maximum of power density. But for electricity generation from these kinds of substrates, DMFCs are more used. In these kinds of DMFCs, the mediator is hydrated Ultrex membrane, and cathode is felt by ferricyanide to accept electrons. The anode is usually made of carbon and felt by a mixture of active microorganisms created by waste which has been entered to the anode chamber (Shrestha et al. 2016).

As mentioned before, here after entering the waste into the anode chamber, it is heated, and the operation is done by occurring anode and cathode reactions too. After oxidizing the waste microorganisms, electrons are released and moved to the

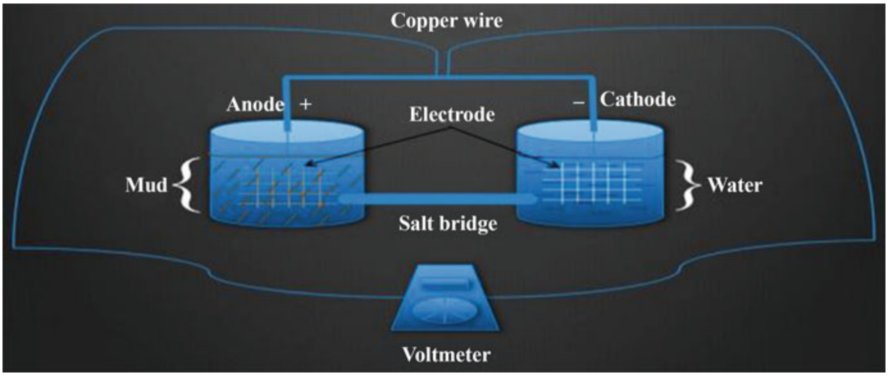

Fig. 9.16 The schematic diagram of the DMFC using mud (Idris et al. 2016)

anode to produce electricity due to creating an external resistance because of carbohydrates, amino acids, etc. formation (Shrestha et al. 2016).

Researchers have shown that the electricity generation from defective onions is higher than tomatoes and potatoes (Rojas Flores et al. 2020).

Overall this kind of electric generation provides the conditions for companies handling the agricultural products to reuse the products unfit for human health and consumption (Rojas Flores et al. 2020).

Using Mud

Chemical waste such as mud can be used in MFCs for converting chemical energy to electrical energy. The mud can be collected from various locations which makes the different results in power generation, because it has various nutrients providing bacteria growth. The MFC uses salt bridge as a membrane transferring proton from cathode to anode, and a longer salt bridge creates bigger voltage than shorter salt bridge (Idris et al. 2016).

In this process two parameters are very important to make high energy efficiency including the length of salt bridge and the type of mud (Idris et al. 2016).

This DMFC has two chambers the same as other DMFCs that one is filled by water and another by mud. Then, anode and cathode electrodes are submerged into these chambers. Afterward, the reaction of water chamber occurs by exposing the aerobic bacteria such as *Proteus vulgaris*, *Rhodoferax ferrireducens*, etc. to oxygen. The water treatment is created by harvesting energy obtained from anaerobic digestion in mud chamber to collect bioenergy from the mud to provide electricity. After a while, the current flow from the mud container is detected and measured (Idris et al. 2016). Figure 9.16 depicts the schematic diagram of this DMFC.

The power density of this DMFC is limited by electrode-base losses and inner resistance created by the electrolyte between the cathode and anode electrodes and membrane resistance (Idris et al. 2016).

Table 9.4 The characteristic of material and wastewater (Wang et al. 2019)

Characteristic	Biogas slurry	Wastewater
Ammonium nitrogen (mg/L)	633.2±16.1	101.5±2.3
COD (mg/L)	4582.5±147.2	106.6±2.0
pH	7.33±0.12	7.58±0.17
Volatile fatty acids (mg/L)	714.2±18.9	–
Soluble cellulose (mg/L)	882.5±59.2	–
Reducing sugar (mg/L)	1015.7±61.9	–

Using Biogas Slurry

Biogas slurry which acts as a microbe in MFCs is used as a anode material to solve the wastewater accumulation produced from plants. It produces hydrolyzed bacteria including *Clostridia* (36%), *Synergistia* (8%), *Bacteroidia* (30%), *Flavobacterium* (7%), *Betaproteobacteria* (2%), *Spirochaetia* (3%), *Methanomicrobia* (1%), and *Gammaproteobacteria* (1%) which can hydrolyze complex organics such as celluloses (Rismani-Yazdi et al. 2013; Jia et al. 2013). In this kind of MFC, the maximal power density is 296 mW/m^2 when the outer resistance is 200 Ω. The removal rate of ammonium nitrogen and COD are 43.9% and 72%, respectively. It is likely to degrade the biogas slurry organics to generate electricity (Wang et al. 2019). Table 9.4 shows the additional parameters of this MFC.

The PEM utilized in DMFC to separate the cathode and anode is salt bridge. The PEM is pretreated by deionized water, H_2O_2 solution (30%, 80 °C), and H_2SO_4 (0.5 mol/L) for 1 h. The cathode and anode are made of carbon. For creating the reactions, firstly, the anode chamber is filled with biogas slurry and wastewater, and the cathode chamber is filled with potassium ferricyanide solution playing an electron acceptor role (Hassan et al. 2014). Then the reactions occur.

This DMFC consumes a big amount of energy because of the hydrolysis reaction which leads to less CE of 4.1%. Researchers have shown that the anode genus can enhance the electricity generation efficiency, for example, by adding *Pseudomonas* or *Hydrogenophaga* to anode chamber (Wang et al. 2019).

9.3.3 Newer Microbial Fuel Cells

Among MFCs, there are ones that use other membranes instead of salt bridge. As mentioned before, these membranes are the substrates which create distance between cathode and anode, and they can act as a place conducive to grow the bacteria by producing enough bacteria in contrast to traditional MFCs that use old suspended bacteria/yeast growth in wastewater, etc. Over a decade, now, they are called 3DEMFC (Dong et al. 2020). This section introduces them.

Using Electronophore (Traditional)

In some MFCs, NR is used known as electronophore made of cation-selective membrane septum and is employed as an electron mediator in MFCs using glucose

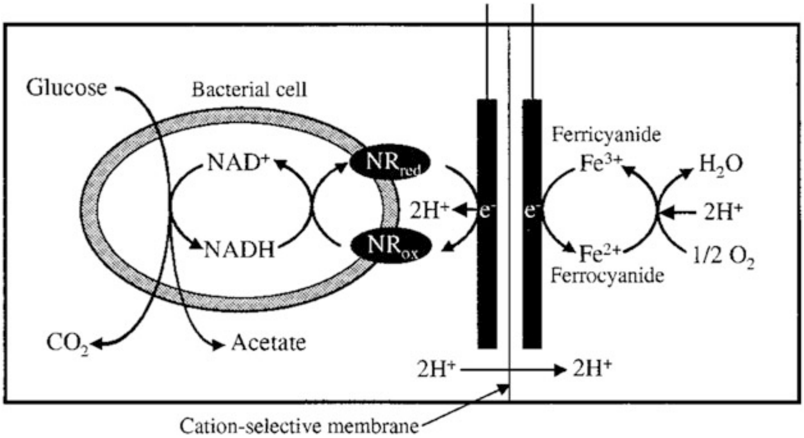

Fig. 9.17 The schematic diagram of the MFC with NR mediator (Park and Zeikus 2000)

up. Glucose plays a key part in changing metabolism as well as anaerobic growth of microorganisms such as *E. coli* and *Actinobacillus succinogenes* (*A. succinogenes*). These microorganisms using NR provide the current tenfold much more than the microorganisms using thionin. In the process, the mud production is also decreased (Park and Zeikus 2000). Figure 9.17 depicts the schematic diagram of this process.

In this kind of MFC, the electrodes are made of woven graphite felt of 12 g. The anode and cathode are moved apart by NR. Here, usually the self-electric resistance between the cathode and anode is nearly 1000 Ω. It can be adjusted by a variable resistance to control the current. The solution of this MFC containing ferricyanide is used as the catholyte and anolyte, respectively, to which glucose is added as an energy source. Oxygen is deleted from the anode by gassing with N_2 during 30 min prior to add NADH, i.e., nicotinamide adenine dinucleotide plus hydrogen. The NADH solution is gassed before with N_2 to delete O_2 (Park and Zeikus 2000).

Table 9.5 shows the electricity generation from glucose when various electron mediators are utilized, and Table 9.6 shows the material consumption and electricity generation by growing the *E. coli* in MFC when NR is used.

These tables depict that NR is the most excellent electron mediator due to increasing the current and coulombic yield (Park and Zeikus 2000).

These kinds of batteries have the good energy efficiency, but their energy efficiency is much less than the chemical fuel cells, due to lowering the metabolic reaction rate. This drawback can be improved by varying the bacterial cell mass, electrode surface area, concentration, and electron mediator type (Park and Zeikus 2000).

Using Biochar (Latest)

As we know, the electron transfer between the cathode and anode in microbial cells is very important in MFCs which makes two features meaning that high specific surface area and good compatibility, essential for them. Now, thanks to new

Table 9.5 Electricity generation from glucose with different mediators (Park and Zeikus 2000)

Microorganism	Potential (V)	Electron mediator	Current (mA)	Energy rate (J/h)	Energy (J)
P. vulgaris	0.3	Thionine	1.25	1.35	5.4
P. vulgaris	0.5	HNQ[a]	0.5	0.9	3.6
E. coli	0.68	NR	4.5	11	44.1

[a]2-Hydroxy-1,4-naphthoquinone

Table 9.6 Electricity generation by growing *E. coli* using NR as an electron mediator (Park and Zeikus 2000)

Cells	Glucose consumption rate (mM/h)	Glucose consumption (mM)	Rate of cell mass increase (g/liter/h)	Electric energy (J/mol of substrate)	Cell mass (g/liter)
Growing	7.52	45.1	0.29	100.8	1.74
Resting	2.59	15.5	0.035	1207.7	0.214

technologies, newer SMFCs have been developed by using biochar as an electrode, which is in the form of particles (Kong et al. 2006), and separate the cathode and anode electrodes. Biochar is obtained from the wasted biomass like crop residues and forestry, so the cost of this kind of MFC reduces (Huggins et al. 2014; Meyer et al. 2011). It has a porous structure having high specific area and good compatibility for electricity generation (Dong et al. 2020).

In addition to biochar, two compounds of biochar can be utilized in the SMFC as a membrane too, i.e., MgO-modified biochar and zeolite mixture (see Fig. 9.18) (Dong et al. 2020).

The power density and overall power generation of this kind of MFC are high because of the biochar conductivity to transfer the electrons which shows the highest removal efficiency of contaminants (Dong et al. 2020). Figure 9.19 shows this SMFC.

9.3.4 Biogas

Biogas is a kind of fuel gas involving of methane, CH_4, CO_2, and other gases, produced through microbial processes under anaerobic condition from biodegradable materials (Shah and Nagarseth 2015). As a renewable energy, biogas has the many applications for power generation and energy efficiency increase. It is one of the natural resources which can decrease the problem of oil prices in the world. Here some applications of it for more efficient electricity generation are defined (Mitan and Badarulzaman 2020).

Fig. 9.18 The kinds of biochar electrodes (Dong et al. 2020)

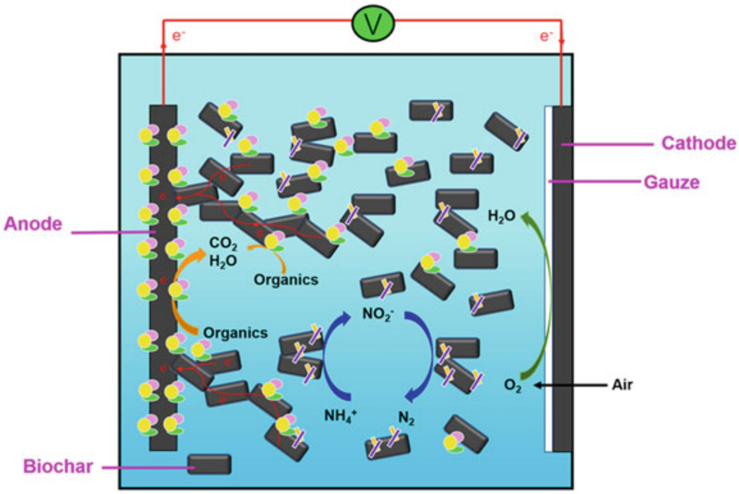

Fig. 9.19 The schema of the SMFC using biochar (Dong et al. 2020)

Using Sewage

For using sewage as a biogas, sewage treatment plants (STPs) have many applications due to sanitary and environmental protection. In fact, sewage is the source of biogas, and these plants play a key role using this potential for energy efficiency. Methane, CH_4, is the main component of biogas generated by these plants which can be utilized for electricity generation, but other gases are also produced (Mattos et al. 2013). The proportion of every gas is dependent on the parameters such as digester type which is anaerobic (Lafratta et al. 2020) or substrate type digesting (Mattos et al. 2013). Anaerobic digestion usually uses thermal hydrolysis process (THP) in the role of pretreatment (Lafratta et al. 2020).

The process of biogas formation in these plants has three steps including fermentation, acetogenesis, and methanogenesis. In the process, the parameters which determine the real electricity generation are heating value, flow rate, and chemical compound (Mattos et al. 2013).

The climate and heat have also an effect on the formation of the biogas, because higher temperature makes this process fast. So most of the plants use heat exchangers for this goal to obtain the heat from exhaust gases and warm the combustion air in turbines to run generators (Mattos et al. 2013).

The biogas obtained from these plants can also be used for engines too due to having low cost (Mattos et al. 2013).

The daily peaks of electricity generation are examined in accordance with how many daily half-hours compose a peak period optimal length. So, given the half-hour generation during a peak period doubles the off-peak generation, the daily peaks are computed, as Eq. (9.8) (Lafratta et al. 2020):

$$\frac{\%\text{GEN}}{\#\text{HH}} = 2 \times \left(\frac{1 - \%\text{GEN}}{48 - \#\text{HH}}\right) \tag{9.8}$$

where $1 - \%$ GEN is the residual daily power produced in the remaining half-hours of a day, 48 in $48 - \#$ HH depicts the total number of half-hours in a day, and % GEN is the share of daily power produced in a number of half-hours, #HH. In this section, two STPs for using biogas from sewage, i.e., animal waste and animal manure/dung, are reviewed.

Using Animal Waste

Biogas can be produced by animal waste such as poultry waste, etc. to generate electricity to overcome the power demands and also supplying it commercially in rural areas. Five tone poultry waste can generate 40–80 m^3/h gas which can easily run a 50 kW biogas generator. The life time of this kind of plants is 20 years with payback of 5 years (Sajib and Hoque 2015).

In these plants, poultry waste is collected in the chamber which mixes the waste with water according to the water content, i.e., the mixing production must be 1:1. Next, this mixing chamber with the help of a pump sends the waste to a digester. Afterward, the gas is produced during the digestion process and stored in the tank (Sajib and Hoque 2015).

These plants used must have the hydrogen sulfide, H_2S, removal unit. This substrate is very metal corrosive. So, the gas passes via this unit to purify. Then the purified gas is sent to the power generation system consisting of a generator and combustion engine to change the mechanical energy into electrical energy and electricity. A hot water tank is also used to maintain the digester temperature hot (Sajib and Hoque 2015). Figure 9.20 shows the diagram of this process.

The H_2S removal unit includes a one-liter sulfide oxidizing unit (SOU) connected to a stirred tank reactor (STR) as an anaerobic digester. The digester effluent is pumped into SOU to create the medium for removing sulfide. Figure 9.21 shows this unit.

The poultry waste is collected from the floor and sheds served for the birds. In total, all around the world, 20% of the GHG is because of the birds' waste which consists of nitrous oxide and methane as the main gases. The parameter of this kind of waste is dependent on the base substrate utilized, bird population density, and the

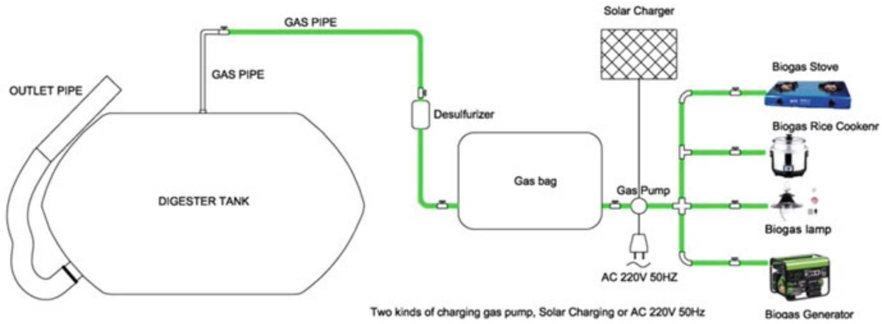

Fig. 9.20 The diagram of the electricity generation from biogas using poultry waste (Sajib and Hoque 2015)

creation time. This waste can be used as a source for ruminants or fertilizers which leads to the nitrogen, N, and phosphorus, P, pollution affecting the water resource (Sajib and Hoque 2015). Table 9.7 shows the poultry waste chemical composition.

For determining the biogas unit size, the below equations are employed (Eqs. 9.9 and 9.10) (Sajib and Hoque 2015):

$$\text{Digester size}\left(\text{m}^3\right) = \text{Daily feed} - \text{in}\left(\text{m}^3/\text{day}\right) \times \text{Retentiontime}(\text{day}) \tag{9.9}$$

$$\text{Daily feed} - \text{in} = \text{Volume of poultry waste} + \text{Volume of water} \tag{9.10}$$

The digester size is defined as the overall size of the biogas unit as follows (Eq. 9.11):

$$\begin{aligned}\text{Digester size} = {} & \text{Any volume occupied by the fermented material} \\ & + \text{Nolume of gas storage}\end{aligned} \tag{9.11}$$

Using Animal Manure

Another waste which can be utilized for biogas generation to produce electricity is animal manure such as cow dung, etc. The small-scale biogas plant using cow dung to produce 1.8 kg of biogas can generate 1400 W power. So financially, application of the large-scale biogas plant has the significant potential for electricity generation. In this plant designed, cow dung is blended with water with ratio of 1:1 and sent to an anaerobic digester to homogenize. The digester inlet is usually covered and padded tightly with rubber for anaerobic digestion. After digesting, the gas is produced and stored in a container/tank. This process is done under high temperature. The gas produced passes through the biogas purification system to be utilized as an effective energy source (Yentekakis and Goula 2017; Müller et al. 2017). This system removes impurities such as H_2S, CO_2, and water vapor to increase the methane concentration in biogas. This causes the calorific value of biogas for energy

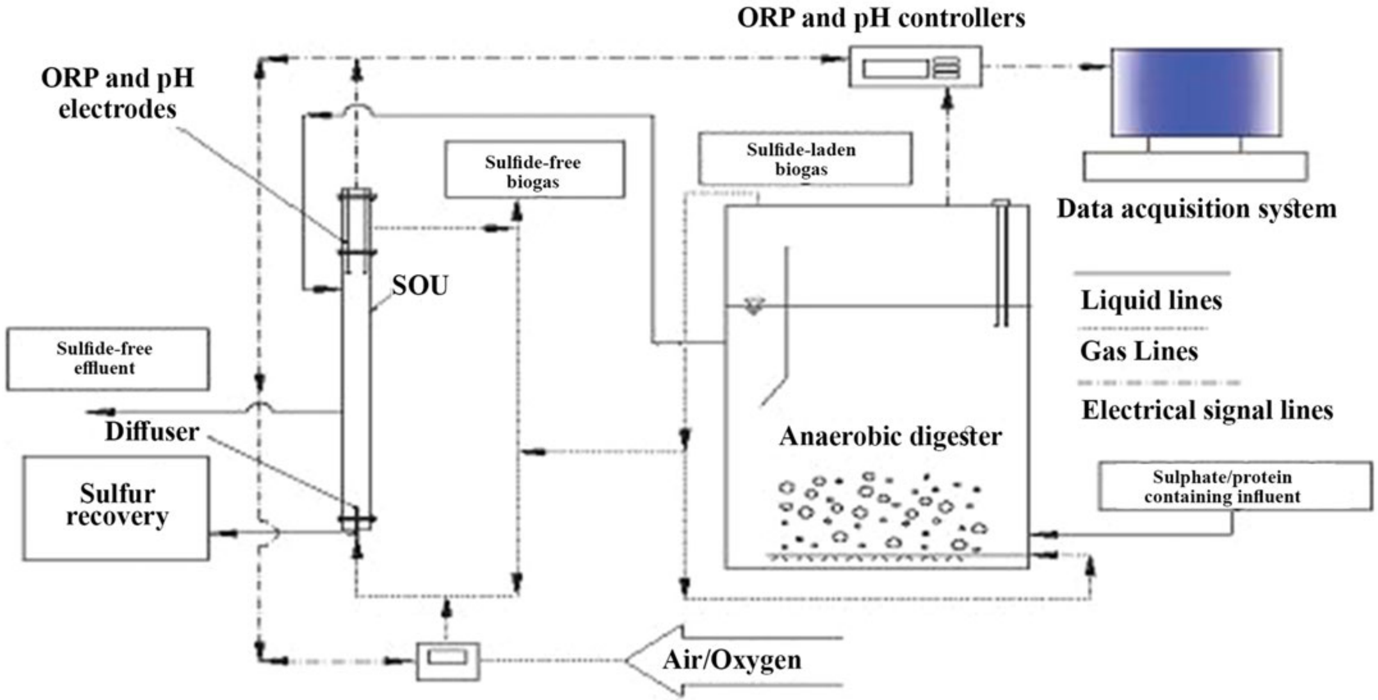

Fig. 9.21 The schematic diagram of the H_2S removal unit (Sajib and Hoque 2015). (ORP is oxidation reduction potential)

Table 9.7 The chemical composition of the poultry waste (Sajib and Hoque 2015)

Microorganisms and metals	μg/g	Microorganisms	μg/100 g
Cu	303	N	2.08
Iron (Fe)	1786	P	1.01
Manganese (Mn)	294	K	2.61
Zn	217	Ca	2.08
Sodium (Na)	2629	Magnesium (Mg)	0.53
Chromium (Cr)	5	S	0.028
Lead (Pb)	22		
Nickel	2		

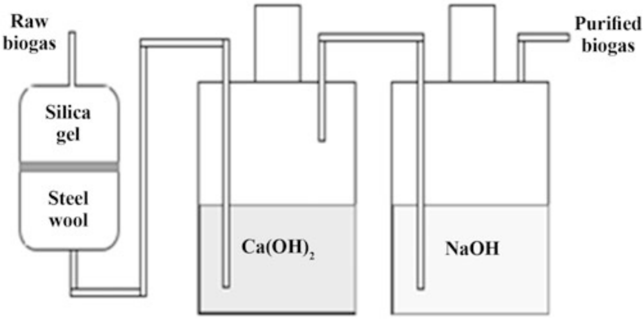

Fig. 9.22 Schematic of the biogas purification system (Akpojaro et al. 2019)

generation to increase (Müller et al. 2017; Akpojaro et al. 2019). Figure 9.22 shows this purification system.

In this system, the biogas is sent to the chamber which contains steel wool and silica gel. Then it is passed for removing H_2S and water vapor. Afterward, the gas is sent to the second chamber. In this chamber, calcium hydroxide, $Ca(OH)_2$, is mixed with water to produce high amount of heat and remove the CO_2. Next, the gas is sent to the third chamber containing sodium hydroxide, NaOH, and water removing the remaining CO_2 and H_2S again (Akpojaro et al. 2019).

The power generation system is made up of a generator and internal combustion engine to generate the mechanical energy to electrical energy and heat (Akpojaro et al. 2019). Figure 9.23 shows this process.

9.3.5 Biohydrogen

Today, the increase of environmental and air pollution leads to increase of the biodiesel production which results in big amounts of glycerol. But what can we do with this production?

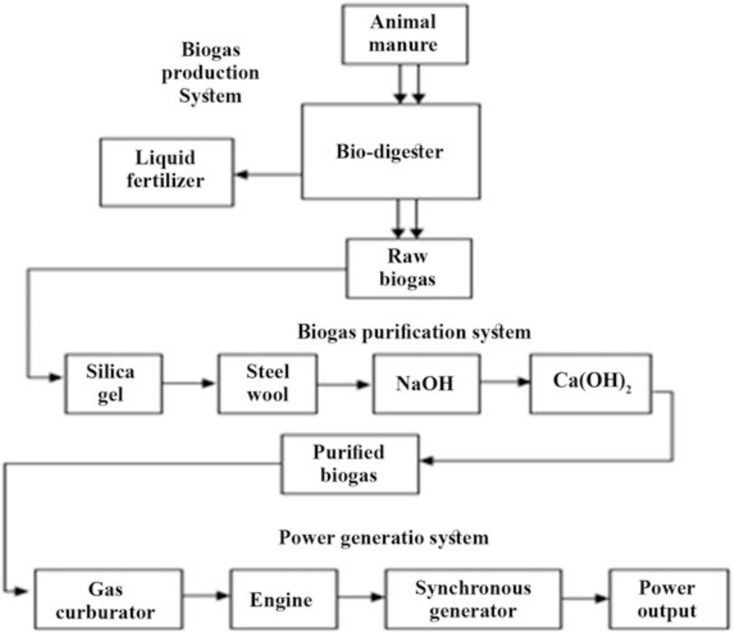

Fig. 9.23 Schematic diagram of the electricity generation from biogas using cow dung (Akpojaro et al. 2019)

The answer is that researchers have found the glycerol have the potential for hydrogen generation through the steam reforming operation which makes it a useful form of a solid oxide fuel cell (SOFC). Glycerol has proved that it can generate much more heat and power energy via the steam reforming process, but its cost is high, and it is an obstacle to make it suitable for practical applications (de Souza et al. 2019).

The reactions created in the steam reforming of the glycerol are as follows (de Souza et al. 2019):

$$C_3H_8O_3 + 3H_2O \leftrightarrows 3CO_2 + 7H_2$$

$$C_3H_8O_3 \leftrightarrows 3CO_2 + 4H_2$$

$$CO + 3H_2 \leftrightarrows CH_4 + H_2O$$

$$CO_2 + 4H_2 \leftrightarrows CH_4 + 2H_2O$$

The electricity production is dependent on every stage of these reactions, which involves three factors, i.e., the diffusion of hydrogen, fuel availability, oxygen, and ion via the porous material, as well as the reaction of the fuel cell. The energy generated by the process will be constrained by one of these factors in accordance with the cell design, composition, and quantity of the input gas (de Souza et al. 2019).

Table 9.8 The types of biomass (Evans et al. 2010)

Dedicated energy crops	Food competitive	
	Short rotation coppice (SRC)	
	Arid/unusable land	
	Mallee	
Residues	Agricultural crop and process residues	
	Bagasse	Others
	Forestry residues	
	Wood wastes	

9.4 Availability

Another application of the microbes in electric generation is availability which this section describes it.

9.4.1 Biomass

Biomass is known because of its availability and sustainability. These are the main features of biomass. The stout crops which are grown on marginal and unused lands are more sustainable source than other lands using fertilizers for biomass (Evans et al. 2010). Table 9.8 depicts the biomass types.

Today electricity creation is very important in the modern societies, because global populations go on increasing and electricity demand goes on growing. So, the energy availability with constant supply and less cost is very important. Currently, with limited fossil fuel supplies with high prices, biomass can be an alternate approach as a renewable and combustion fuel. It is an organic material which can be changed to other forms of energy. It can be generated in every environment as well as also reproduced fast (Evans et al. 2010). Table 9.9 shows the global distribution of the biomass consumption.

For combustion and conversion of biomass into electricity, there are three technologies utilized for this process including (a) pyrolysis which is the biomass thermal destruction in an anaerobic environment without adding air/steam to generate vapors/gases which happens in a gas turbine (Vochozka et al. 2017), (b) gasification which is biomass oxidized by oxygen control and adding steam to generate high calorific combustible gases, and (c) direct gasification which is the full biomass oxidation in the excess air to generate CO_2 and water (Rumão et al. 2014). Hot flue gases are consumed to warm water to be converted to steam to drive a gas turbine. This technology is old and simple but among others is very inefficient. The (a) and (b) have the most efficient technologies, but they need control and investment (Evans et al. 2010). Figure 9.24 depicts the technologies utilized for electricity creation from biomass reactions, and Table 9.10 depicts the CO_2 emissions from the biomass power generation.

Table 9.9 Global distribution of biomass use (Lamers et al. 2015)

Countries	Co-firing capacity	Solid biomass installations	NREAP projections (2020)				NPOL scenario (2020)			
			Mtonnes				Mtonnes			
	MWe (by 2012)	MWe (by 2010)	MWe	GWh	WPe	PJ	MWe	GWh	WPe	PJ
Belgium	280	727	2007	9575	5.8	102.1	910	4341	2.6	45.8
Germany	(n/a)	3179–3650	4792	24,569	14.8	260.5	4313	22,112	13.3	234.1
Denmark	996	1168	2404	6345	3.8	66.9	1814	4788	2.9	51.0
The Netherlands	413–551	992	2253	11,975	7.2	126.7	1306	6942	3.7	65.1
United Kingdom	208–338	2097	3140	20,590	12.4	218.2	3895	25,541	15.4	271.0
Sum	1897–2165	8163–8634	14,596	73,054	44	774.4	12,238	63,724	38	668.8

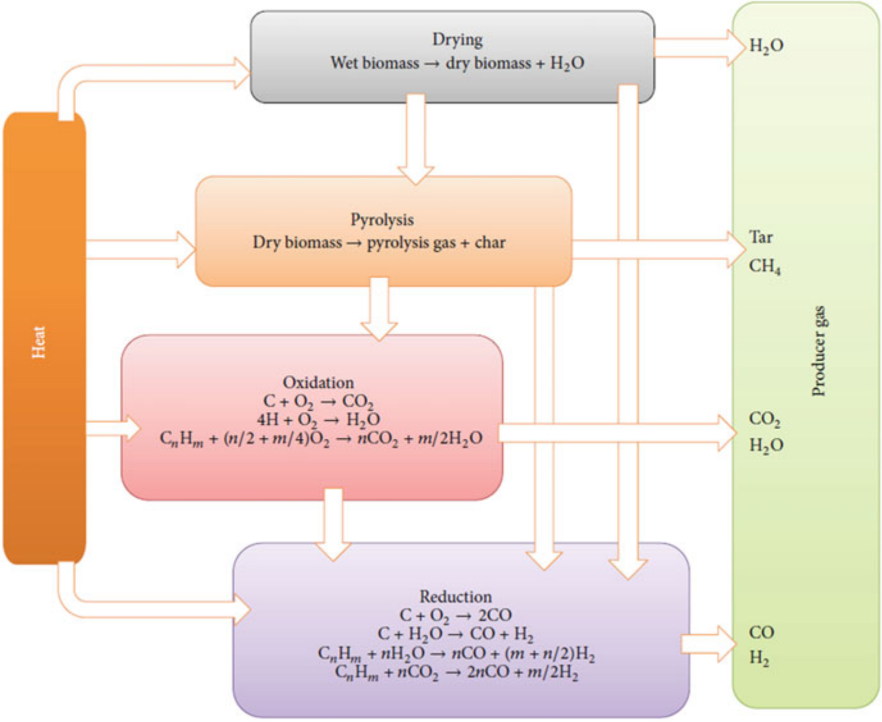

Fig. 9.24 Technologies utilized for conversion and combustion of biomass into electricity (Das and Hoque 2014)

Table 9.10 CO_2 emissions form the biomass power generation (Evans et al. 2010)

Year	gCO_2/kWh	Power generation
1998	24	Steam turbine
1399	30–40	
2003	48	
2003	37	Combined circle (CC)
2007	58	
2007	131	SRC
2007	132	

An electricity generation system by biomass can be designed such an integrated combined cycle plant as depicted in Fig. 9.25. It consists of handling equipment and fuel storage; gasifier; boiler; furnace/combustor; fans; pumps; generator; steam turbine; cooling tower; condenser; emissions/exhaust controls; as well as automated system controls (Alidrisi and Demirbas 2016).

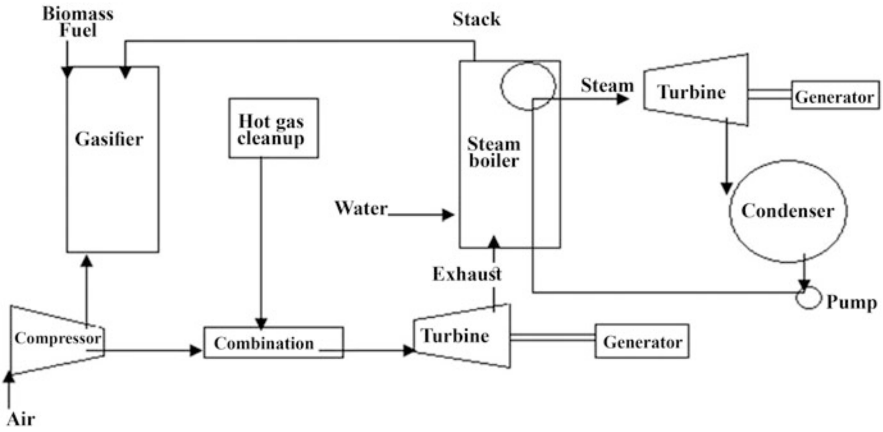

Fig. 9.25 The integrated combined cycle plant for power generation (Alidrisi and Demirbas 2016)

9.5 Clean Energy

One of the most important properties of microbes in electricity generation is that they can pollute the air and the environment less. Even microbial contamination can be reduced to zero which this section describes it.

9.5.1 Algae

One of the processes that may seem amazing at best is electricity generation from algae. This process is really clean because it does not release any CO_2 into the air; thus it does not play any role in global warming. This source of energy is cheap, while it can play an important role in economic potential due to fuel the engines (O'Sullivan 1993).

London is one of the cities around the world which utilizes this potential by installing Biocoil units designed for mass production of algae with capacity of approximately 3000 gal. They are employed for sewage treatment (O'Sullivan 1993).

These Biocoils involve of a 26 high $\times$ 16 wide feet metal frame. They have two unique features: (1) self-cleaning to avoid creation of algae on the inner wall and (2) having common, single intake, and outlet manifold joined into bands of tubes to avoid from internal pressure and decrease the light transmission throughout it. For drying the chamber, solutions include using dissolved CO_2 from the engine exhaust and *Chlorella* which must be heated by algae vapor and passed throughout the coil. The liquid wastes from the last stage of the sewage treatment are the source for the growth of nitrates and phosphates (O'Sullivan 1993).

A Biocoil unit can produce up to 15 tons *Chlorella* a year. So these units together can generate enough fuel to run a power plant. Since these units have a simple structure, installing these units is not difficult and expensive (O'Sullivan 1993).

9.5.2 Microbial Biophotovoltaic Cells

Microbial biophotovoltaic cells are a green-energy device which can convert light/solar energy to electricity through a biological process (Asiri 2019; Kusmayadi et al. 2020). In these cells, the unicellular chlorophyll-including algal cells or cyanobacterial cells change CO_2 into biomass with the help of solar/light energy (Lee et al. 2015; Sawa et al. 2017). This reaction is as follows:

$$CO_2 + H_2O + \text{light} \rightarrow \text{biomass} + O_2$$

All CO_2 generated by anodic chamber can be used by cathodic chamber, i.e., microalgal cells or cyanobacterial cells, because of light, but all O_2 obtained can be employed as electron acceptors required for cathodic chamber (Lee et al. 2015; Sawa et al. 2017). So, the total reaction is as follows:

$$\text{organics} + \text{light} \rightarrow \text{biomass} + \text{external power}$$

Figure 9.26 shows the cycle of microbial biophotovoltaic cells use for power generation.

Using Algae

The microbial biophotovoltaic cells which use algae to convert solar/light energy into electricity are known as microalgae-microbial fuel cells (mMFCs) (Lee et al. 2015; Kusmayadi et al. 2020). As mentioned before, algae are a promising source for biochemical production and biofuel production (Chisti 2007; Delrue et al. 2012; Liu et al. 2012; Chen et al. 2013; Lin et al. 2015; Show et al. 2013, 2015; Tran et al. 2013; Yen et al. 2013). It can be generated much more oil from algae than biomass (Lee et al. 2015).

The mMFC uses the photosynthetic microorganisms to change solar energy into electrical energy via the metabolic reactions (Bombelli et al. 2011) (see Fig. 9.27). Also, mMFC has the capable of removing nitrogen pollution from water by separating CO_2 form the air (Wang et al. 2010; Xiao et al. 2012). With this, it has a potential in deleting the CO_2 from the air.

In Fig. 9.27, dash lines depict the anodic/cathodic integration of the chambers with carbon flows (Lee et al. 2015).

Using Cyanobacteria

The cyanobacteria are like algae. They have the ability to generate light/solar energy to electricity in the microbial biophotovoltaic cells. These cells use water as the electron source. In these kinds of cells, the biophotovoltaic cell is a cyanobacterial

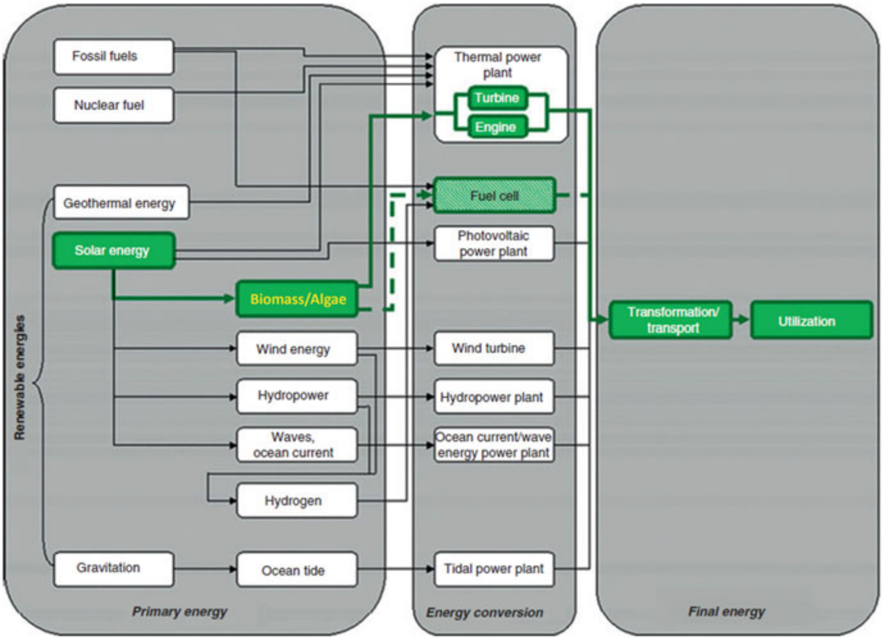

Fig. 9.26 The cycle of microbial biophotovoltaic cell use for the power generation (Wiese 2017)

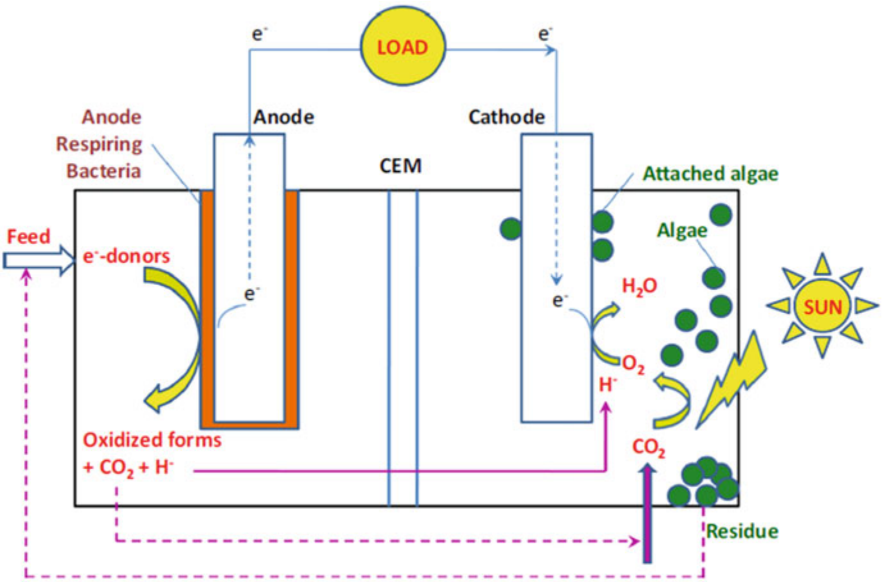

Fig. 9.27 Schematic diagram of mMFC work (Lee et al. 2015)

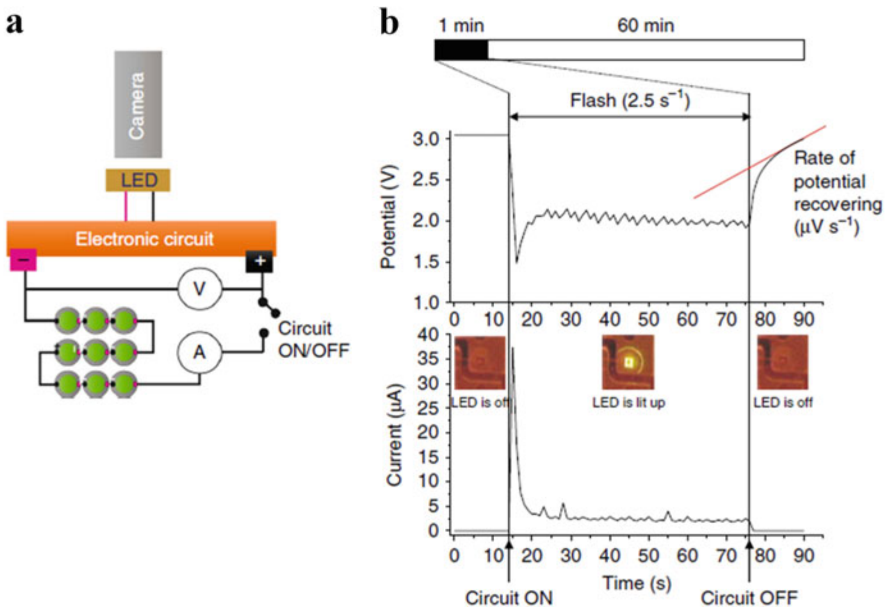

Fig. 9.28 Schema of the electricity generation by microbial biophotovoltaic cells using cyanobacteria, (a) the organized cells in series, and (b) electricity generation analysis by LED (Sawa et al. 2017)

cell. The potential of these MFCs can be utilized in low-power devices such as LEDs and biosensors. Also, they are not expensive. The power generation process does not have any effect on the cell viability. The Fig. 9.28 depicts the electricity generation by these kinds of MFCs. To produce the voltage, an array involving nine cells connected in series generates the output voltage for electricity. It means that the output voltage is equal to sum of voltage of these microbial biophotovoltaic cells (Sawa et al. 2017).

Using Plant Rhizodeposition

The microbial biophotovoltaic cells have potential for using the paddy fields (see Fig. 9.29) of non-tidal wetlands to generate in situ electricity from solar energy without gathering the biomass as a PMFC (Sudirjo et al. 2018). They are installed in paddy fields such as rice paddy fields, etc., and the electricity is generated electrochemically by active bacteria/yeast, e.g., supplied by plants, rhizodeposits, and plant residues with the help of a bioanode (Sudirjo et al. 2018; Matsumoto et al. 2020).

Regardless of these PMFCs producing clean energy (Regmi and Nitisoravut 2020), using big amounts of electrodes overcoming the weak conductivity is expensive (Matsumoto et al. 2020). However, it is possible to decrease the amounts of electrodes by changing the soil compound and adding AC for better conductivity (Sudirjo et al. 2018). Iron is a corrosive substrate which can be existed in soil. If this

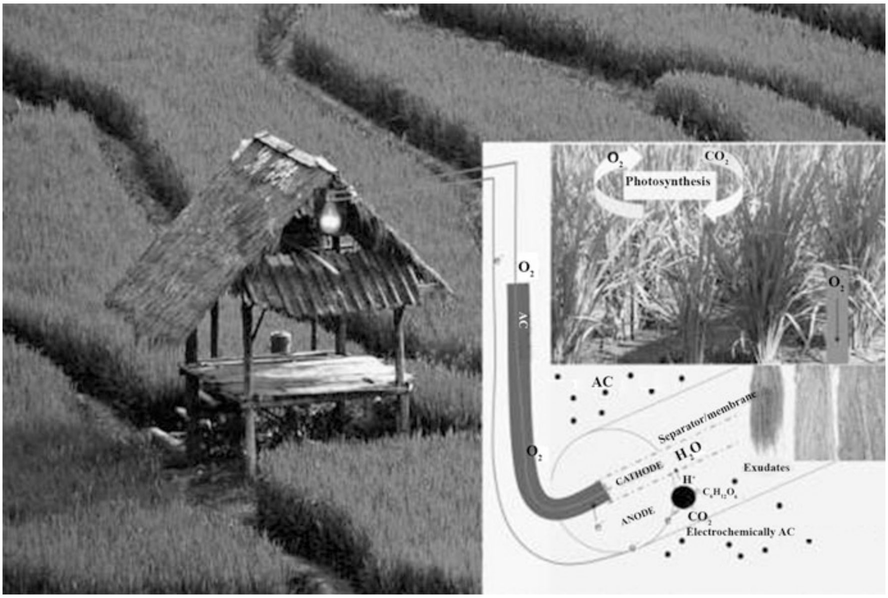

Fig. 9.29 Electricity generation from the plant rhizodeposition

substrate is removed from the soil, the power density increases (Matsumoto et al. 2020).

Today nanostructured catalysts have been introduced instead of ACs that can have the best electrical conductivity (Mangeli et al. 2020).

A PMFC is a flat plate reactor made of plexiglas material. Each PMFC has two chambers consisting of the cathode and anode electrodes made of graphite detached by a CEM. The anode electrode is placed nearly 5 cm below the soil, and the cathode electrode is floated above the water. The anode chamber is filled with AC or nanostructured catalysts as a plant growth medium for growing microorganisms such as *Spartina anglica* (Sudirjo et al. 2018; Matsumoto et al. 2020). The cathode chamber is usually filled with platinum catalysts. The cathode and anode can make an outer resistor of 1000 Ω through epoxy-encapsulated wires after being connected (Matsumoto et al. 2020).

These kinds of plants must monitor some parameters such as electric signal nature, daily power output, plant growth, polarization method, and changing the soil physiochemical features (Regmi and Nitisoravut 2020).

By adding the *Azolla* to the soil, the current of paddy field is improved. It also plays a useful role in biomass growth and is placed in the cathode (Fig. 9.30). The maximum power generated is up to 84% (Regmi and Nitisoravut 2020).

The current generation of these PMFCs at night is less than the daytime, because during daytime, photosynthesis causes the carbohydrates formation to increase (Regmi and Nitisoravut 2020).

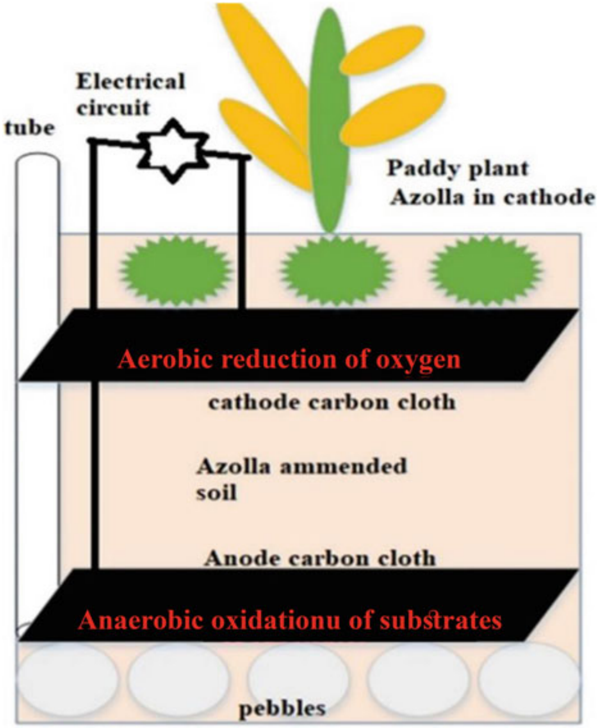

Fig. 9.30 Schematic diagram of the plant rhizodeposition by PMFC adding *Azolla* to the soil (Regmi and Nitisoravut 2020)

9.6 Sustainability

Sustainability is the ability to be constantly. In fact, it is a socioeconomic process achieved through balance of resources within the environment with suitable benefits such as cost, etc. One of the applications of microbes in electric generation is sustainability. So, this section describes this application of microbes in detail.

9.6.1 Biomass

As mentioned before, biomass to electricity can decrease the dependency on fossil fuel. The electricity generation from biomass is sustainable thermochemical process (Das and Hoque 2014; Bhavirisetti et al. 2017). In this regard, forest residues are major sources of biomass and could contribute in this process which this subsection describes it.

Crop Residue

Among the biomass materials, crop residues such as rice residue, etc. play a key role in electricity production. In fact, the power generation from crop residues is considered as a source of income for farmers which creates other opportunities such as economic activities and employment based on sustainability. It can decrease the foreign exchange requirements for furnace fuel/fuel imports.

This conversion can be performed by using two technologies known as biochemical and thermochemical (Jiang et al. 2012) in plants. The thermochemical conversion is the direct combustion of fuels generating thermal energy for electricity and steam generation using converters such as steam turbines/engines, etc. These plants can produce electricity from kilowatts to megawatts (Ahmed and Ahmad 2014).

For estimation of the power potential, Eq. (9.12) is used as follows (Ahmed and Ahmad 2014):

$$\mathrm{RRPP}_j = \frac{K \times \mathrm{ACR_J} \times \mathrm{WAQRB} \times \mathrm{LHVR}}{T} \tag{9.12}$$

where RRPP_j is the crop residue power potential of the Jth region, K the overall energy conversion efficiency, ACR_J the rice acreage in acres in the Jth region, *WAQRB* the weighted average quantity of crop residue burnt per acre, *LHVR* the less heating value of the crop, and T the annual operating duration in seconds (Ahmed and Ahmad 2014).

In addition to income opportunities, this conversion has a high impact on the physical characteristics of the environment as well as soil and also crops yield (Ahmed and Ahmad 2014).

Table 9.11 illustrates the percentage of the residue production for some agricultural crops. Table 9.12 depicts some forest residue production utilized for this conversion.

9.6.2 Camphor

The camphor is a biodegradable matter which can be made artificially too. For power generation, it is usually used in solid form to result in maximum capacity when it is burnt, but its shape does not affect the electricity generation. It can be found in any shape in the markets. Also, the calorific value of that is high due to having carbon. It

Table 9.11 The percentage of the residue production for some agricultural crops (Das and Hoque 2014)

Crop residue	Production in 2011 (million tons)	Fractions	Number of fractions	Crop residue (million tons)
Maize	1.02	Stalks	200.00	2.04
		Cobs	30.00	20.3104
Rice	50.63	Straw	50.00	25.31
		Husk	20.00	10.13
Jute	1.52	Stalk	58.84	0.90
		Leaves	13.91	0.21
Wheat	0.97	Straw	65.00	0.63
Mustard	0.23	Straw	75.00	0.17
Sugarcane	4.67	Bagasse	36.00	1.68
Lentil	0.081	Straw	72.46	0.058
Coconut	0.08	Husk	31.00	0.024
		Shell	24.40	0.019

Table 9.12 Forests residues utilized for biomass to electricity generation (Das and Hoque 2014)

Forest residues used for power generation	Forest product	Production in 2011 (m^3)
	Sawlogs and veneer logs	174,000
	Plywood	1000
	Sawnwood	388,000
	Wood fuel	27,286,834
	Industrial roundwood	282,000
	Particle board	2200
	Pulpwood round and splits	18,000
	Hardboard	5100
	Paper and paperboard	8000
	Wood charcoal	326,684
	Newsprint	20,000
	Writing and printing paper	30,000
	Fiber pulp	18,000

is flammable, white transparent, and waxy with a strong odor. The chemical formula of camphor is $C_{10}H_{12}O$. It can be obtained from the wood of cinnamomum tree which is found in some areas such as sea (Santhosh et al. 2014).

It has more benefits than other fuel like diesel or petrol such burning completely and its availability in every country as a cheap fuel (Santhosh et al. 2014) (see Fig. 9.31).

Also camphor gases are toxic, and its GHG emissions are also less than other fuels (see Fig. 9.32).

The camphor plant is like a coal plant. This plant includes six parts as fuel storage and duel conveyer belt, boiler, condenser, turbine, transmission lines, and cooling tower (Hensley 1985) (see Fig. 9.33).

Camphor generates clean energy with less cost as well as high efficiency. The electricity produced by camphor is very eco-friendly and economical (Santhosh et al. 2014).

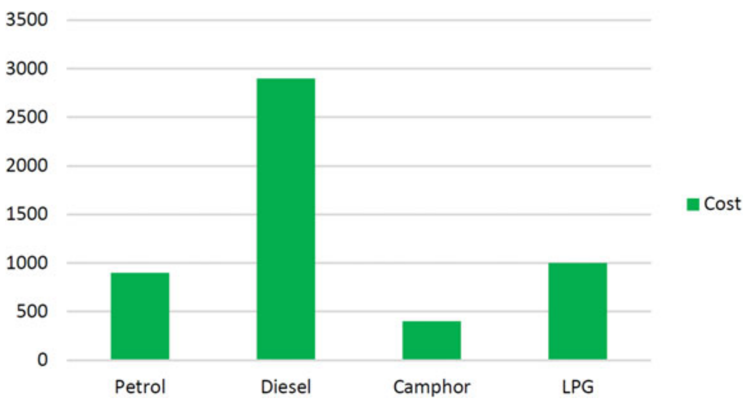

Fig. 9.31 The camphor cost (LPG is liquefied petroleum gas) (Santhosh et al. 2014)

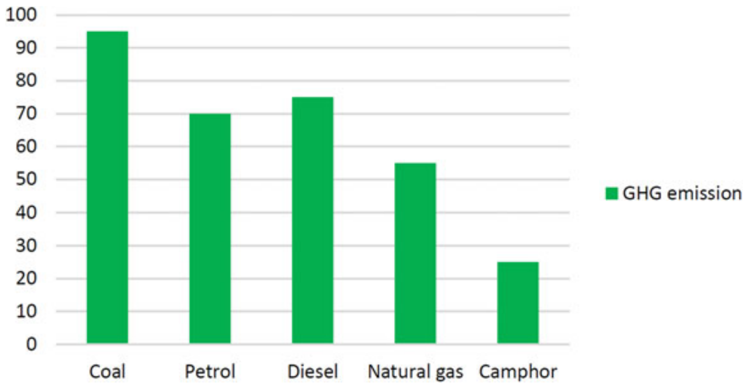

Fig. 9.32 GHG emission of camphor (Santhosh et al. 2014)

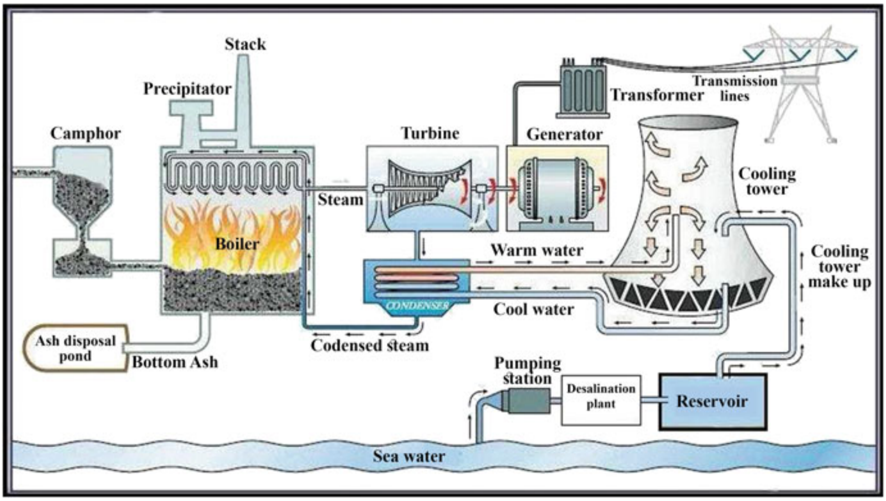

Fig. 9.33 Schema of the camphor plant (Santhosh et al. 2014)

9.7 Conclusion

This chapter investigates the application of microbes in electric generation which has a great potential for producing electricity. Microbes can be obtained from various sources and used as a new resource of energy and also renewable energy. In the meantime, instead of installing plants, the technologies such as MFCs and microbial biophotovoltaic cells are able to create electricity easier straightforward from different types of (in)organic compounds, using these microbes. Table 9.13 shows this chapter in summary.

Table 9.13 Application of microbes in electric generation

Applications	Major source of microbe production	Price of electricity generation	Social impacts	Limitations	Water use	Ref.
Reduction of the environmental and air pollution						
Natural aerosols from vegetation	Blue haze above heavily forested regions	–	Toxicity reduction of aerosols	The major factor in blue haze production is the particles having the size range less than 0.6μm	Yes	Fish (1972)
LFG	Waste-filled parts of land	Expensive	GHG emission reduction	It is very important to have the field measurement before using LFG	No	Morgan and Yang (2001), Salihoglu (2018), Christensen et al. (1996), Zuberi and Ali (2015), Aghdam et al. (2018), Calabrò (2009), Calabrò et al. (2011)
Biogas	Leachate of the waste	–	GHG and PM reduction	A plant reactor must be constructed for the leachate treatment	Yes	Rashidi et al. (2012), American Public Health Association, American Water Works Association, Water Pollution Control Federation,, and Water Environment Federation (1915)
Biodiesel	Fats and oils	Cheap	GHG and global warming reduction	Obeying the limits and parameters of standards	No	Mittelbach (2013), Di Pascoli et al. (2001)

(continued)

Table 9.13 (continued)

Applications	Major source of microbe production	Price of electricity generation	Social impacts	Limitations	Water use	Ref.
Bioethanol	Celluloses	Cheap	GHG and global warming reduction	Solid residue combustion for generation of electricity and steam is required	Yes	Galbe and Zacchi (2012)
	Starch	Cheap	GHG reduction	Solid residue combustion for generation of electricity and steam is required	Yes	Friedl (2012)
	Sugar	Cheap	GHG and global warming reduction	Cogeneration process for mechanical/electrical as well as thermal energy generation is required	Yes	Coelho et al. (2013)
Sewer	Wastewater and refuse from animal or human	Cheap	Environmental and air pollution reduction	Sewer electrical generation apparatus is required	No	Gotay (2013)
Energy efficiency						
Microorganisms	Exoelectrogens, electrogens, and anode-respiring bacteria	Cheap	Generating light and heat from lamps or LEDs	–	No	Logan (2009), Logan and Regan (2006), Lovley (2006), Torres et al. (2008), Bennetto (1990), Lithgow et al. (1986), Vega and Fernández (1987), Kreysa and Krämer (1989), Kim et al. (1999a, b, c, 2000), 2002, Yamazaki et al. (2002), Jang et al. (2004)

MFCs	Natural fermentation	–	Collecting the energy and to power and manage devices	This system is utilized for remote areas such as bottom of the oceans more	No	Khan (2009)
	Biomass	Cheap	Using biomass maintains the chemical process and conducts the generated electrons to the anode straightaway	Current density decreases	No	Fu and Wu (2010). Liu (2010)
	Domestic wastewater	Expensive	Enhancing electricity generation	The total power output depends on the types of mediators and electrode materials, distance between the cathode and anode, and oxygen reaction of the cathode	Yes	Zhao et al. (2019), Njoku et al. (2020), Ogugbue et al. (2015), Luo et al. (2017), Gao et al. (2020)
	Industrial wastewater	Expensive	Enhancing efficiency of 60–71%	It is an expensive process	Yes	Mohamed et al. (2017), Ma'arof et al. (2020)
	Sewage	Cheap	Producing high voltage and current for generating electricity	- The sewage sterilization reduces the current and voltage, because of killing the microorganisms. - As the H_2S and ammonia creates air and environmental problems, so it is better to pretreatment is done before using this kind of waste.	Yes	El-Nahhal et al. (2020)

(continued)

Table 9.13 (continued)

Applications		Major source of microbe production	Price of electricity generation	Social impacts	Limitations	Water use	Ref.
		Crop residue	Cheap	Generating electricity with maximum of power density	Using inorganic matter due to not needing oxygen for the degradation process is better	Yes	Takahashi et al. (2016), Shrestha et al. (2016), Logan and Rabaey (2012), Rojas Flores et al. (2020)
		Mud	–	It makes high energy efficiency by longer membrane	The power density is limited by electrode-base losses and internal resistance	Yes	Idris et al. (2016)
		Biogas slurry	–	Enhancing the electricity generation efficiency	It consumes a big amount of energy because of the hydrolysis reaction which leads to less CE	Yes	Wang et al. (2019), Rismani-Yazdi et al. (2013), Jia et al. (2013), Hassan et al. (2014)
Newer MFCs	Electronophore membrane	Glucose	–	Having the good energy efficiency	Their energy efficiency is much less than the chemical fuel cells, due to lowering the metabolic reaction rate	No	Park and Zeikus (2000)
	Biochar membrane	Biomass	Cheap	The power density and overall power generation is high	–	No	Dong et al. (2020), Kong et al. (2006), Huggins et al. (2014), Meyer et al. (2011)

Biogas	Sewage	Cheap	High energy efficiency	The climate and heat have also an effect on the formation of the biogas, because higher temperature makes the process fast	Yes	Mattos et al. (2013), Lafratta et al. (2020)
	Animal waste	Cheap	High energy efficiency	H_2S removal unit is required	Yes	Sajib and Hoque (2015)
	Animal manure	Cheap	High energy efficiency	The biogas purification system is required	Yes	(Yentekakis and Goula (2017), Müller et al. (2017), Akpojaro et al. (2019)
Biohydrogen	Glycerol	Expensive	Generating much more heat and power energy	The electricity generation depends on the diffusion of hydrogen, fuel availability, oxygen and ion via the porous material, as well as the reaction of the fuel cell	Yes	de Souza et al. (2019)
Availability						
Biomass	Marginal and unused lands	Cheap	High energy efficiency	The combustion and conversion are required	Yes	(Evans et al. (2010), Lamers et al. (2015), Vochozka et al. (2017), Rumão et al. (2014), Das and Hoque (2014), Alidrisi and Demirbas (2016)

(continued)

Table 9.13 (continued)

Applications	Major source of microbe production	Price of electricity generation	Social impacts	Limitations	Water use	Ref.
Clean energy						
Algae	Algae	Cheap	Zero emissions	–	No	O'Sullivan (1993)
Microbial biophotovoltaic cells	Algae	–	Zero emissions	–	Yes	Lee et al. (2015), Kusmayadi et al. (2020), Chisti (2007), Yen et al. (2013), Bombelli et al. (2011), Wang et al. (2010), Xiao et al. (2012)
	Cyanobacteria	Cheap	Zero emissions	It can be utilized in low-power devices such as LEDs and biosensors	Yes	Sawa et al. (2017)
	Plant rhizodeposition	Expensive	Zero emissions	Using big amounts of electrodes overcoming the weak conductivity is expensive	Yes	Sudirjo et al. (2018), Matsumoto et al. (2020), Regmi and Nitisoravut (2020), Mangeli et al. (2020)
Sustainability						
Biomass	Crop residue	Cheap	Economic activities and employment based on sustainability	–	Yes	Das and Hoque (2014), Jiang et al. (2012), Ahmed and Ahmad (2014)
Camphor	Camphor	Cheap	Economic activities and clean energy based on sustainability	–	No	Santhosh et al. (2014)

9.8 Future Approach

Researchers believe that using microbes to generate electricity has mush more beneficial applications for the country's industry and economy than conventional energy, like natural gas, oil, and coal. Therefore, in order to realize this belief as much as possible, future research should proceed it based on the following studies:

1. Assessment of the electricity generation during the anaerobic treatment for reducing the amount of mud placed in the treatment systems must be accomplished more (Park and Zeikus 2000).
2. The contaminant rate of some gases such as NO_2 is usually higher than permitted limit in digester reactors which must be solved by technical approaches (Rashidi et al. 2012; Li and Yu 2014).
3. More investigation for minimizing the energetic and physical losses must be performed in the treatment process, for example, reusing and confirming the clean water for STPs (Mattos et al. 2013).
4. During the camphor process for electric generation, the water must be filtered to not damage the boilers (Santhosh et al. 2014).
5. Further research is need for decrease of crop residue price due to using biomass much more for electric generation (Lamers et al. 2015; Bhavirisetti et al. 2017; Atănăsoae et al. 2018).
6. Using biomass for electric generation must be implemented for both urban and remote regions as a sustainable and clean energy (Matsumoto et al. 2020; Atănăsoae et al. 2018).
7. Using compact fuels such as pellets and briquettes with high energy density can enhance the overall efficiency and energy supply (Wiese 2017).
8. For enhancing the calorific value and mechanical features of animal manure, further investigation is required (Mitan and Badarulzaman 2020).
9. Future studies must evaluate the optimal amount of iron and its environmental effect on soil (Matsumoto et al. 2020).
10. Much more research is needed to be done due to enhancing the MFC functionality for wastewater treatment and electric generation (Njoku et al. 2020).
11. The newer studies must focus on biosensors for detecting the various contaminants (Yaqoob et al. 2020).

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References

Aghdam EF, Fredenslund AM, Chanton J, Kjeldsen P, Scheutz C (2018) Determination of gas recovery efficiency at two Danish landfills by performing downwind methane measurements and stable carbon isotopic analysis. Waste Manag 73:220–229. https://doi.org/10.1016/j.wasman.2017.11.049

Ahmed T, Ahmad B (2014) Burning of crop residue and its potential for electricity generation. Pak Dev Rev:275–292. https://doi.org/10.2307/24398410

Akiba THPB, Bennetto HP, Stirling JL, Tanaka K (1987) Electricity production from alkalophilic organisms. Biotechnol Lett 9(9):611–616. https://doi.org/10.1007/BF01033196

Akpojaro J, Ofualagba G, Akpojaro MA (2019) Electricity generation from cow dung biogas. J Appl Sci Environ Manag 23(7):1301–1307. https://doi.org/10.4314/jasem.v23i7.17

Alidrisi H, Demirbas A (2016) Enhanced electricity generation using biomass materials. Energy Sources A 38(10):1419–1427. https://doi.org/10.1080/15567036.2014.948647

American Public Health Association, American Water Works Association, Water Pollution Control Federation, & Water Environment Federation (1915). Standard methods for the examination of water and wastewater (2). American Public Health Association USA

Ardeleanu I, Mârgineanu DG, Vais H (1983) Electrochemical conversion in biofuel cells using Clostridium butyricum or Staphylococcus aureus oxford. J Electroanal Chem Interfacial Electrochem 156:273–277. https://doi.org/10.1016/S0022-0728(83)80678-0

Inamuddin, Ahmer M.F, & Asiri A.M. (2019) Microbial fuel cells – materials and applications. LLC, 46., 364 Pages. ISBN: 9781644900116

Atănăsoae P, Pentiuc RD, Milici MR, Hopulele E, Mihai I (2018) Promoting the electricity generation from biomass in Romania. In: 2018 international conference and exposition on electrical and power engineering (EPE). IEEE, Washington, DC, pp 0373–0376. https://doi.org/10.1109/ICEPE.2018.8559890

Bacenetti J, Fiala M, Baboun SH, Demery F, Aburdeineh I (2016) Environmental impact assessment of electricity generation from biogas in Palestine. Environ Eng Manag J 15(9). https://doi.org/10.30638/eemj.2016.206

Bennetto HP (1990) Electricity generation by microorganisms. Biotechnol Educ 1(4):163–168

Bhavirisetti M, Tallapragada VS, Pasula A, Tatituri S (2017) Thermochemical conversion of biomass into gaseous fuel for electricity generation, Biofuels and bioenergy (BICE2016). Springer, Cham, pp 83–92. https://doi.org/10.1007/978-3-319-47257-7_9

Bombelli P, Bradley RW, Scott AM, Philips AJ, McCormick AJ, Cruz SM et al (2011) Quantitative analysis of the factors limiting solar power transduction by Synechocystis sp. PCC 6803 in biological photovoltaic devices. Energy Environ Sci 4(11):4690–4698. https://doi.org/10.1039/C1EE02531G

Calabrò PS (2009) Greenhouse gases emission from municipal waste management: the role of separate collection. Waste Manag 29(7):2178–2187. https://doi.org/10.1016/j.wasman.2009.02.011

Calabrò PS, Orsi S, Gentili E, Carlo M (2011) Modelling of biogas extraction at an Italian landfill accepting mechanically and biologically treated municipal solid waste. Waste Manag Res 29 (12):1277–1285. https://doi.org/10.1177/0734242X11417487

Chen CY, Kao PC, Tsai CJ, Lee DJ, Chang JS (2013) Engineering strategies for simultaneous enhancement of C-phycocyanin production and CO_2 fixation with Spirulina platensis. Bioresour Technol 145:307–312. https://doi.org/10.1016/j.biortech.2013.01.054

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25(3):294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Christensen TH, Kjeldsen P, Lindhardt B (1996) Gas-generating processes in landfills. In: Landfilling of waste: biogas. E & FN Spon, London, pp 27–50

Coelho ST, Gorren R, Guardabassi P, Grisoli R, Goldemberg J (2013) Bioethanol from sugar: the Brazilian experience. In: Kaltschmitt M, Themelis NJ, Bronicki LY, Söder L, Vega LA (eds) Renewable energy systems. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-5820-3_312

Das BK, Hoque SM (2014) Assessment of the potential of biomass gasification for electricity generation in Bangladesh. J Renew Energy 2014. https://doi.org/10.1155/2014/429518

Delrue F, Setier PA, Sahut C, Cournac L, Roubaud A, Peltier G, Froment AK (2012) An economic, sustainability, and energetic model of biodiesel production from microalgae. Bioresour Technol 111:191–200. https://doi.org/10.1016/j.biortech.2012.02.020

Di Pascoli S, Femia A, Luzzati T (2001) Natural gas, cars and the environment. A (relatively) 'clean'and cheap fuel looking for users. Ecol Econ 38(2):179–189. https://doi.org/10.1016/S0921-8009(01)00174-4

Dong J, Wu Y, Wang C, Lu H, Li Y (2020) Three-dimensional electrodes enhance electricity generation and nitrogen removal of microbial fuel cells. Bioprocess Biosyst Eng:1–10. https://doi.org/10.1007/s00449-020-02402-9

El-Nahhal YZ, Al-Agha MR, El-Nahhal IY, Nabil A, El-Nahal FI, Alhalabi RA (2020) Electricity generation from animal manure. Biomass Bioenergy 136:105531. https://doi.org/10.1016/j.biombioe.2020.105531

Evans A, Strezov V, Evans TJ (2010) Sustainability considerations for electricity generation from biomass. Renew Sust Energ Rev 14(5):1419–1427. https://doi.org/10.1016/j.rser.2010.01.010

Fish BR (1972) Electrical generation of natural aerosols from vegetation. Science 175 (4027):1239–1240. https://doi.org/10.1126/science.175.4027.1239

Friedl A (2012) Bioethanol from starch. In: Meyers RA (ed) Encyclopedia of sustainability science and technology. Springer, New York, NY. https://doi.org/10.1007/978-1-4419-0851-3_432

Fu CC, Wu WT (2010) Electricity generation by photosynthetic biomass. Biomass 125. https://doi.org/10.5772/9771

Galbe M, Zacchi G (2012) Bioethanol from celluloses. In: Meyers RA (ed) Encyclopedia of sustainability science and technology. Springer, New York, NY. https://doi.org/10.1007/978-1-4419-0851-3_521

Gao N, Fan Y, Long F, Qiu Y, Geier W, Liu H (2020) Novel trickling microbial fuel cells for electricity generation from wastewater. Chemosphere 248:126058. https://doi.org/10.1016/j.chemosphere.2020.126058

Gotay V (2013) U.S. Patent No. 8,344,536. U.S. Patent and Trademark Office, Washington, DC

Hassan SH, El-Rab SMG, Rahimnejad M, Ghasemi M, Joo JH, Sik-Ok Y et al (2014) Electricity generation from rice straw using a microbial fuel cell. Int J Hydrog Energy 39(17):9490–9496. https://doi.org/10.1016/j.ijhydene.2014.03.259

Hensley JC (ed) (1985) Cooling tower fundamentals. Marley Cooling Tower Company, Stockton, CA

Huggins T, Wang H, Kearns J, Jenkins P, Ren ZJ (2014) Biochar as a sustainable electrode material for electricity production in microbial fuel cells. Bioresour Technol 157:114–119. https://doi.org/10.1016/j.biortech.2014.01.058

Idris SA, Esat FN, Abd Rahim AA, Rizzqi WZ, Ruzlee W, Razali WZ (2016) Electricity generation from the mud by using microbial fuel cell. In: MATEC web of conferences, vol 69. EDP Sciences, France, p 02001. https://doi.org/10.1051/matecconf/20166902001

Ilieva R, Iliev M, Angelova B, Groudeva V, Spasova I, Groudev S, Georgiev P (2018) Isolation of the Iron reducing Bacteria and application in microbial fuel cell for electricity generation. In: 3rd international conference on applied biotechnology, Tirana, Albania

Jang JK, Pham TH, Chang IS, Kang KH, Moon H, Cho KS, Kim BH (2004) Construction and operation of a novel mediator-and membrane-less microbial fuel cell. Process Biochem 39 (8):1007–1012. https://doi.org/10.1016/S0032-9592(03)00203-6

Jia J, Tang Y, Liu B, Wu D, Ren N, Xing D (2013) Electricity generation from food wastes and microbial community structure in microbial fuel cells. Bioresour Technol 144:94–99. https://doi.org/10.1016/j.biortech.2013.06.072

Jiang D, Zhuang D, Fu J, Huang Y, Wen K (2012) Bioenergy potential from crop residues in China: availability and distribution. Renew Sust Energ Rev 16(3):1377–1382. https://doi.org/10.1016/j.rser.2011.12.012

Jin YZ, Wu YC, Li BQ, Zhu HD, Li YP, Zhuang MZ, Fu HY (2020) Study on the electricity generation characteristics of microbial fuel cell with different substrates. In: IOP conference series: earth and environmental science, vol 435, no 1. IOP Publishing, Bristol, p. 012036. https://doi.org/10.1088/1755-1315/435/1/012036

Khan AM (2009) Electricity generation by microbial fuel cells. Adv Nat Appl Sci 3(2):279–286

Kim BH, Ikeda T, Park HS, Kim HJ, Hyun MS, Kano K et al (1999a) Electrochemical activity of an Fe (III)-reducing bacterium, Shewanella putrefaciens IR-1, in the presence of alternative electron acceptors. Biotechnol Tech 13(7):475–478. https://doi.org/10.1023/A:1008993029309

Kim BH, Kim HJ, Hyun MS, Park DH (1999b) Direct electrode reaction of Fe (III)-reducing bacterium, Shewanella putrefaciens. J Microbiol Biotechnol 9:127–131

Kim HJ, MOON SH, BYUNG HK (1999c) A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, Shewanella putrefaciens. J Microbiol Biotechnol 9(3):365–367

Kim N, Choi Y, Jung S, Kim S (2000) Effect of initial carbon sources on the performance of microbial fuel cells containing Proteus vulgaris. Biotechnol Bioeng 70(1):109–114. https://doi.org/10.1002/1097-0290(20001005)70:1<109::AID-BIT11>3.0.CO;2-M

Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzym Microb Technol 30 (2):145–152. https://doi.org/10.1016/S0141-0229(01)00478-1

Kim BH, Park HS, Kim HJ, Kim GT, Chang IS, Lee J, Phung NT (2004) Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. Appl Microbiol Biotechnol 63(6):672–681

Kim JR, Min B, Logan BE (2005) Evaluation of procedures to acclimate a microbial fuel cell for electricity production. Appl Microbiol Biotechnol 68(1):23–30

Kim GT, Webster G, Wimpenny JWT, Kim BH, Kim HJ, Weightman AJ (2006) Bacterial community structure, compartmentalization and activity in a microbial fuel cell. J Appl Microbiol 101(3):698–710

Kim JR, Jung SH, Regan JM, Logan BE (2007) Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresour Technol 98(13):2568–2577

Kong W, Wang B, Ma H, Gu L (2006) Electrochemical treatment of anionic surfactants in synthetic wastewater with three-dimensional electrodes. J Hazard Mater 137(3):1532–1537. https://doi.org/10.1016/j.jhazmat.2006.04.037

Kreysa G, Krämer P (1989) Macrokinetics and mathematical modelling of quinone reduction by cyanobacteria. J Chem Technol Biotechnol 44(3):205–217. https://doi.org/10.1002/jctb.280440305

Kusmayadi A, Leong YK, Yen HW, Huang CY, Dong CD, Chang JS (2020) Microalgae-microbial fuel cell (mMFC): an integrated process for electricity generation, wastewater treatment, CO2 sequestration and biomass production. Int J Energy Res. https://doi.org/10.1002/er.5531

Lafratta M, Thorpe RB, Ouki SK, Shana A, Germain E, Willcocks M, Lee J (2020) Dynamic biogas production from anaerobic digestion of sewage sludge for on-demand electricity generation. Bioresour Technol 123415. https://doi.org/10.1016/j.biortech.2020.123415

Lamers P, Hoefnagels R, Junginger M, Hamelinck C, Faaij A (2015) Global solid biomass trade for energy by 2020: an assessment of potential import streams and supply costs to North-West Europe under different sustainability constraints. GCB Bioenergy 7(4):618–634. https://doi.org/10.1111/gcbb.12162

Lee DJ, Chang JS, Lai JY (2015) Microalgae–microbial fuel cell: a mini review. Bioresour Technol 198:891–895. https://doi.org/10.1016/j.biortech.2015.09.061

Lewis K (1966) Symposium on bioelectrochemistry of microorganisms. IV. Biochemical fuel cells. Bacteriol Rev 30(1):101

Li WW, Yu HQ (2014) Utilization of microbe-derived electricity for practical application. Environ Sci Technol 48(1):17–18. https://doi.org/10.1021/es405023b

Lin JH, Lee DJ, Chang JS (2015) Lutein in specific marigold flowers and microalgae. J Taiwan Inst Chem Eng 49:90–94. https://doi.org/10.1016/j.jtice.2014.11.031

Lithgow AM, Romero L, Sanchez IC, Souto FA, Vega CA (1986) Interception of the electron-transport chain in bacteria with hydrophilic redox mediators. I: selective improvement of the performance of biofuel cells with 2, 6-disulphonated thionine as mediator. J Chem Res. *Synopses* (print) (5):178–179

Liu H (2010) Microbial electricity generation from cellulosic biomass. Bioenergy and Biofuel from Biowastes and Biomass:116–129. https://doi.org/10.1061/9780784410899.ch06

Liu H, Logan BE (2004) Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ Sci Technol 38 (14):4040–4046. https://doi.org/10.1021/es0499344

Liu H, Ramnarayanan R, Logan BE (2004) Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ Sci Technol 38(7):2281–2285. https://doi.org/10.1021/es034923g

Liu X, Clarens AF, Colosi LM (2012) Algae biodiesel has potential despite inconclusive results to date. Bioresour Technol 104:803–806. https://doi.org/10.1016/j.biortech.2011.10.077

Liu Y, Sun X, Yin D, Cai L, Zhang L (2020) Suspended anode-type microbial fuel cells for enhanced electricity generation. RSC Adv 10(17):9868–9877. https://doi.org/10.1039/C9RA08288C

Logan BE (2009) Exoelectrogenic bacteria that power microbial fuel cells. Nat Rev Microbiol 7 (5):375–381

Logan BE, Rabaey K (2012) Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies. Science 337(6095):686–690. https://doi.org/10.1126/science.1217412

Logan BE, Regan JM (2006) Electricity-producing bacterial communities in microbial fuel cells. Trends Microbiol 14(12):512–518. https://doi.org/10.1016/j.tim.2006.10.003

Lovley DR (2006) Microbial fuel cells: novel microbial physiologies and engineering approaches. Curr Opin Biotechnol 17(3):327–332. https://doi.org/10.1016/j.copbio.2006.04.006

Luo H, Xu G, Lu Y, Liu G, Zhang R, Li X et al (2017) Electricity generation in a microbial fuel cell using yogurt wastewater under alkaline conditions. RSC Adv 7(52):32826–32832. https://doi.org/10.1039/C7RA06131E

Ma'arof MIN, Chala GT, Chaudhry MB, Premakumar BK (2020) A comparative study of electricity generation from industrial wastewater through microbial fuel cell. Platform 4(2):70–75

Mangeli A, Mostafavi A, Shamspur T, Fathirad F (2020) Binary nanostructured catalysts to facilitate electricity generation from ethylene glycol electrooxidation. Inorg Chem Commun 118:108038. https://doi.org/10.1016/j.inoche.2020.108038

Matsumoto A, Nagoya M, Tsuchiya M, Suga K, Inohana Y, Hirose A et al (2020) Enhanced electricity generation in rice paddy-field microbial fuel cells supplemented with iron powders. Bioelectrochemistry:107625. https://doi.org/10.1016/j.bioelechem.2020.107625

Mattos AP, Veloso TGC, Sotomonte C, Lopes AO, Coronado CR, Rosa do Nascimento MA (2013) Electricity generation from biogas produced in sewage treatment plant. In: 22nd international congress of mechanical engineering (COBEM 2013), Ribeirão Preto, SP, Brazil

Metcalf L, Eddy HP, Tchobanoglous G (1979) Wastewater engineering: treatment, disposal, and reuse, vol 4. McGraw-Hill, New York

Meyer S, Glaser B, Quicker P (2011) Technical, economical, and climate-related aspects of biochar production technologies: a literature review. Environ Sci Technol 45(22):9473–9483. https://doi.org/10.1021/es201792c

Mitan NMM, Badarulzaman S (2020) Preliminary observation on temperature effect of briquetting cow manure as a solid biofuel. In: Proceedings of the 6th international conference and exhibition on sustainable energy and advanced materials. Springer, Singapore, pp 689–693. https://doi.org/10.1007/978-981-15-4481-1_65

Mittelbach M (2013) Biodiesel. In: Kaltschmitt M, Themelis NJ, Bronicki LY, Söder L, Vega LA (eds) Renewable energy systems. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-5820-3_311

Mizil SN (2016) Electricity generating by microbial fuel cell. Al-Mustansiriyah J Sci 27(4):1–3. https://doi.org/10.23851/mjs.v27i4.11

Mohamed HO, Obaid M, Sayed ET, Liu Y, Lee J, Park M et al (2017) Electricity generation from real industrial wastewater using a single-chamber air cathode microbial fuel cell with an activated carbon anode. Bioprocess Biosyst Eng 40(8):1151–1161. https://doi.org/10.1007/s00449-017-1776-0

Morgan SM, Yang Q (2001) Use of landfill gas for electricity generation. Pract Period Hazard Toxic Radioact Waste Manag 5(1):14–24. https://doi.org/10.1061/(ASCE)1090-025X(2001)5:1(14)
Müller FP, Maack GC, Buescher W (2017) Effects of biogas substrate recirculation on methane yield and efficiency of a liquid-manure-based biogas plant. Energies 10(3):325. https://doi.org/10.3390/en10030325
Njoku I, Eke B, Onyeocha E (2020) Electricity generation from a microbial fuel cell using abattoir waste water. Phys J 5:41–50. Retrieved from https://purkh.com/index.php/tophy/article/view/683
O'Sullivan D (1993) Algae fuel clean electricity generation. Chemical and Engineering News, United States, 71(6)
Ogugbue CJ, Ebode EE, Leera S (2015) Electricity generation from swine wastewater using microbial fuel cell. J Ecol Eng 16(5). https://doi.org/10.12911/22998993/60450
Park DH, Zeikus JG (2000) Electricity generation in microbial fuel cells using neutral red as an electronophore. Appl Environ Microbiol 66(4):1292–1297. https://doi.org/10.1128/AEM.66.4.1292-1297.2000
Pezeshki Z, Bielefeldt AR, Rezakazemi M (2021) Optimization, modelling, prediction, and kinetics of the best factors that support the biodegradation process after treatment with biosurfactant. https://www.researchgate.net/publication/343684863_Optimization_Modelling_prediction_and_kinetics_of_the_best_factors_that_support_the_biodegradation_process_after_treatment_with_biosurfactant
Raeburn S, Rabinowitz JC (1971) Pyruvate: ferredoxin oxidoreductase: II. Characteristics of the forward and reverse reactions and properties of the enzyme. Arch Biochem Biophys 146 (1):21–33. https://doi.org/10.1016/S0003-9861(71)80037-1
Rahayuningwulan D, Permana D, Putra HE (2014) Performance of microbes consortium on single-chamber microbial fuel cell as electricity generation. In: The ASEAN conference on science and technology 2014, Bogor, West Java, Indonesia
Rashidi Z, Karbassi AR, Ataei A, Ifaei P, Samiee-Zafarghandi R, Mohammadizadeh MJ (2012) Power plant design using gas produced by waste leachate treatment plant. Int J Environ Res 6 (4):875–882
Regmi R, Nitisoravut R (2020) Azolla enhances electricity generation of Paddy microbial fuel cell. ASEAN Eng J 10(1):55
Reimers CE, Tender LM, Fertig S, Wang W (2001) Harvesting energy from the marine sediment–water interface. Environ Sci Technol 35(1):192–195. https://doi.org/10.1021/es001223s
Rismani-Yazdi H, Carver SM, Christy AD, Yu Z, Bibby K, Peccia J, Tuovinen OH (2013) Suppression of methanogenesis in cellulose-fed microbial fuel cells in relation to performance, metabolite formation, and microbial population. Bioresour Technol 129:281–288. https://doi.org/10.1016/j.biortech.2012.10.137
Rojas Flores S, Naveda RN, Paredes EA, Orbegoso JA, Céspedes TC, Salvatierra AR, Rodríguez MS (2020) Agricultural wastes for electricity generation using microbial fuel cells. Open Biotechnol J 14(1). https://doi.org/10.2174/1874070702014010052
Rumão AS, Jaguaribe EF, Bezerra AF, Oliveira BLN, Queiroga BLC (2014) Electricity generation from biomass gasification. Revista de Engenharia Térmica 13(1):28–31. https://doi.org/10.5380/reterm.v13i1.62065
Sajib HM, Hoque SM (2015) Electricity generation from poultry waste in Bangladesh. http://icmime-ruet.ac.bd/2015/DIR/Contents/Technical%20Papers/Energy%20Technology/ET-18.pdf
Salihoglu NK (2018) Electricity generation from landfill gas in Turkey. J Air Waste Manage Assoc 68(10):1126–1137. https://doi.org/10.1080/10962247.2018.1474145
Sam AM, Mercy D (2013) Developments in microbial fuel cell system for electricity generation. Trends Biosci 6(6):701–704
Santhosh G, Palaniappan PR, Krishnan NS, Kumar RD, Muruganandam D (2014) Electricity generation with camphor as fuel. In: Applied mechanics and materials, vol 575. Trans Tech

Publications Ltd, Switzerland, pp 644–648. https://doi.org/10.4028/www.scientific.net/AMM.575.644
Sawa M, Fantuzzi A, Bombelli P, Howe CJ, Hellgardt K, Nixon PJ (2017) Electricity generation from digitally printed cyanobacteria. Nat Commun 8(1):1–10. https://doi.org/10.1038/s41467-017-01084-4
Shah D, Nagarseth P (2015) Low cost biogas purification system for application of bio CNG as fuel for automobile engines. IJISET 2(6). http://ijiset.com/vol2/v2s6/IJISET_V2_I6_46.pdf
Show KY, Lee DJ, Chang JS (2013) Algal biomass dehydration. Bioresour Technol 135:720–729. https://doi.org/10.1016/j.biortech.2012.08.021
Show KY, Lee DJ, Tay JH, Lee TM, Chang JS (2015) Microalgal drying and cell disruption–recent advances. Bioresour Technol 184:258–266. https://doi.org/10.1016/j.biortech.2014.10.139
Shrestha N, Fogg A, Wilder J, Franco D, Komisar S, Gadhamshetty V (2016) Electricity generation from defective tomatoes. Bioelectrochemistry 112:67–76. https://doi.org/10.1016/j.bioelechem.2016.07.005
de Souza TAZ, Coronado CJR, Silveira JL, Pinto GM (2019) Biohydrogen production from glycerol for SOFC electricity generation in the biodiesel industry. https://www.researchgate.net/profile/Tulio-A-Zucareli-De-Souza/publication/337402612_Biohydrogen_production_from_glycerol_for_SOFC_electricity_generation_in_the_biodiesel_industry/links/5dd57dc0a6fdcc37897d7ee2/Biohydrogenproduction-from-glycerol-for-SOFC-electricity-generation-in-the-biodiesel-industry.pdf
Sudirjo E, Buisman CJN, Strik DPBTB (2018) Electricity generation from wetlands with activated carbon bioanode. In: IOP conference series: earth and environmental science, vol 131, no 1. IOP Publishing, Bristol, p. 012046. https://doi.org/10.1088/1755-1315/131/1/012046
Sulonen ML, Kokko ME, Lakaniemi AM, Puhakka JA (2015) Electricity generation from tetrathionate in microbial fuel cells by acidophiles. J Hazard Mater 284:182–189. https://doi.org/10.1016/j.jhazmat.2014.10.045
Takahashi S, Miyahara M, Kouzuma A, Watanabe K (2016) Electricity generation from rice bran in microbial fuel cells. Bioresour Bioprocess 3(1):1–5. https://doi.org/10.1186/s40643-016-0129-1
Torres CI, Kato Marcus A, Rittmann BE (2008) Proton transport inside the biofilm limits electrical current generation by anode-respiring bacteria. Biotechnol Bioeng 100(5):872–881. https://doi.org/10.1002/bit.21821
Tran DT, Le BH, Lee DJ, Chen CL, Wang HY, Chang JS (2013) Microalgae harvesting and subsequent biodiesel conversion. Bioresour Technol 140:179–186. https://doi.org/10.1016/j.biortech.2013.04.084
Vega CA, Fernández I (1987) Mediating effect of ferric chelate compounds in microbial fuel cells with Lactobacillus plantarum, Streptococcus lactis, and Erwinia dissolvens. Bioelectrochem Bioenerg 17(2):217–222. https://doi.org/10.1016/0302-4598(87)80026-0
Vochozka M, Stehel V, Maroušková A, Majerník J, Karková M, Kolář L, Žák J (2017) Alternatives for the use of solid pyrolysis by-products for electricity generation. Energy Sources A 39(17):1875–1878. https://doi.org/10.1080/15567036.2017.1381782
Wang X, Feng Y, Liu J, Lee H, Li C, Li N, Ren N (2010) Sequestration of CO2 discharged from anode by algal cathode in microbial carbon capture cells (MCCs). Biosens Bioelectron 25(12):2639–2643. https://doi.org/10.1016/j.bios.2010.04.036
Wang F, Zhang D, Shen X, Liu W, Yi W, Li Z, Liu S (2019) Synchronously electricity generation and degradation of biogas slurry using microbial fuel cell. Renew Energy 142:158–166. https://doi.org/10.1016/j.renene.2019.04.063
Wiese A (2017) Biomass combustion for electricity generation. In: Meyers R (ed) Encyclopedia of sustainability science and technology. Springer, New York, NY. https://doi.org/10.1007/978-1-4939-2493-6_254-3
Xiao L, Young EB, Berges JA, He Z (2012) Integrated photo-bioelectrochemical system for contaminants removal and bioenergy production. Environ Sci Technol 46(20):11459–11466. https://doi.org/10.1021/es303144n

Yamazaki SI, Kaneko T, Taketomo N, Kano K, Ikeda T (2002) Glucose metabolism of lactic acid bacteria changed by quinone-mediated extracellular electron transfer. Biosci Biotechnol Biochem 66(10):2100–2106. https://doi.org/10.1271/bbb.66.2100

Yang Y, Jiang J, Liu X, Si Y (2020) Effect of sulfonamides on the electricity generation by Shewanella putrefaciens in microbial fuel cells. Environ Prog Sustain Energy:e13436. https://doi.org/10.1002/ep.13436

Yaqoob AA, Khatoon A, Mohd Setapar SH, Umar K, Parveen T, Mohamad Ibrahim MN et al (2020) Outlook on the role of microbial fuel cells in remediation of environmental pollutants with electricity generation. Catalysts 10(8):819. https://doi.org/10.3390/catal10080819

Yen HW, Hu IC, Chen CY, Ho SH, Lee DJ, Chang JS (2013) Microalgae-based biorefinery–from biofuels to natural products. Bioresour Technol 135:166–174. https://doi.org/10.1016/j.biortech.2012.10.099

Yentekakis IV, Goula G (2017) Biogas management: advanced utilization for production of renewable energy and added-value chemicals. Front Environ Sci 5:7

Zhao N, Treu L, Angelidaki I, Zhang Y (2019) Exoelectrogenic anaerobic granular sludge for simultaneous electricity generation and wastewater treatment. Environ Sci Technol 53 (20):12130–12140. https://doi.org/10.1021/acs.est.9b03395

Zuberi MJS, Ali SF (2015) Greenhouse effect reduction by recovering energy from waste landfills in Pakistan. Renew Sust Energ Rev 44:117–131. https://doi.org/10.1016/j.rser.2014.12.028

Applications of Microbes in Food Industry 10

Narayana Saibaba KV

Abstract

Microorganisms have been playing a significant role in the production of useful substances to the mankind. They have been in use in domestic applications such as yoghurt, bread and wine production since ancient times. Ever since the Louis Pasteur explained role of microorganism in food fermentation, scientists have been trying to discover the microorganisms that can be used for various industrial applications. They have been developing new technologies for the isolation of microbes which can be used in the production of specific desired products with high yields. Many organisms from yeast, bacteria and fungi are found to be useful in the production of numerous varieties of foodstuff-related applications like bread, dairy products, alcohol and beverages, enzymes, organic acids, food colours, etc. These microorganisms are added to the food production process to impart attractive colour, flavour, aroma and texture and enhance the marketability of the products. With the advancement of technology, novel methods have been developed which opens extensive applications of microorganisms in food and beverage industries. Comprehensive list of various microorganisms and their uses in food production are highlighted in this chapter.

Keywords

Microorganisms · Food industry · Baking industry · Alcohol production · Dairy products

N. S. KV (✉)
Department of Biotechnology, GITAM Institute of Technology, GITAM University, Visakhapatnam, Andhra Pradesh, India
e-mail: skvn@gitam.edu

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10.1 Introduction

Microorganisms are omnipresent, i.e., they exist everywhere: in air, soil, water, human body, on plants and animals. General presumption is that microorganisms are harmful to humans; however, there are many organisms which are useful in many ways to mankind. Microorganisms that live in association with humans (live on various surfaces of the human body) protect them from infections and other diseases. For example, *Lactobacillus* and *Bifidobacterium* present on the human body restrict the growth of pathogenic microorganisms, they are used in wastewater treatment, and help to reduce the atmospheric nitrogen and transform them into useful ammonia. Many fungi and bacteria plays significant role in the ecological balance of the environment, they are considered as planets major composters and recyclers. They release digestive enzymes to degrade the highly complex materials and help in the environmental sustainability.

Microorganisms are one way responsible for the food spoilage and diseases, on the other way they are used for the manufacture of valuable substances. Many types of microbes from yeast, bacteria and fungi are found to be useful to the mankind. Yeast is the most extensively absorbed microorganism in the food-processing industry followed by bacteria. Microorganisms impart desired physico-chemical, biological characteristics and enhance flavour, aroma and shelf life of the products at very low cost. Many microorganisms have industrial importance; some of them are tabulated in Table 10.1 (Bintsis 2018).

Microorganisms, despite their small size, have been playing a key role in the food processing and manufacturing industries. Microorganisms not only help in the production of desired products but also are used to restrict the growth of pathogenic organisms. Therefore, microorganisms are considered as very important elements in the food production, maintenance of food quality and safety.

10.2 Applications of Microorganisms in Food Industry

Microorganisms produce various food products by a process known as fermentation. Fermentation is a biochemical conversion of simple sugars into desired products such as acid, alcohol, carbon dioxide through various metabolic pathways. For example, *Lactobacillus bulgaricus* and S*treptococcus thermophilus* convert lactose to lactic acid during the production of yoghurt. Yeast (*Saccharomyces calbergeneis* and *Saccharomyces cerevisiae*) decomposes glucose to ethanol and carbon dioxide during bear, rum, cider and other alcoholic making process. Microorganisms further help in cost saving and revenue creation in food industry. These organisms can be genetically manipulated to produce the product with required characteristics in large scale. In addition to the production of desired products, microorganisms also help to ensure the quality and safety of the products. For example, *lactobacillus* bacteria produce lactic acid during yoghurt production which restrict the growth of pathogenic organisms and keeps the product safe for human consumption. Enzyme micro

Table 10.1 Microorganisms used in the food industry applications

Product	Microorganism used
Acetic acid	*Acetobacter aceti*, *Acetobacter orleansis*, *Acetobacter schutzenbachi*
Lactic acid	*Lactobacillus plantarum*, *Pediococcus cerevisiae*, *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus brevis*
Beer, whiskey, wine, cider, sake, etc.	*Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, *Saccharomyces cidri*, *Acetobacter* spp., *Aspergillus oryzae*, *Lactobacillus* spp.
Bread, cake	*Saccharomyces cerevisiae*
Dairy products such as acidophilus milk, Bulgarian milk, cheese, yoghurt, kefir	*Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Streptococcus lactis*, *Saccharomyces cremoris*, *cheddar*, *Saccharomyces durans*, *P. candidum; Lactobacillus caseiroqueforte*, *Penicillium camemberti*, *Penicillium roqueforti*
Meat and fish processing	*Aspergillus*, *Penicillium* spp., *Pediococcus cerevisiae*, *LActobacillus plantarum*, *Lactobacillus reuteri*
Mushrooms	*Agaricus bisporus*, *Morchella hortensis*
Enzymes such as amylases, cellulases, glucose oxidases, catalase, lipase, invertase, proteases, pectinases	*Bacillus spp.*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Trichoderma viride*, *Corynebacterium* spp., *Saccharomyces cerevisiae*, *Saccharomycopsis lipolytica*, *Aspergillus* spp., *Bacillus licheniformis*, *Bacillus subtilis; Aspergillus* spp., *Saccharomyces cerevisiae*, *Candida utilis*
Organic acids such as acetic acid, citric acid, lactic acid	*Acetobacter aceti*, *Candida aceticum*, *Aspergillus niger*, *Saccharomycopsis lipolytica*, *Lactobacillus delbrueckii*
Amino acid and vitamins: riboflavin, Vit. B-12, Pro-Vit. A	*Eremothecium ashbyi*, *Bacillus megaterium*, *Streptomyces olivaceus*, *Propionibacterium*, *Rhodotorula gracilis*
Pigments	*Bradyrhizobium Sepp*, *Serratia marcescens*, *Dunaliella Salina*, *Blakeslea trispora*, *Fusarium sporotrichioides*, *Mucor circinelloides*, *Neurospora crassa*, *Phycomyces blakesleeanus*

catalase produced from the *Micrococcus lysodeikticus* is used in the raw milk treatment.

Microorganisms find applications in the foodstuff, medical, drug, textile and dyeing industries. They are the fundamental components in the food preparation. They are used in many applications in food industry. Microorganisms alter the characteristics of the food and improve the quality, quantity, and availability. They can convert food from one form to other, e.g. milk to yoghurt and cheese, and sugar to alcohol and bread, etc. by various reactions. Various application areas of microbes in food industry are dairy products, bread baking, alcohol and beverage industry, organic acid production, enzyme production, steroid production, sewage treatment, production of insecticides, vitamins, antibiotics, fertility of soil and other biotechnological applications.

10.2.1 Baking Industry Applications

Enzymes derived from the microbes are extensively used for applications like fruit juice production, syrup production, bread making, brewing of coffee, etc. During bread making, enzymes are mixed with the wheat flour to convert the complex starch molecules into simpler molecules and help in the production of the desired product. Supplementation of enzymes during bread making enhances the flavour, increases the volume and imparts the required texture to the bread. In addition it increases the sugar concentration in dough which helps in improving the flavour and colour of the product. Microbes also help to improve the storage life of the bread. Enzymes derived from the *Bacillus stearothermophilus* are found to be very efficient in bread making and used widely in baking industry (van der Maarel et al. 2002). Many bacterial and fungal species are used in bread making for imparting flavour, aroma, shelf life and nutritional quality.

Microorganisms such as *Saccharomyces cerevisiae* and *Streptococcus* are widely used in the production of bread as leavening agents. Yeast is a single-cell fungus used widely in bread production. It is added to the dough to bring the required fermentation for the production of bread. *Saccharomyces cerevisiae*, commercially known as baker's yeast, provides flavour to the bread. During fermentation, yeast converts the sugars present in the substrate into carbon dioxide gas and ethanol. CO_2 produced in the process traps in the dough and causes bread rising and provides the required softness, texture, holes and rise of volume in bread.

10.2.2 Alcohol and Beverage Industry Applications

Alcohol production is the process in which microorganisms convert the carbon sources into alcohol and carbon dioxide through microbial fermentation. Most commonly used microorganism in the production of alcohol is yeast. *Saccharomyces cerevisiae*, commonly known as brewer's yeast, is used to produce alcohol from various malted cereals and fruit juices. Bacteria and fungus are also used to some extent. Enzymes produced from the bacteria and fungus such as amylases are used in the clarification of beer and other fruit juices. These enzymes are also used for improving digestive characteristics of animal feed during pre-processing of feed.

Beer is the most consumed alcoholic beverage in the world. It is commonly produced from the carbon sources such as malted barley and malted wheat. Maize, potatoes, millets, etc. are also mixed with barley or wheat to reduce the cost of alcohol production. Beer making involves two important stages: brewing and fermentation. In the brewing process, starch molecules are broken and converted into wort; then in fermentation process, wort is converted into alcohol and carbon dioxide by microbes. Yeast species such as *Saccharomyces cerevisiae*, *Saccharomyces diastaticus* and *Saccharomyces uvarum* are generally used in the commercial bear production. Microorganisms added to fermentation culture impart additional aroma and flavour to the alcohol.

In general, alcoholic beverages are produced from the fermentation of fruits, barley, molasses, etc. Alcohols produced from the different origins have different taste and flavour. Different alcoholic drinks such as bear, wine, rum, vodka can be produced depending on the substrate used in the fermentation and type of processes used. For example, *Aspergillus oryzae* is commonly used in the production of sake for converting starch from the rice into alcoholic product. Kombucha tea can be brewed with mixed species of bacteria and yeast such as *Acetobacter xylinum*, *Acetobacter xylinoides*, *Saccharomycodes ludwigii*, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* found to be of medicinal value. This brewed tea is also popular with different names as Manchurian mushroom tea, fungus japonicus and tea fungus.

10.2.3 Enzyme Production and Its Applications

Enzymes are commonly produced from plant and animal sources by either submerged or solid-state fermentation methods; however, solid-state fermentation is widely used for enzyme production. For decades, enzymes have been exploited in applications such as leavening of bread, production of yoghurt, fermentation of fruits for juice, alcohol and other beverage production, brewing. Food, textile, paper and pulp industries have large demand for enzymes. World market for enzymes used in food industry has been growing due to the advancement of technology and invention of new processes. It was estimated that market value of commercial enzymes was 4.61 billion US dollars during 2017, it was predicted to reach $6.3 billion in 2022 with the growth rate of 5.8% (Global Forecast Report 2016; Chapman et al. 2018). Enzymes are consumed in food, beverage, detergent, paper, textile and chemical industries; however, food industry is dominating the other industries in enzyme usage. Over 45% of the total enzymes produced are consumed in this sector alone. Enzyme rennet has the largest global market followed by gluco amylase, alpha amylase and glucose isomerase (Godfrey and West 1996; Ratledge and Kristiansen 2001; Pai 2003).

Enzymes are commonly produced from animal, plant and microbial sources; however, microbial production has been gaining importance due to its cost efficiency, and technical and ethical advantages. Cheese production using calf rennet, protease enzyme, has been replaced by microbial enzymes since 1970s. However, its commercial production started in India in 1980. In India cheese is produced only from microorganisms due to the ban of use of calf rennet. Microbes such as *Rhizomucor miehei*, *Rhizomucor pusillus*, *Escherichia coli*, *Aspergillus niger*, *K. lactis*, *Endothia parasticia* are used in the cheese production.

Table 10.2 Applications of microbial enzymes in food applications

Microbial enzyme	Applications
α-Amylases	Liquefying agent in starch processing, used in baking and brewing industries, clarifying agent in fruit juice
Proteases	Fish meal production, cheese making, meat tenderiser, bread making, brewing, clarification of juices
Pectinase	Clarification of juices, softness of fruits, oil extraction
Cellulase	Oil extraction, animal feed, clarification of fruit juices
Glucose oxidase	Dehydrating egg powder
Lipase	Flavour enhancer, cheese production
Lactase	Probiotic
Esterase	Flavour and aroma enhancer
Xylanase	Clarification of juices, beer production
Laccase	Wine making, baking
Peroxidase	Food additive, flavour, colour and aroma enhances

Enzymes derived from the *Bacillus* species source are found to be very stable in many applications. Enzymes convert complex substrates into smaller molecules and improve the reaction yields. Enzymes such as pullulanase, α-amylase, glucose isomerase derived from extracellular *Bacillus* species have lot of industrial importance. For example, α-amylases produced from the *Bacillus* species is highly thermo stable. These enzymes liquefy the substrates and improve the reaction yields of maltopentose in starch processing. They are applied for stabilization of volatile flavours. *Bacillus* species also produce industrially important proteases. These protease enzymes are used in cheese making and fish meat processing (Godfrey and West 1996; Ratledge and Kristiansen 2001; Cocconcelli et al. 1991; Madden 1995). Pectinase enzyme is industrially used in clarification of juices (apple, guava, etc.), softening of fruits (apple, peaches, tomatoes, avocadoes, etc.) and thereby produces high yields of fruit juices and pulp extracts. Mixture of pectinase enzyme and cellulase enzyme is used in oil industry for the extraction of oil from vegetables and fruits such as olive. Glucose oxidase enzyme is used to enhance the dehydrating egg power by removing glucose from egg white. Protease enzymes such as papain are used in the clarification of juices. It is also used to remove cloudiness in beer and wines. β-Galactosidase used in the hydrolysis of lactose is derived from yeast *Kluyveromyces lactis* and *Kluyveromyces fragilis.*

Enzymes produced from the microbial origin have wide applications due to their thermal stability and availability. The major advantages of microbial-based enzymes are due to their flexibility in manipulating to produce enzymes with desired characteristics and production in large scale and economic production (Tanyildizi et al. 2005). Applications of enzymes derived from various microorganisms are tabulated in Table 10.2. Many enzymes such as amylases, lipase, lactase, protease, peptidase are derived with the help of microorganisms. One fourth of enzymes market in the world belongs to amylases. Amylases are obtained through sources such as plant origin, animal origin and microbial origin; however, amylases obtained from the bacterial and fungal sources are dominating the industry (Rao and Satyanarayana 2007; Rajagopalan and Krishnan 2008). Applications of enzymes have been growing due to the advancement of technology, and their application has been extended to textile, brewing and distillation industries (Pandey et al. 2000; Gupta et al. 2003; Kandra 2003).

Starch is an essential component present in almost all human diet foods. It is processed into syrups, ethanol, organic acids, and other products through chemical or enzymatic processes (de Souza and de Oliveira e Magalhães 2010). Enzymes such as amylases are used in the hydrolysis of starch molecules into glucose containing polymers such as oligosaccharides. α-Amylase can be produced from different bacterial species; however, *Bacillus* is widely used for the commercial production of α-amylase. Bacterial species such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus stearothermophilus* and *Bacillus amyloliquefaciens* are found to be very efficient in the production of enzymes (Pandey et al. 2000; Konsoula and Liakopoulou-Kyriakides 2007). Enzymes derived from the bacterial species have commercial importance due to their thermal stability and efficient expression characteristics. Starch processing is conducted at high temperatures such 100–110°C; therefore, thermal stability of enzymes is a very vital requirement in industrial applications.

Mesophilic fungi are found to be very efficient in the enzyme production. Fungi such as *Aspergillus* species is used in the production of wide range of extracellular enzymes; however, amylases are very prominent in commercial applications. Significant quantities of enzymes are produced industrially through various filamentous fungal organisms. Fungi such as *Aspergillus oryzae* are widely applied in organic acids production, high value proteins, enzymes production, soy sauce production, etc. *Aspergillus niger* has good hydrolytic capacitance and acidity tolerance ($pH < 3$) and helps in the reduction of bacterial contamination (Jin et al. 1998; Djekrif-Dakhmouche et al. 2006; Kammoun et al. 2008).

10.2.4 Production of Amino Acids

Amino acids are considered as building blocks of protein structure. Amino acids have been used in many applications in food, pharmaceutical, medical, cosmetic, polymer and leather industries. They are used as food additives, nutrients, rejuvenators, personal care, drugs, etc. Many amino acids have great demand in various applications of food industry. For example, L-glutamate is used for enhancing flavour of food, glycine is used as sweetening agent, aspartate and phenylalanine are used as substitutes of sugar for sweetening of food products. Market demand for amino acids has been increasing and posing a challenge for the producer to meet that demand. In general, large-scale production of amino acids from carbon sources such as glucose, fructose, ethanol, glycerol is carried out using microorganisms.

About 20 amino acids are being produced industrially; however, L-glutamic acid (also known as glutamate) and L-lysine are found to have great commercial demand.

Glutamate is the most produced amino acid which was also the first amino acid produced by the microorganisms. L-Glutamate and its salt mono sodium glutamate (MSG), commonly called as tasting salt, are used in most of the Chinese food products as flavour enhancer. Glutamate was first produced from *Corynebacterium glutamicum* bacterium and has been using till today. It is also used for the production of lysine, threonine, phenylalanine, etc. Microorganisms such as *Microbacterium*, *Brevibacterium* and *Arthrobacter* are also being used for the L-glutamate production to some extent. Organisms such as *Escherichia*, *Candida*, *Bacillus*, *Saccharomyces* species are also used in the amino acid production. L-Phenylalanine is commercially produced by species such as *Escherichia coli*, *Corynebacterium glutamicum*. L-Lysine has been used as feed additive and infusion solution. It is found to have diversified applications like feed, alcoholic and pharmaceutical, etc. It is found to be important limiting amino acid in the growth of chicken and pigs. It is used as important diet supplement in poultry and pig farming industries. Methionine is commonly applied in most of the livestock formulations. It is used as feed in the diets of humans and other livestock. Methionine is a natural lipotropic agent that removes the fats from the liver and acts as natural detoxifying agent. Moreover, it has excellent antioxidant properties. L-Aspartic acid is used as low calorie sweetener in soft drinks.

10.2.5 Microbial Detergents as Food Stain Removers

Application of detergents is very essential in human life for washing hands, body, cloths, household utensils etc. Many detergent products such as washing powders, dish washing liquids, body creams, shower gels and shampoos are available in the market. Detergents are made either from synthetic chemicals or from microbial sources. Microbial enzymes have been used in the detergents for over 50 years. Enzymes are used in detergent industry to increase the stain removal efficiency of detergents and also to make detergents environment friendly. Detergent industry is one of the major enzyme-consuming industries. Ninety percent of the all liquid detergent production use enzymes to impart necessary thermal stability and removal efficiency (Gupta et al. 2003; Mitidieri et al. 2006; Hmidet et al. 2009). They are used widely in detergents for laundry and dish washing purposes. They help the detergent in the degradation of starchy food residues such as gravies, sauces, chocolate strains into smaller oligosaccharides.

Microorganisms find its applications in detergent industry as stain removers. Chemical surfactant-based detergents remove the dirt and stains from the cloths and release them into water, thus creating water pollution. In contrast, microbial detergents decompose the organic matter present in the dirt through their metabolic/enzymatic reactions, thereby reducing pollution. Enzymes produced by many organisms are used to make detergents. These enzymes digest the fat contained in the dirt and make them easily removable from the cloths. Microorganisms such as *Bacillus natto*, *Aspergillus*, lactic fermentation bacteria and yeast cells have the capacity to decompose the dirt through their metabolic activities. In addition to dirt, microbial enzymes remove the proteinaceous stains such as milk, blood, meat, fish and egg. In addition, microbial detergents clean the water pathways all the way from sewage line to the end points until their decomposition power lasts. Microbial detergents are harmless to humans and animals, soft on hands and safe to use.

10.2.6 Dairy Industry Applications

Dairy products such as milk, cheese, yoghurt have been used as good source of nutrition since ancient times. Many varieties of dairy products have been available to humans. Although these dairy products are being domestically produced to some extent, most of these products are industrially produced to meet the high demand. Many microorganisms are used in the dairy industry; however, bacteria are the prominent microorganisms used in dairy industry. Bacteria help in fermentation process which facilitates the wide varieties of cheese, curd, cream and other dairy products. *Streptococcus* is the most widely used organism in the dairy industry.

Bacterial species convert the lactose into lactic acid during fermentation of milk. Bacterial species such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were added to the pasteurized milk and maintained at a temperature of 40 °C. Then lactose present in the milk converts into lactic acid through bacterial respiration and lowers the pH. The lactic acid produced in the fermentation process precipitates the milk proteins and prevents the growth of other microorganisms. More than 2000 varieties of cheeses are available. Lactic acid bacteria impart the required texture and flavour to the yoghurt, cheese, etc. These are added to the fermentation culture to improve the flavour, aroma, texture of various products butter, buttermilk and cereal and legume fermentation products such as idli, dosa, vegetable pickles. Some of the fermentation products can be used as probiotics which improves the health of the consumer. For example, lactic acid bacteria convert milk into yoghurt, cheese, etc. by various biochemical reactions. These reactions produce enzymes such as proteases as intermediate products which helps the formation of yoghurt, cheese, etc. with required quality, aroma, flavour and texture. Bacteriocins derived from bacterial species act as antimicrobial agents and restrict the growth of unwanted microorganisms during the production of dairy products (such as cheese, yoghurt, butter) (Pai 2003; Miwa et al. 1983; Eikmanns et al. 1991).

10.2.7 Pigment Production

Colours show the freshness, quality, safety and aesthetic value of the food. Colour pigments are widely used in food, pharmaceutical, textile, dyeing and tanning industries as they impart colours to the products and make them attractable and marketable. Pigments either can be produced from natural sources or can be prepared synthetically. Toxicity problems associated with the synthetic colours restricting their use in food industry and opened the new worldwide market for pigments derived from natural sources. In general, natural pigments are derived from the plant and microorganisms; however, microbial-based pigments are gaining interest owing to their high stability, biodegradable, eco-friendly and availability of cultivation technology (Kim et al. 1999; Parekh et al. 2000; Raisainen et al. 2002; Kumar et al. 2015). Pigments derived from the microorganisms provide good appearance to the product; in addition, they provide nutritional and medicinal values. Microbial pigments have many advantages over synthetic colours. Microbial pigments production is easy, fast and efficient, cheap and independent of weather conditions. Different shades of colours can be produced from microbial pigments. Microbial pigments are used as antioxidant, anticancer agent, antiproliferative, immunosuppressive and diabetes treatment agent.

Microbial colours are used in fish industry for enhancing the pink colour of salmon. Pigments derived from the fermentation process such as β-carotene derived from the fungus *Blakeslea trispora* and red pigments derived from *Monascus* are widely used in food industry. *Monascus* fungi originated pigments are used for producing Red Yeast Rice (RYR). Monocolin present in these microbial colours plays a vital role in the reduction of LDL cholesterol and increase in HDL cholesterol (Vidyalakshmi et al. 1999). Monoscus is used to produce red colour, yellow colour and orange colour pigments that are used as colouring and flavouring agents in food industry. Yellow-coloured β-carotene pigment also known as pro-vitamin A is used as antioxidant and has ability to cure obesity and many other diseases. Industrially β-carotene is produced from microorganisms such as *Blakeslea trispora*, *Mucor circinelloides*, *Phycomyces blakesleeanus* and shows high carotegenic and antioxidant properties (Kumar et al. 2015). Arpink Red pigment produced from the *Penicillium oxalicum* strain is recommended to use in various food products such as meat, confectionary and ice creams. Many microorganisms (e.g., *Ashbya gossypii)* have the potential to produce yellow-coloured pigment called as riboflavin, also known as vitamin B_2. It is used in the treatment of migraine headache, mouth ulcers, burning of eyes, cataract, glaucoma, cancer, etc.

Both inorganic and organic pigments can be produced from microbial sources; however, organic pigments are widely used as food colourants. Many of microbial pigments are not only used as food colourants and food additives but also used as health beneficial components. Many microbial pigments possess biological activity and are used as antioxidant, anticancer, immunoregulatory and anti-inflammatory agents. Some of the industrially important pigments derived from the microbial sources are tabulated in Table 10.3.

10.2.8 Organic Acid Production

Most of the commercially important organic acids such as citric, acetic, lactic acid are widely produced from fungal species. Fermentation of fruits and sugar syrup substrates with the help of fungal species such as *Acetobacter*, *Rhizopus*, *Penicillium*, etc. produces commercially important organic acids. For example, it is reported that 5×10^5 tonnes citric acid is being produced annually which amount to more than 0.5 billion dollars. Citric acid is produced from the fermentation of citrus fruits

Table 10.3 Application of microbial pigments in food industry and their sources

Food colourant	Source of microorganism	Application	Reference
Canthaxanthin	*Bradyrhizobium Sepp*	Food colourant for poultry and fish products	Surai (2012), Chuyen and Eun (2017)
Astaxanthin	*Basidiomycetous yeast*	Food colourant for fish and animal products	Zuluaga et al. (2017), Pogorzelska et al. (2018)
Prodigiosin	*Serratia marcescens*	Colouring agent in yoghurt, milk, carbonated drinks	Bennett and Bentley (2000), Namazkar and Ahmad (2013)
Phycocyanin	*Aphanizomenon flos-aquae*, *SPirulina*	Colouring agent in sweets and beverage industry	Eriksen (2008), Barsanti et al. (2008)
Beta-carotene	*Dunaliella salina*, *Blakeslea trispora*, *Fusarium sporotrichioides*, *Mucor circinelloides*, *Neurospora crassa*, *Phycomyces blakesleeanus*	Food additive, antioxidant, cholesterol-suppressive agent	Fabio et al. (2021), Sen et al. (2019)
Riboflavin	*Ashbya gossypii*	Food colourant in diary items, breakfast cereals, baby foods, sauces, fruit drinks, and energy drinks. Antioxidant, anticancer agent, protects against cardiovascular diseases	Powers (2003)
Melanin	*Saccharomyces neoformans*	Antimicrobial agent, antioxidant	Vinarov et al. (2003)
Lycopene	*Fusarium sporotrichioides, and Blakeslea trispora*	Meat colorant, antioxidant, anti-cancer agent	Di Mascio et al. (1989), Giovannucci et al. (2002)
Arpink red	*Penicillium oxalicum*	Food additive in meat, confectionary and ice creams	Kumar et al. (2015)
Monascus red	*Monascus*	Monascus fermented rice	Kumar et al. (2015)

using *Aspergillus niger*. *Candida* species also found to be efficient in the citric acid production (Roehr et al. 1996). Gluconic acid is primarily produced by fermentation using *Aspergillus niger*. *Aspergillus niger* converts the glucose to gluconic acid with efficiency of more than 97–99%. It is also produced using *Acetobacter suboxidans* and *Penicillium* species (Milsom and Meers 1985). Lactic acid is other commercially important microbial product emulsifiers and additives industry. Microorganisms such as *Lactobacillus delbrueckii*, *Lactobacillus casei*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus amylophilus* and *Lactobacillus amylovorus* are used for its production (Zhang and Cheryan 1991; Kascak et al. 1996).

10.2.9 Aroma and Flavouring Agents Production

Development of aroma and enhancement of flavour is a very important stage in the food production and process industries. Microorganisms are added to the food products to retain the natural characteristics of the food and develop aroma and flavour to the food product. Lactic acid and carbonyl compounds produced during the yoghurt production enhance the aroma and flavour of the yoghurt.

Soy sauce is a salty condiment commonly used in the countries China, Korea, Japan and other Asian countries. Soy sauce is traditionally produced from the fermentation of soy beans. It is an aroma and flavour enhancer, used in a variety of food products such as frozen foods such as meat, sea foods, baked foods, dairy products, salad dressings, soups. Funguses such as *Aspergillus oryzae*, *Aspergillus sojae*, *Aspergillus tamarii* are added for the brewing of soy sauce. *Saccharomyces cerevisiae* added to the soy culture converts sugars into ethanol which further produces flavour compounds. *Bacillus* spp. are added to enhance the aroma and *Lactobacillus* species are added to the brewing of soy sauce for lactic acid production which further inhibits the growth of pathogens.

10.2.10 Miscellaneous Applications

There are many applications of microorganisms in food and beverage industries. Microorganisms are used in food industry for enhancing flavour, texture and quality of the product. In addition, they are used for making better improvements in the food processes. For example, they have been using in many food applications such as xanthan gum production, food ripening process, food grade paper production, single-cell protein production, meat processing, chocolate production.

Xanthan Gum Production

Xanthan gum, also called as Ticaxam, is a popular food additive, commonly used as thickener and stabilizer to prevent ingredients from separation. It is produced from the fermentation of simple sugars using *Xanthomonas campestris* strains. It helps to improve viscosity of the products, prevents moisture loss from the baked foods, inhibits syneresis in fruit blends, acts as stabilizer in beverages, adds smoothness to cream cheese and controls the formation and growth of ice crystals in frozen foods (Umo 1997).

Ripening Process

Microorganisms are used in the food ripening process and make the products ready for eating. Adding microorganisms to food products reduces the food ripening time and saves lot of money in food ripening process. For example, *Penicillium roqueforti*, *Penicillium camemberti*, *Brevibacterium linens* are used to get brownish red surface on cheese, hardness and also to get subtle, pleasing flavour to the cheese by converting cheese proteins into amino acids. In butter production microorganisms such as *Lactococcus lactic* subsp. *Cremoris lactic Leuconostoc mesenteroides* subsp. *Cremoris* are added to the cream to ripen the butter.

Food Grade Paper Production

Food grade paper used in the packing and storage of food materials is commonly prepared with the help of microorganisms. Starch liquefaction and conversion into smaller molecules are very important step in paper production. Enzymes originated from the microorganisms liquefy the starch and help in producing good quality food grade paper. Enzyme modification facilitates the smooth and strong surface of the paper. Enzymes also improve the stiffness and strength of paper (Gupta et al. 2003).

Single-Cell Protein

Single-cell protein is a rich source of protein made from algae, yeast and bacteria. It also contains good amount of essential nutrients such as minerals, vitamins, fat and carbohydrates. It is easily produced since they grow very fast, does not require large space and grows on wide range of substances such as domestic waste, agriculture wastes, industrial wastes; moreover, their yield is very high. It can be safely used as human and animal diet. The composition and nutritional value depend on the type of substrate used and the type of organism used. Pruteen was the first major single-cell protean produced by bacterium, *Methylophilus methylotrophus*. Pruteens are rich in essential amino acids and vitamins and more nutritious than soybean.

Applications in Other Foods

Mushrooms are cultivated throughout the world due to its high protein values. Mushrooms are derived from edible fungi. Microorganisms such as *Lactobacilli*, *Bacillus cereus*, *Bacillus coagulans*, *Bacillus pumilus*, *Acetobacter aceti*, *Acetobacter pasteurianus*, *Acetobacter fabarum*, *Acetobacter pasteurianus*, *Acetobacter tropicalis* are commonly used in chocolate production. They are used to ferment the cacao seeds for the separation of seeds from the cacao pods. Fungi are very important for the manufacture of vitamin B_{12}. B_{12} is a very essential vitamin for immunity and digestion. Microorganisms such as *Lactobacilli*, *Micrococci*, *Pediococcus*, *and Saccharomyces cerevisiae* are used in the sausage and salami production.

10.3 Summary

Microorganisms have been used in food applications since ancient times. Importance of microorganisms has been increasing due to the growth of food manufacturing and processing industries. Food and its associated products manufacturing through microbial processes are cheaper and easier since large-scale production and genetic modification for better quality products generation are easier. Advancements in science and technology particularly in biotechnology, systems biology, genetic engineering, etc. have propelled the growth of microbial usage in food industry. Latest developments in the fermentation technology associated with the use of genetically modified microorganisms are able to reduce food production cost with enhanced desired characteristics such as texture, flavour, aroma, shelf life.

In the present day busy life, most of the people are forced to take the processed food; therefore, demand for the processed foods has been increasing. This situation necessitates the large-scale production of food products with low cost and high shelf life. With the developments of technologies, discovery of useful microorganisms becomes easier; therefore, research should be focused on the discovery of new natural sources for microbial production, existing process developments, finding new methods for large-scale production of foods with nutritional and health benefits.

References

Barsanti L, Coltelli P, Evangelista V, Frassanito AM, Passarelli V, Vesentini N et al (2008) Oddities and curiosities in the algal world. In: Evangelista V, Barsanti L, Frassanito AM, Passarelli V, Gualtieri P (eds) Algal toxins: nature, occurrence, effect and detection. Springer, New York, pp 353–391

Bennett JW, Bentley R (2000) Seeing red: the story of prodigiosin. Adv Appl Microbiol 47:1–32. https://doi.org/10.1016/S0065-2164(00)47000-0

Bintsis T (2018) Lactic acid bacteria: their applications in foods. J Bacteriol Mycol Open Access 6(2):89–94. https://doi.org/10.15406/jbmoa.2018.06.00182

Chapman J, Ismail AE, Dinu CZ (2018) Industrial applications of enzymes: recent advances, techniques, and outlooks. Catalysts 8:238

Chuyen HV, Eun JB (2017) Marine carotenoids: bioactivities and potential benefits to human health. Crit Rev Food Sci Nutr 57:2600–2610. https://doi.org/10.1080/10408398.2015.1063477

Cocconcelli PS et al (1991) Single stranded DNA plasmid, vector construction and cloning of Bacillus stearothermophilus α-amylase in Lactobacillus. Res Microbiol 142:643–652

Di Mascio P, Kaiser S, Sies H (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538. https://doi.org/10.1016/0003-9861(89)90467-0

Djekrif-Dakhmouche S, Gheribi-Aoulmi Z, Meraihi Z, Bennamoun L (2006) Application of a statistical design to the optimization of culture medium for α-amylase production by Aspergillus niger ATCC16404 grown on orange waste powder. J Food Process Eng 73:190–197

Eikmanns BJ et al (1991) Amplification of three threonine biosynthesis genes in Corynebacterium glutamicum and its influence on carbon flux in different strains. Appl Microbiol Biotechnol 34:617–622

Eriksen NT (2008) Production of phycocyanin – a pigment with applications in biology, biotechnology, foods and medicine. Appl Microbiol Biotechnol 80:1–14. https://doi.org/10.1007/s00253-008-1542-y

Fabio C, Maranhao T, Gisele Zenker J, Nelson Eduardo Duran C, Paulo Afonso N, Stefanie Costa Pinto L (2021) Use of violacein in free form or encapsulated in antimalarial polymeric systems. Universidade Estadual deCampinas – Unicamp, Brazil. PIBr 56399

Giovannucci E, Rimm EB, Liu Y, Stampfer MJ, Willett WC (2002) A prospective study of tomato products, lycopene, and prostate cancer risk. J Natl Cancer Inst 94:391–398. https://doi.org/10.1093/jnci/94.5.391

Global Forecast Report. Industrial enzymes market by type (Amylases, Cellulases, Proteases, Lipases, and Phytases), application (Food & Beverages, Cleaning Agents, and Animal Feed), source (Microorganism, Plant, and Animal), and region—global forecast to 2022. Market Research Report. 2016. Available online: https: //www.marketsandmarkets.com/Market-Reports/industrial-enzymes-market-237327836.html. Accessed 18 Aug 2020

Godfrey T, West S (1996) Industrial enzymology, 2nd edn. Macmillan Press, London

Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B (2003) Microbial α-amylases: a biotechnological perspective. Process Biochem 38:1599–1616

Hmidet N, El-Hadj Ali N, Haddar A, Kanoun S, Alya S, Nasri M (2009) Alkaline proteases and thermo stable α-amylase co-produced by Bacillus licheniformis NH1: characterization and potential application as detergent additive. Biochem Eng J 47:71–79

Jin B, van Leeuwen HJ, Patel B, Yu Q (1998) Utilisation of starch processing wastewater for production of microbial biomass protein and fungal α-amylase by Aspergillus oryzae. Bioresour Technol 66:201–206

Kammoun R, Naili B, Bejar S (2008) Application of a statistical design to the optimization of parameters and culture medium for alpha amylase production by Aspergillus oryzae CBS 819.72 grown on gruel (wheat grinding by-product). Bioresour Technol 99:5602–5609

Kandra L (2003) α-Amylases of medical and industrial importance. J Mol Struct (THEOCHEM) 666–667:487–498

Kascak K et al (1996) Lactic acid in biotechnology. In: Rehm HJ, Reed G (eds) Products of primary metabolism, vol VI, 2nd edn. Verlag Chemie, Weinheim, pp 294–306

Kim CH, Kim SW, Hong SI (1999) An integrated fermentation separation process for the production of red pigment by Serratia sp. KH-95. Process Biochem 35:485–490

Konsoula Z, Liakopoulou-Kyriakides M (2007) Co-production of alpha-amylase and beta-galactosidase by Bacillus subtilis in complex organic substrates. Bioresour Technol 98:150–157

Kumar A, Vishwakarma HS, Singh J, Dwivedi S, Kumar M (2015) Microbial pigments: production and their applications in various industries. Int J Pharm Chem Biol Sci 5(1):203–212

van der Maarel MJ, van der Veen B, Uitdehaag JC, Leemhuis H, Dijkhuizen L (2002) Properties and applications of starch-converting enzymes of the alpha-amylase family. J Biotechnol 94: 137–155

Madden D (1995) Food biotechnology. ILSI Press, Washington, DC

Milsom PE, Meers JL (1985) In: Blanch HW, Drew S, Wang DIC (eds) Gluconic and itaconic acids in comprehensive biotechnology: the principles, applications, and regulations of biotechnology industry, agriculture and medicine, vol III. Pergamon Press, Oxford, pp 681–700

Mitidieri S, Souza Martinelli AH, Schrank A, Vainstein MH (2006) Enzymatic detergent formulation containing amylase from Aspergillus niger: a comparative study with commercial detergent formulations. Bioresour Technol 97:1217–1224

Miwa K et al (1983) Construction of L-threonine overproducing strains of Escherichia coli K-12 using recombinant DNA technique. Agric Boil Chem 47:2329–2334

Namazkar S, Ahmad WA (2013) Spray-dried prodigiosin from Serratia marcescens as a colorant. Biosci Biotechnol Res Asia 10:69–76. https://doi.org/10.13005/bbra/1094

Pai JS (2003) Applications of microorganisms in food biotechnology. Indian J Biotechnol 2:382–386

Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000) Advances in microbial amylases. Biotechnol Appl Biochem 31(Pt 2):135–152

Parekh S, Vinci VA, Strobel RJ (2000) Improvement of microbial strains and fermentation processes. Appl Microbiol Biotechnol 54:287–301

Pogorzelska E, Godziszewska J, Brodowska M, Wierzbicka A (2018) Antioxidant potential of Haematococcus pluvialis extract rich in astaxanthin on colour and oxidative stability of raw ground pork meat during refrigerated storage. Meat Sci 135:54–61. https://doi.org/10.1016/j.meatsci.2017.09.002

Powers HJ (2003) Riboflavin (vitamin B-2) and health. Am J Clin Nutr 77:1352–1360. https://doi.org/10.1093/ajcn/77.6.1352

Raisainen R, Nousiainen P, Hynninen PH (2002) Dermorubin and 5-chlorodermorubin natural anthrax quinone carboxylic acids as dyes for wool. Textile Res J 72:973–976

Rajagopalan G, Krishnan C (2008) Alpha-amylase production from catabolite derepressed Bacillus subtilis KCC103 utilizing sugarcane bagasse hydrolysate. Bioresour Technol 99:3044–3050

Rao JLUM, Satyanarayana T (2007) Improving production of hyper thermo stable and high maltose-forming α-amylase by an extreme thermophile Geobacillus thermoleovorans using response surface methodology and its applications. Bioresour Technol 98:345–352

Ratledge C, Kristiansen B (2001) Basic biotechnology, 2nd edn. Cambridge University Press, UK

Roehr M et al (1996) Citric acid in biotechnology. In: Rehm HJ, Reed G (eds) Products of primary metabolism, vol VI, 2nd edn. Verlag Chemie, Weinheim, pp 308–345

Sen T, Barrow CJ, Deshmukh SK (2019) Microbial pigments in the food industry—challenges and the way forward. Front Nutr 6:7. https://doi.org/10.3389/fnut.2019.00007

de Souza PM, de Oliveira e Magalhães P (2010) Application of microbial α-amylase in industry – a review. Braz J Microbiol 41:850–861

Surai PF (2012) The antioxidant properties of canthaxanthin and its potential effects in the poultry eggs and on embryonic development of the chick. Part 2. Worlds Poultry Sci J 68:717–726. https://doi.org/10.1017/S0043933912000840

Tanyildizi MS, Ozer D, Elibol M (2005) Optimization of α-amylase production by Bacillus sp. using response surface methodology. Process Biochem 40:2291–2296

Umo HE (1997) The economic importance of microorganism in food processing. Nig J Biotechnol 8:1

Vidyalakshmi R, Paranthaman R, Murugesh S, Singaravadivel K, O'Carroll P (1999) Microbial bioconversion of rice broken to food grade pigments naturally exciting colours. World Ingred:39–42

Vinarov A, Robucheva Z, Sidorenko T, Dirina E (2003) Microbial biosynthesis and making of pigment melanin. Commun Agric Appl Biol Sci 68(2 Pt A):325–326

Zhang DX, Cheryan M (1991) Direct fermentation of starch to lactic acid by Lactobacillus amylovorus. Biotechnol Lett 13:73–738

Zuluaga M, Gregnanin G, Cencetti C, Di Meo C, Gueguen V, Letourneur D et al (2017) PVA/Dextran hydrogel patches as delivery system of antioxidant astaxanthin: a cardiovascular approach. Biomed Mater 13:015020. https://doi.org/10.1088/1748-605X/aa8a86

Applications of Microbes in Human Health 11

Sharmila Jasmine, Vidya Sankarapandian, Vijayakumar Natesan, Rajapandiyan Krishnamoorthy, and Annamalai Thangavelu

Abstract

Beneficial microbes play a major role in human health with spectacular symbiotic relationship. The understanding of microbe interaction with human is essential for the novel personalized health care strategies. In this relation, application of probiotics is proposed to account for human health for the prevention and treatment of various diseases. This has driven the researchers with the aspirations to uncover the health impacts of various probiotic bacteria. Furthermore, probiotics have shown promising results as anti-inflammatory, anticancer, anti-microbial, antioxidant, and immunomodulatory potential. Henceforth, probiotics application is a simple, low-cost, receptive, and intrinsic approach to achieve better health outcome in human. In this chapter we have described the current evidences of beneficial bacteria and their influence on human health in the medical sector.

S. Jasmine (✉) · A. Thangavelu
Department of Oral Maxillofacial Surgery, Rajah Muthiah Dental College and Hospital, Annamalai University, Chidambaram, Tamil Nadu, India

V. Sankarapandian
Department of Microbiology, Srimad Andavan Arts and Science College, Trichy, Tamil Nadu, India

V. Natesan
Department of Biochemistry and Biotechnology, Annamalai University, Chidambaram, Tamil Nadu, India

R. Krishnamoorthy
Nanobiotechnology and Molecular Biology Research Lab, Department of Food Science and Nutrition, College of Food Science, King Saud University, Riyadh, Kingdom of Saudi Arabia

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Human gut microbiome · Probiotics · Immunomodulators · Dental caries · Oral mucositis · Prebiotics · Bioactive metabolites

11.1 Introduction

The microbial ecosystem in human body encompasses microbiome that maintains health status via symbiotic relationship. In humans, the count of microbial cells is more than the cells of the body (Shreiner et al. 2015). Microbiome unveils the microhabitats of the body; mouth, skin, gut, etc., each of these microhabitats upholds a distinct ecosystem with discrete environment and nutritional components. The harmonizing effects of microbiome ascribed to the host immune response is by augmenting the number of immune cells and also the levels of antibodies, cytokines, and interferon (Meurman 2005). Thus, they prevent inflammation and result in betterment of the systemic status (Sanders 2008). Prebiotics is described as indigestible substances which influence the activity, proliferation and composition of the inhabitant bacteria and favors health to the host (Roberfroid 2007). They also support the survival and (Gibson and Roberfroid 1995) balance of the gut microbiota and protect against various diseases (Bengmark 2001). Bacteriotherapy is an assuring replacement therapy that uses harmless bacteria to replace pathogenic microorganisms and in such a way resist infection. Maladaptation and imbalance of the gut microbes lead to dysbiosis. On the otherhand, dysbiosis decreases the amount of beneficial microbes and significantly increases the pathogenic organisms (Li et al. 2015). These pathogenic strains disturb the integrity of the enterocyte tight junction of gastrointestinal tract and promote the entry of bacterial products into the systemic circulation that might cause systemic inflammation (Clements et al. 2012). Further, the metabolites of pathogenic organisms have been shown to enhance the secretion of proinflammatory cytokines and reactive oxygen species production (Ye et al. 2015). The oral microbiome being a part of human microbiome, impacts both the systemic and oral health significantly. The inhabitant of oral microbiome forms a composite environment that includes bacteria, viruses, archaea, and protozoa. It is considered as the second most complex microbiota of the human body (Wade 2013). Since the correlation between microbiota and human health becomes more evident, the research about microbiome is a glooming field for the disease diagnosis and therapeutics as well as the development of personalized medicine (Zarco et al. 2012). This chapter has discussed the applications of microbes in human health and emphasis was given to human microbiome.

11.2 Human Microbiome

Since birth, human body both inside and outside is covered with microorganisms called microbiome. They are otherwise known as the normal body flora. Human microbiome is a beneficial and desirable source with tremendous metabolic and chemical diversity. Moreover, they act as the potential natural source for drugs such as antibiotics, antioxidants, enzymes, enzyme inhibitors, colors, hypocholesterolemic agents, vitamins, and immunosuppressant's and help in preventing and treating diseases like diarrhea, cancer, atopic dermatitis, diabetes, anemia, obesity, etc. (Gupta et al. 2014).

Microbiome through various mechanisms influences the well-being of the humans. Pathogen associated molecular patterns (PAMPs) are microbial components such as lipopolysaccharide (LPS), endotoxins, (1–3)-β-D glucans (from fungi), and flagellin (from bacteria). They interact with the human cells thus influence the innate immune system (Lambrecht and Hammad 2014). The presence of microbiome and their relationship with the host is a finely tuned ecosystem. It is being altered by the lifestyle (diet, stress, alcohol, and tobacco consumption) of the individual. These factors as such may influence the properties and the virulence of the microbiome, no more the ecosystem is in balance. Hence, knowing about the microecology balance and interactions is crucial to combat and protect against the pathogens with the intension to improve the health. Existence of some precise organisms within the ecological niches is termed as keystone pathogen. This low abundance pathogen remodels the eubiosis microbial environment into a dysbiotic one, by altering the number and proportions of other microorganisms in the normal benign microbiota (Hajishengallis and Lambris 2011). An example of such one is *Porphyromonas gingivalis* which enables to provoke a chronic inflammatory condition of the oral periodontium (Holt and Ebersole 2005). In addition, *P. gingivalis* has developed complex mechanisms that evade the host immune system and cause adverse systemic effects (Darveau 2009).

11.3 Probiotics

Scientist Werner Kollath coined the term probiotic in 1953. The term "Pro" means "for" and "biotic" means "live" (Hamilton-Miller et al. 2003). Insight into the theory of probiotics was elucidated by Elie Metchnikoff in 1900. He stated that, "the intestinal microbes rely on the food, adopt themselves to modify the flora of the body and replaces the harmful microbes by useful microbes." Moreover, he proposed a scientific hypothesis; *Lactobacillus* is a probiotic that has valuable impact on health and helps in preventing aging. Each scientist has their own insights on probiotics in various time periods (Table 11.1) (McFarland 2015).

In fact, Bulgarians survived longer than citizens of other nations because they consumed much of fermented milk products which comprise numerous viable bacteria that compete with pathogenic organisms of GIT (Ljungh and Wadstrom

Table 11.1 Definition of probiotics by different scientist

Year	Scientist name/Organization	Probiotics definition
1953	Werner Kollath	"Active substances that are essential for a healthy development of life"
1965	Lilly and Stillwell	"Substances secreted by one organism which stimulate the growth of another"
1992	Fuller	"Live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance"
2001	World Health Organization/Food and Agricultural Organization	"Live microorganisms which when administered in adequate amounts confer a health benefit on the host"

Table 11.2 List of probiotic microorganisms and their sources

Probiotic organisms	Name of the organisms	Source
Lactic acid producing bacteria	*L. bulgaricus*, *L. casei* *L. fermentum* *L. acidophilus* *L. paracasei*, *L. rhamnosus* *L. delbrueckii* subsp. *bulgaricus*, *L. brevis* *L. johnsonii*, *L. reuteri* *L. plantarum* *L. salivarius*, *L. fermentum* *L. kefir* *L. lactis* *L. sakei* *L. sake* *L. sanfranciscensis* *L. pontis* *L. mucosae* *L. gallinarum* *L. crispatus* *L. amylovorus*	Yogurt, cheese Milk Kefir Human breast milk Barley Oat groat Molasses Grains Marine fish Smoked salmon Cabbage Wheat flour Sourdough Goat milk Diary products Chicken crop Porcine (Nguyen et al. 2017)
Bifidobacterium species	*B. breve* *B. bifidum*, *B. infantis* *B. longum*, *B. animalis* *B. lactis* *B. adolescentis* *B. Crudilactis*	Breast Milk Fermented Milk Yogurt Raw Milk cheese (Sichetti et al. 2018)
Propionibacterium sp.	*P. freudenreichii*, *P. shermanii*, *P. jensenii* *P. cyclohexanicum*	Skim Milk Orange (Rossi et al. 2007)
Saccharomyces sp.	*S. boulardii*, *S. cerevisiae* *S. carlsbergensis*	Kafir Craft Beers (Diosma et al. 2014)
Enterococcus sp.	*E. faecium* *E. hhirae*, *E. faecalis*	Turkey Poults Rumen—*Bos primigenius* (Arokiyaraj et al. 2014)

2006). Various gut microbes that are used as probiotics and their sources are listed in Table 11.2.

11.4 Properties of Probiotics

Several studies have unleashed the properties of an ideal probiotic candidate. The essential properties of probiotics are illustrated in Fig. 11.1.

- It should be of non-pathogenic microorganism
- It should tolerate the bile acid
- Adherence to the mucus membrane of the gastrointestinal tract
- Produce antimicrobial substance like bacteriocin
- Produce extracellular substances like organic acids and enzymes
- Possess immune-modulatory effects (Parvez et al. 2006).

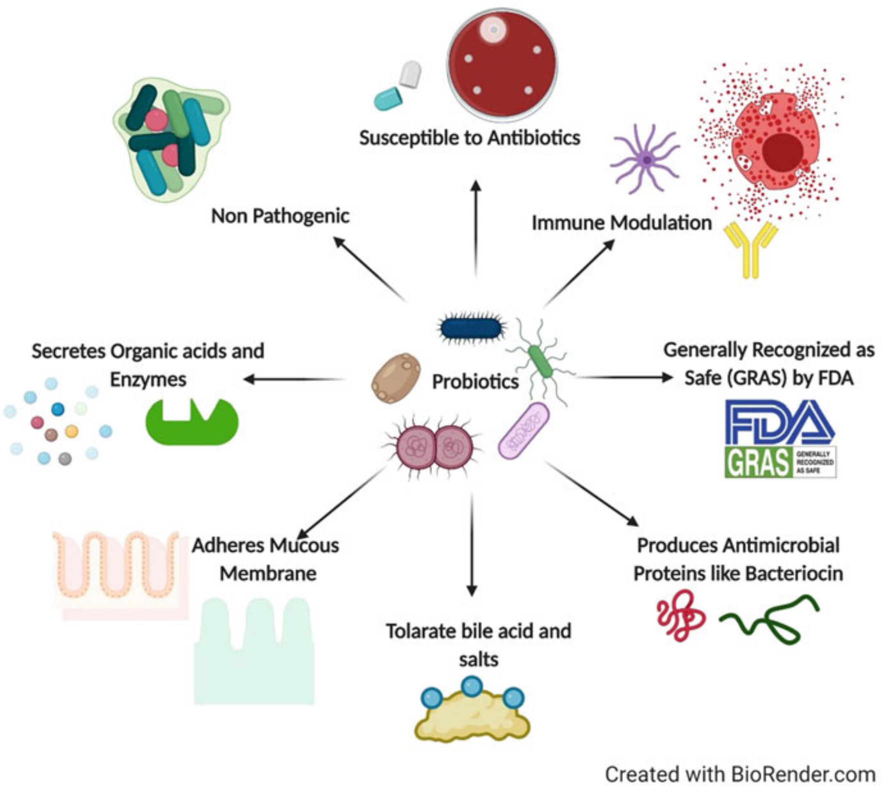

Fig. 11.1 Properties of probiotics

11.5 Probiotics Mechanism of Action

1. Contest with pathogens for attachment sites and nourishment.
2. Inhibits and kills the pathogens through production of bacteriocins, acids, and peroxides.
3. Alters the pH and oxidation-reduction potential and modifies the surrounding environment that compromises the potency of the pathogens.
4. Modulates cell proliferation and apoptosis of the mucosal system.
5. Stimulates and modulates the mucosal immune system.
6. Upregulates mucin production and improves intestinal barrier integrity.
7. Induces cytoprotective protein expression on host cells.
8. Inhibits collagenases activity.
9. Stimulates the secretion of IFN-Ɣ, IL-10, and IgA to improve the gut mucosal barrier (Alkaya et al. 2017).

Probiotics exhibit a variety of health purposes that include improvement in oral and intestinal health, prevention of diarrhea, increase the immune response, antimicrobial, and anti-biofilm properties, reduction of cholesterol level in serum, antioxidant property, anti-inflammatory and anti-diabetic properties. These health properties are specific to a particular strain of probiotics and are executed by different mechanisms.

11.6 Oral Probiotics

Oral probiotics are not supposed to ferment sugars and have to be part of biofilm, so that they may not lower the pH or otherwise may promote caries. Besides, they should have the ability to adhere and colonize all the tissues of the oral cavity (Teughels et al. 2011). It has been showed that oral cavity is being dominated by the following phyla that account for about 94% among the bacterial taxa detected (Wade 2013). Among them, *Firmicutes* sp., *Bacteroidetes* sp., *Proteobacteria* sp., *Actinobacteria* sp., *Spirochaetes* sp., and *Fusobacteria* sp. are some of the healthy microbiota. Indeed, presence of up to 101 fungal species has been documented. They are present widely as normal commensals in healthy individuals and the observed variations in the number of fungal species between persons ranged between 9 and 23. Among them most frequently observed organisms are *Candida* sp. (Ghannoum et al. 2010).

Application of probiotics in the oral cavity was piloted by Kragen in 1954. Lactobacillus and Bifidobacterium are the common strains from which probiotics are obtained. Theses strains include *L. acidophilus*, *L. johnsonii*, *L. rhamnosus*, *L. casei*, *L. reuteri*, *and L. gasseri in Lactobacillus species.* Likewise, *B. bifidum*, *B. infantis*, and *B. longum* are included in Bifidobacterium species. The commensal microbiota through toll-like receptors signaling (TLRs) are associated with epithelial homeostasis through production of epithelial repair factor, immune regulation and thereby protect from epithelial injury. Further, they also regulate the release of inflammatory cytokines like IL-1β and TNF-α and lessen the gingival inflammation (Jones and Versalovic 2009).

11.6.1 Probiotics in Preventing Dental Caries Progression

Probiotics in oral health include the use of Bifidobacterium such as *B. dentium*, *B. breve*, *B. scardovii*, *and B. longum* that play a crucial role in preventing deep dentine caries progression (Becker et al. 2002). *L. lactis* has decreased *Streptococcus oralis*, *Veillonella dispar*, *Actinomyces naeslundii*, and cariogenic *S. sobrinus* colonization by modifying the growth of normal oral microbiota (Comelli et al. 2002). Research study on *L. rhamnosus* GG (LGG) stated that milk with LGG has beneficial effect in caries reduction (Nase et al. 2001). It also inhibits the colonization of caries forming streptococci pathogens and lowered the incidence of caries in children even after the discontinuation of yogurt consumption. Besides, the presence of LGG was observed in the saliva for 2 more weeks. Likewise, brief consumption of cheese containing LGG has significantly reduced the caries associated microbes especially *S. mutans* (Ahola et al. 2002). Probiotic yogurt of *Bifidobacterium* and *Lactobacilli* when consumed daily was shown to reduce the resistant *Streptococci* sp. (Caglar et al. 2005). *Lactobacillus* and *Bifidobacterium* were proved to decrease the caries associated *Streptococci* sp. (Stamatova et al. 2007). In patients with fixed appliances, intake of fruit yogurt along with Bifidobacterium for a short period has reduced the levels of resistant *Streptococci* (Rosenbloom and Tinanoff 1991).

11.6.2 Probiotics in Prevention of Gingival Inflammation

Bifidobacterium such as *B. dentium*, *B. breve*, *B. scardovii*, *and B. longum* inhibit the adhesion of the pathogens to the oral sites hence found to be effective in reducing plaque and gingival bleeding (Alkaya et al. 2017). Krillase is a proteolytic enzyme that consists of both endo and exopeptides which significantly reduced the plaque formation and resulted in decreased gingival inflammation (Hellgren 2009). Fragmentation of surface adhesive proteins impedes the aggregation of oral pathogenic microorganisms on the dental surfaces, thus inhibits the formation of biofilm.

Studies have shown the potency of *L. salivarius* against subgingival microbiota. Current studies have focused on *L. reuteri* since it has antimicrobial substance reuterin that has decreased gingivitis and related gingival bleeding (Krasse et al. 2006). The use of *L. reuteri* containing probiotics significantly decreased the periodontal pathogens like *P. gingivalis* and considerably reduced the total bacterial counts (Iniesta et al. 2012). Few studies revealed that, *L. reuteri* significantly reduced the *Aggregatibacter actinomycetemcomitans*, *P. intermedia*, *P. gingivalis*, *Treponema denticola*, and *T. forsythia* pathogens following consumption of probiotics containing *L. reuteri* for 4 weeks (Mayanagi et al. 2009). Previous researches found that *L. reuteri* has the capacity to inhibit proinflammatory cytokines by secreting bacteriocins, reuterin, and reutericyclin that are recognized to prevent the development of various pathogens (bacteria, viruses, and fungi) (Schaefer et al. 2010).

11.6.3 Probiotics in Prevention of Periodontal Diseases

Recent studies revealed the predominant presence of *L. fermentum and L. gasseri* in healthy individuals. They have the potency of inhibiting the pathogens such as *P. gingivalis*, *A. actinomycetemcomitans*, and *Prevotella intermedia* of periodontium through production of either hydrogen peroxide, antibacterial substance bacteriocins and inorganic acids (Koll-Klais et al. 2005). Thereby maintain the dynamic equilibrium of the normal microbiota and restore the homeostasis (Krasse et al. 2006).

The effective anti-inflammatory action of *Lactobacillus brevis* in chronic periodontitis is mainly due to the observed significant reduction of prostaglandin E2 (PGE2), matrix metalloproteinases (MMPs) as well as prevention of nitric oxide production that decrease the plaque accumulation (Riccia et al. 2007). Studies on *Lactobacillus salivarius* have shown that intake of *L. salivarius* probiotics on regular basis (thrice a day for 8 weeks) benefits positively in probing depth of periodontal pocket and plaque index (Shimauchi et al. 2008). In addition, it resulted in production of acid that prevents the anaerobes *P. gingivalis*, *P. intermedia*, *P. nigrescens* (Ishikawa et al. 2003). Bacteriocin, a peptide toxin released from *L. casei* was found to be a novel therapeutic agent in killing *P. gingivalis* (Pangsomboon et al. 2006). *L. helveticus* increased the osteoblastic activity and favored bone formation and counteract pathogen associated bone resorption in individuals with periodontitis (Narva et al. 2004).

11.7 Probiotics in Halitosis

Halitosis is the presence of volatile molecules due to lack of oral hygiene, periodontal diseases, scab in oral tissues, food impaction, dirty dentures, broken restorations, carcinomas of oral cavity, respiratory and GIT infections. In addition, altered commensal microbiota results in generation of vaporous sulfur components such as hydrogen sulfide, dimethyl sulfide, and methyl mercaptan (Yoo et al. 2019). More precisely, anaerobic organisms degrade food and salivary proteins that generate amino acids which get converted into volatile sulfur compounds (Young et al. 2003). *S. salivarius* was detected to produce salivaricin, a lantibiotic which attributes to the inhibition of most of the *S. pyogenes* that are responsible for throat infections and oral malodour (Abdelahhad et al. 2020). Oral rinse with probiotic suspension of *Weissella cibaria* was found to reduce malodour caused by *F. nucleatum* (Kang et al. 2006). *W. cibaria* produces peroxide that competes with the secondary colonizers for coaggregation sites, thus reduce the reservoir of periodontal pathogens that produce volatile sulfur compounds and subgingival plaque (Persson et al. 1990).

11.7.1 Probiotics in Oral Mucositis

Incidence of oral cancer is strongly associated with the alteration of both oral and gut microbiota. Variation in the concentration of vitamins and nutrients stimulates production of inflammatory cytokines that have led to different pathology (Kany et al. 2019). Microbes such as *Fusobacterium nucleatum*, *P. gingivalis*, and *P. intermedia* are strongly correlated with the development of oral cancer (Atanasova and Yilmaz 2014). The most demonstrated species are *Actinomyces*, *Clostridium*, *Enterobacteriaceae*, *Fusobacterium*, *Haemophilus*, *Prevotella*, *Veillonella*, and *Streptococcus* sp., that have strong correlation with pre-cancerous lesions and oral cancer (Hu et al. 2016).

Inflammation of the oral mucosa is otherwise known as oral mucositis (OM) causes erythema, ulceration, pain, dysphagia, and malnutrition. OM is the common sequel in patients receiving radiotherapy and chemotherapy (Lalla et al. 2014). It is due to basal stem cell death by reactive oxygen species, damaged DNA strands, and abundant release of inflammatory cytokines (Ronai et al. 2007).

LGG is the normal commensal of the gut, describes anti-inflammatory properties, and remains the first explored bacteria used in oncology (Banna et al. 2017). It preserves the intestinal mucosal homeostasis by neutralizing the pathogens and the toxins produced and prevents breach of the mucosal through high affinity binding system (Okumura and Takeda 2018). The prime mechanism of action is, LGG is able to regulate IgA production (Wang et al. 2017). *L. rhamnosus* GG (LGG) produces increased geniposide, an anticancer molecule and is proven to have beneficial adjuvant effect during cancer treatment. Research on probiotics revealed that, lipoteichoic acid in LGG protects epithelial stem cells from radiation injury and can reduce their apoptosis. Other mechanism of action includes modulation of cyclooxygenase-2 and stimulation of PGE2 release (Desai et al. 2018). Moreover, LGG modulates the immune system through secretion of cytokines such as IL-1β, TNF-α, IL-6, IL-10, IL-12, and p40, thus reduces the inflammation, regulates epithelial function, and maintains the intestinal mucosa integrity (Andrews et al. 2018).

L. brevis CD2 lozenges in patients undergoing high dose chemotherapy have reduced the occurrence of oral mucositis (Sharma et al. 2017). In addition, application of *L. brevis* lozogenes was also beneficial in reducing oral ulcers of Behçet's syndrome (Tasli et al. 2006). The mechanism of action of *L. brevis* is via production of arginine deiminases and sphingomyelinase. It inhibits the conversion of arginine into nitric oxide and downregulates the expression of proinflammatory cytokines. Thus, it significantly reduces the incidence of chronic radiotherapy induced oral mucositis and intake of analgesics in head and neck cancer patients (Sharma et al. 2005). Moreover, it assists the patient to withstand the anticancer treatment and further aids in completion of the treatment. *L. brevis* is cost-effective and is shown to be safe in developing countries. Pathogen associated molecular patterns (PAMPs) stimulate NK cells, mast cells, dendritic cells, and macrophages thus activate innate immunity. In addition, secretion of proinflammatory cytokines activates NF-κB which leads to apoptosis of epithelial cells. Probiotics containing *L. brevis* or combination of *Lactobacillus* sp., *Bifidobacterium* sp., and *Enterococcus* sp. act via toll-like receptors (TLRs) and prevent epithelial cells apoptosis (Hughes et al. 2017).

11.7.2 Benefits of Probiotics in General Health

- Reduce the vulnerability towards infection.
- Decrease lactose intolerance, alleviate allergic episodes and respiratory infections.
- Reduce serum cholesterol and blood pressure.
- Prevent the gut from gastritis and diarrhea.
- Prevent urogenital and vaginal infections.
- Reduce the chance of colon cancer (Reid et al. 2003).

11.7.3 Anti-Inflammatory Property

Several studies proved that probiotic bacteria act as a potent anti-inflammatory agent especially in chronic intestinal inflammatory diseases (Grimoud et al. 2010). It was stated that the probiotic strains such as *L. plantarum*, *L. acidophilus*, *B. breve*, and *B. lactis* upon oral administration has lowered the level of proinflammatory cytokines via toll-like receptors signaling. Beneficial mechanisms through which probiotics influence chronic intestinal diseases are illustrated in Fig. 11.2. It demonstrates the potential of probiotics and prebiotics combination and the decrease of inflammatory bowel disease (IBD) incidence in 2,4,6 trinitrobenzenesulfonic acid (TNBS) and dextran sulfate sodium (DSS) induced animal models. Similarly, under in vitro cell line condition they decrease the activity of NF-κB—nuclear factor kappa, MAPK—mitogen-activated protein kinase, and TLR—toll-like receptor mediated pathways and secrete short-chain fatty acids (Plaza-Díaz et al. 2017). Indeed, the probiotic intervention has strain-specific anti-inflammatory effects in healthy adults (Kekkonen et al. 2008).

The commensal microbiota in the maintenance of epithelial homeostasis are through TLRs signaling and stimulation of epithelial repair factor, immune regulation and thus protect from epithelial injury. Regulation of proinflammatory cytokines by probiotics lessens the gingival inflammation (Jones and Versalovic 2009). The commonly used probiotic strains are Lactobacilli and Bifidobacterium; *L. acidophilus*, *L. casei*, *L. rhamnosus*, *L. reuteri*, *L. johnsonii*, and *L. gasseri*, *B. longum*, *B. bifidum*, *and B. infantis.* Recent study revealed that the predominant presence of *L. fermentum* and *L. gasseri* in healthy individuals are responsible for the inhibition of periodontal pathogens such as *P. gingivalis*, *Prevotella intermedia*, *and A. actinomycetemcomitans* via production of hydrogen peroxide/antibacterial substance bacteriocins and inorganic acids (Koll-Klais et al. 2005). Thus the dynamic equilibrium of normal microbiota are maintained and restores the homeostasis (Krasse et al. 2006).

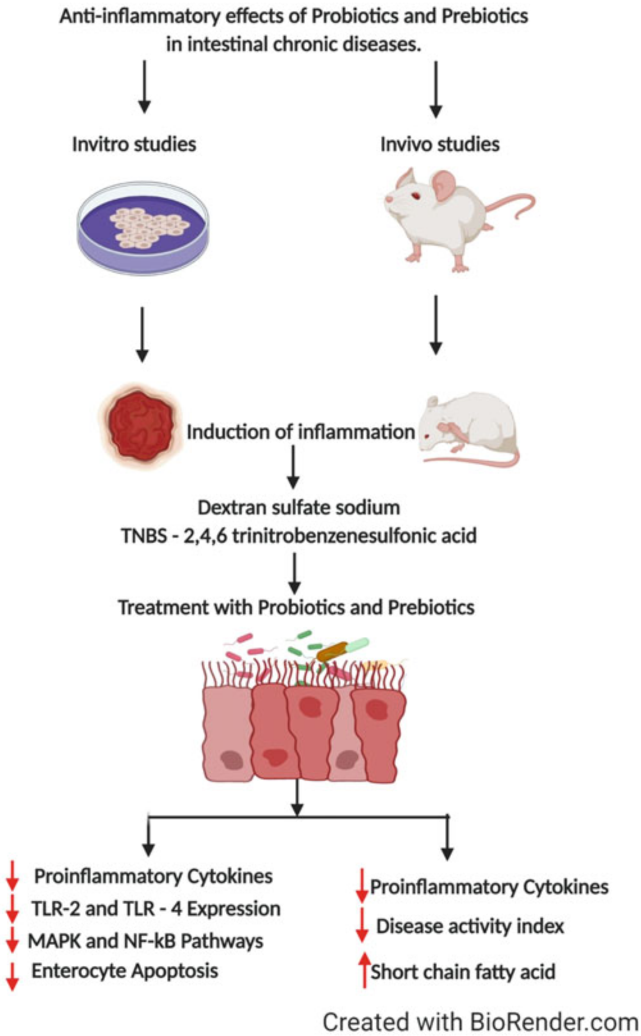

Fig. 11.2 Influence of Probiotics and prebiotics in chronic intestinal diseases

11.8 Antimicrobial Properties

Competitive exclusion of pathogens through production of antimicrobial peptides is the crucial step for the antimicrobial activity of probiotics. *Lactobacillus* strains demonstrated antimicrobial activity against pathogens such as *klebsiella* sp., *C. difficile*, *Shigella* sp., *E. coli*, *P. aeruginosa*, *S. mutans*, and *S. aureus* (Chuayana et al. 2003). They compete with pathogens for nutrients, produce lactic acid,

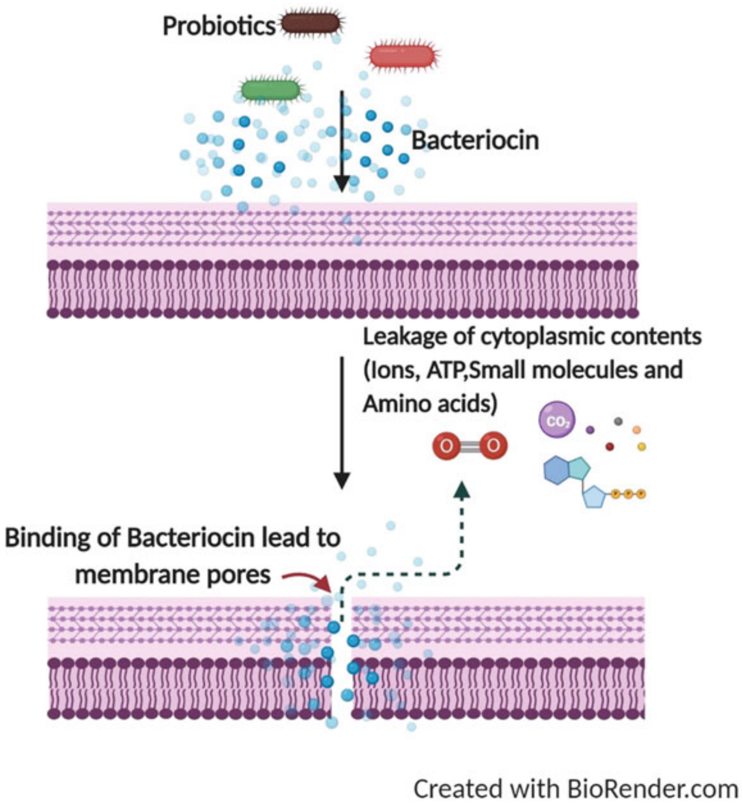

Fig. 11.3 Diagrammatic representation of Bacteriocin action

bacteriocin, and hydrogen peroxide, inhibit pathogenic bacterial adhesion to the mucosa, and enhance the immune response (Plaza-Diaz et al. 2019).

The antimicrobial activity of lactobacillus is attributed mainly due to the release of Bacteriocins (La Storia et al. 2020). Bacteriocins are antimicrobial peptides, which act against both Gram-positive and Gram-negative bacteria, but the producing bacteria carry specific immune mechanisms that protect it from its own bacteriocin (Oelschlaeger 2010). Both Bifidobacterium and Lactobacillus produce bacteriocin, which crosses the cell wall and inhibits the cell membrane lipid II and prevents the synthesis of peptidoglycan, the cell wall component. Another mechanism of bacteriocin action is migration into the cell wall and forms pores through pore-forming receptor in the mannose-phosphotransferase system and is shown in Fig. 11.3 (Martinez et al. 2013).

11.9 Antioxidant Properties

Oxidative stress is either due to the cumulative effect of reactive oxygen species (ROS) production or ineffective scavenging activity of antioxidant system to detoxify the oxidative stress that leads to redox imbalance (Pizzino et al. 2017). Being beneficial to health, the ingestion of probiotics either alone or along with food showed to have an increased antioxidant activity thus able to reduce the oxidation related tissue damage (Wang et al. 2017). Among the antioxidant activity of Lactobacillus, Bifidobacterium, and Propionibacterium probiotics strains; *P. freudenreichii* was found to exhibit maximum antioxidant activity (Amaretti et al. 2013). Through the release of potent antioxidant compounds such as vitamin E, vitamin C, glutathione, beta-carotene, superoxide dismutase (SOD), polysaccharide, prototype coenzyme I (NADH), and some unknown substances, probiotics promote intestinal health (Hemarajata and Versalovic 2013). Probiotics may reduce oxidative stress via suppression of cytokine production, decreasing interleukin 1, tumor necrosis factor-alpha and increasing glutathione (GSH) levels (Bahrami et al. 2011).

The antioxidant property of probiotics is mainly due to the following actions (Fig. 11.4).

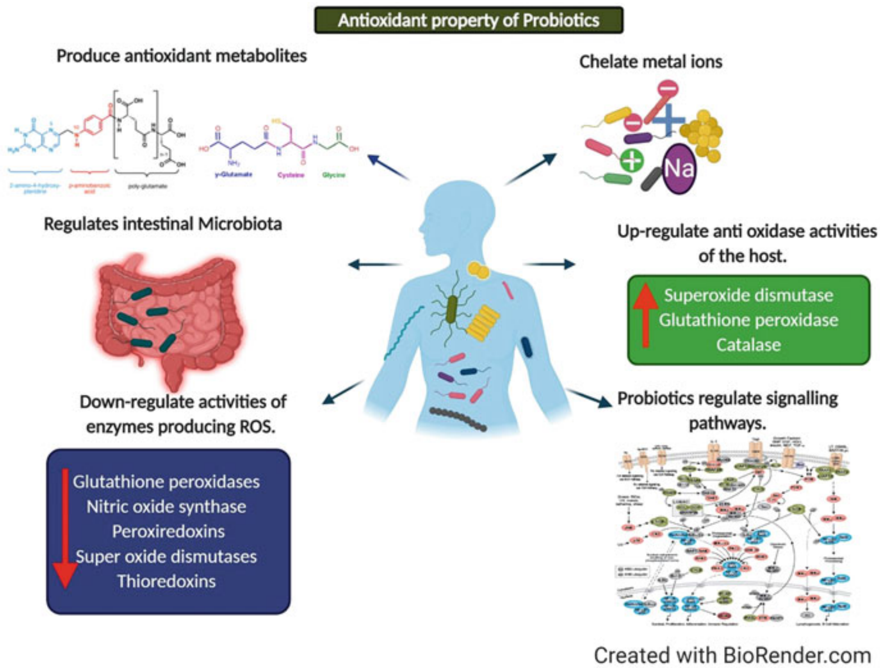

Fig. 11.4 Antioxidant potential of probiotics

- Probiotics chelate metal ions.
- Probiotics produce antioxidant metabolites.
- Probiotics up-regulate the hosts antioxidant levels.
- Probiotics modulate the signaling pathways.
- Downregulation of ROS producing enzymes.
- Probiotics govern the intestinal microbiota (Wang et al. 2017).

11.10 Anticancer Properties

Probiotics can act as potent anticancer agent and have been proved both in vitro and in vivo. The probiotic strains of *L. paracasei* SR4, *L. casei* SR1, and *L. casei* SR2 exhibit anticancer activity against cervix cancer (HeLa) cells via upregulation of BAX BAD, caspase8, caspase3, and caspase9 apoptotic genes and downregulation of BCl-2gene (Riaz Rajoka et al. 2018). The study on *Enterococcus thailandicus* revealed that, the bacteriocin from *Enterococcus thailandicus* has significant anticancer activity against liver cancer HepG2 cell line. The study by Yazdi et al. proved that administration of 0.5 mL of *L. casei* (2.7×10^8 CFU/mL^{-1}) to breast cancer induced mice showed decrease in tumor growth rate and has prolonged the survival rate significantly in comparison to control (Yazdi et al. 2013).

The yogurt enriched with probiotics exhibits significant inhibition on 1, 2-dimethylhydrazine induced colon tumors in BALB/c mice. The induced apoptosis of non-small cell lung cancer cells by aqueous extract of Bifidobacterium sp. has led to the inhibition of cancer invasiveness (Ahn et al. 2020). Probiotic (*L. casei*-01) combinations with dairy beverages are potential candidate against human prostate cell lines. This mixture exhibits both anti-proliferative and apoptotic effects (Rosa et al. 2020).

The bioactive compounds; bacterial proteins, and peptides exhibit excellent anticancer activity (Karpiński and Adamczak 2018). Actinomycin D, doxorubicin, mitomycin C, and bleomycin are some of them that are used as anticancer antibiotics. Peptides like non-ribosomal peptides (NRPs), toxins, azurin, p28, Entap, and Pep27anal2 originated from bacteria act as antimicrobial peptides. The mechanisms of probiotics that inhibit the cancer cells are illustrated in Fig. 11.5.

11.10.1 Probiotics in Treatment of Upper Respiratory Tract Infections

Upper respiratory infections (URTIs) are most probably caused by viruses than bacteria. The common URTIs include acute pharyngitis, acute sinusitis, common cold, and acute otitis media (Morris 2009). Morbidity associated with acute URTIs is quite high and is not cured with antibiotics. Probiotics offer promising benefits in inhibiting URTIs. *S. salivarius* and *L. helveticus* M1MLh5 are known to adhere effectively to pharyngeal epithelial cells, promote the expression of TNF-α, modulate the host innate immunity and thus antagonize *S. pyogenes* (Taverniti et al. 2012).

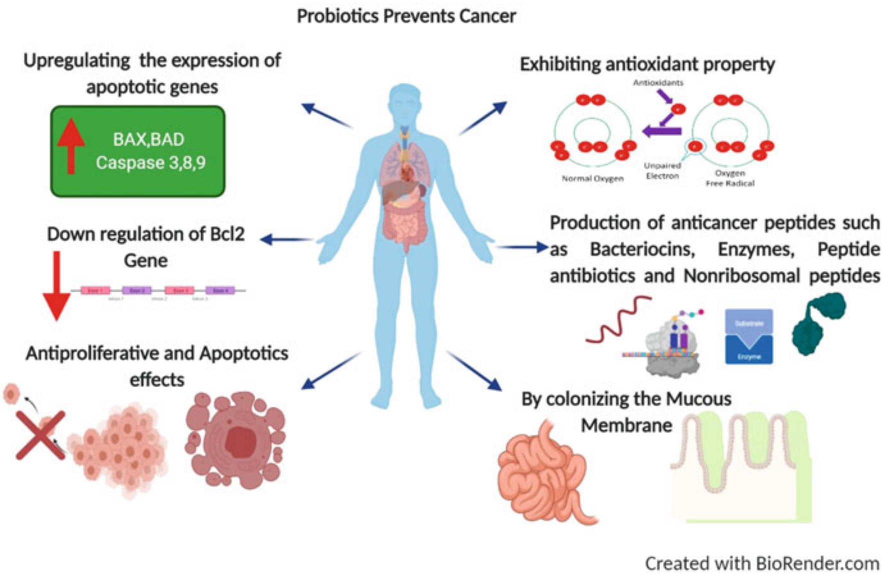

Fig. 11.5 Probiotics on treatment and prevention of cancer

Bacteriocidins producing *S. salivarius* 24 SMB were found to inhibit the common respiratory pathogen, Streptococcus pneumonia (Santagati et al. 2012).

L. rhamnosus alone or combined with *B. animalis* subsp. or Lactis Bb-12 has decreased the occurrence of upper respiratory infections and acute otitis media in children (Nase et al. 2001). It modulates the immune system and reduced the nasal colonization of *S. aureus* and *S. pneumoniae* in adults. The use of *S. salivarius* K12 in group A beta-hemolytic streptococci induced pharyngo-tonsillar infections and in children with otitis media has shown to decrease the incidence of infectious episodes significantly. It also prevents the recurrence of tonsillitis and pharyngitis (Di Pierro et al. 2016).

11.10.2 Probiotics in Treatment of Urogenital Infections

Urogenital infections such as yeast vaginitis, bacterial vaginosis (BV), urinary tract infections (UTI), and non-sexually transmitted urogenital infection are the common cause for a woman to visit the gynecologist (Al-Badr and Al-Shaikh 2013). The normal vaginal commensals of healthy premenopausal women and those who attained menopause vary. Lactobacillus species are generally predominant in healthy premenopausal women that include *L. delbrueckii*, *L. brevis*, *L. crispatus*, *L. casei*, *L. jensenii*, *L. fermentum*, *L. plantarum*, *L. reuteri*, *L. salivarius*, *L. rhamnosus*, *L. gasseri*, and *L. vaginalis* (Petrova et al. 2015). The probable cause for such differences are: vaginal pH, glycogen content, hormonal changes (estrogen), and menstrual cycle. These factors facilitate the colonization and adherence of the pathogens to the epithelial cells of the vagina. Increase in estrogen levels in healthy premenopausal women increases the adherence of lactobacillus and prevents colonization of other pathogens. Vice-versa occurs in postmenopausal women and causes urogenital infections.

Bacterial vaginosis (BV) the commonest of all urogenital infections (UGI) is mainly due to the depletion of *Lactobacillus* species and further population by Gram-negative anaerobes. Failure in antimicrobial treatment and the risk of recurrent infections are mostly due to antibiotic and biofilm resistance, suboptimal defense mechanism, elimination of commensal organisms by repeated antimicrobial therapy, and recurrent attack with virulent pathogens perhaps from their sexual partners or from the individuals own gut (Reid et al. 2006). Probiotics are the plausible way to replenish the commensal organisms that disturb the growth of pathogenic organisms in vagina and inhibit the biofilm formation (Cribby et al. 2008). The concept of repopulating vagina with lactobacilli through oral probiotics was first reported by Reid et al. (2001). The possible mechanisms by which lactobacilli fight against vaginal pathogens is by production of antimicrobial agents (bacteriocins) (Gaspar et al. 2018) and biosurfactants that modify the surface tension of the environment and prevent the adherence of pathogens that further inhibit their spread to bladder and also maintain the vaginal pH ≤ 4.5 (Aroutcheva et al. 2001). Research study revealed that, administration of *L. rhamnosus* GR-1 probiotic to premenopausal women raised the expression levels of antimicrobial defenses (Cribby et al. 2008). Among all the lactobacillus strains, *L. gasseri* 335 and *L. salivarius* FV2 were proficient of coaggregating *G. vaginalis* and prevent their adherence (Mastromarino et al. 2002). Further, these strains when combined with *L. brevis* CD2 were shown to reduce *G. vaginalis* up to 57.7% . Augmentation efficacy of antibiotic treatment with the inclusion of *L. reuteri* RC-14 and *L. rhamnosus* 1 probiotics to BV women had reduced the Nugent scores significantly (Hummelen et al. 2010). Treatment of BV with vaginal probiotics preceded by metronidazole therapy has reduced the recurrence rate with acceptable cure rate.

Preterm birth (PTB) is the greatest challenge and the second most reason for neonatal death all over the world (Keelan and Newnham 2017). Studies revealed a strong relation between BV and PTB. Consumption of *Lactobacillus* in early and late pregnancy has decreased the incidence of infection and inflammation mediated PTB and preeclampsia (Zheng et al. 2019). The predominance of less diverse *Lactobacillus* community is believed as the characteristic feature of healthy female reproductive tract (Madhivanan et al. 2015). In contrast, high diverse vaginal microbiome is associated with BV and also the prime cause for acquisition of sexually transmitted diseases, PTB and pelvic inflammatory diseases.

11.10.3 Probiotics in Improvement of Intestinal Health

Currently, both in vivo and in vitro studies have proven that probiotics consumed in adequate amount act as an effective barrier against pathogenic and opportunistic microorganisms. Probiotics restore the normal gastrointestinal tract flora which is usually disturbed by diet, surgery, and antibiotics that are used for treating intestinal disorders. Effective inhibition of *H. pylori* and rota viral infections by probiotic strains has been reported in few studies (Sullivan and Nord 2005). The strains predominantly used are L*actobacillus* sp. and *Bifidobacterium* sp. (Mercenier et al. 2004). It is also efficient in preventing traveler's diarrhea. In addition, *Saccharomyces boulardii* CNCM I-745 also significantly reduces traveler's diarrhea (Black et al. 1989). Probiotics are also proved to be helpful in chronic pouchitis, inflammatory bowel disease, and ulcerative colitis in animal models (Schultz and Sartor 2000). The research conducted by Vanderpool, et al. states the following mechanisms of probiotics in preventing intestinal disorders: (Vanderpool et al. 2008).

- Probiotics produce bactericidal substances and compete with pathogens for the binding sites.
- Probiotics modulate pathogen-induced inflammation via toll-like receptor-regulated signaling pathways thus enhance the innate immunity.
- Probiotics promote survival of intestinal epithelial cells and regulate the intestinal epithelial homeostasis by stimulating the protective responses thus enhance the intestinal barrier function (Sheu et al. 2002).

11.10.4 Probiotics in Treatment of Chemotherapy and Radiotherapy Induced Diarrhea

Radiotherapy and chemotherapy are the most common therapeutic measures provided to all types of cancer patient that eventually results in diarrhea which exactly leads to poor quality of life and negatively affects the treatment outcome. Probiotics play crucial role in inflammatory bowel syndrome and reduce the incidence of cancer associated with chronic inflammation (Delia et al. 2007). WHO defines diarrhea as passing of watery stools for more than three times in 24 h.

In developing countries, diarrhea is one among the cause for childhood morbidity and mortality (Giannattasio et al. 2016). Rehydration methods do not reduce the infectious symptoms and restore the gut microbiota. Bacillus Calmette-Guerin (BCG) has been employed as preventive immunotherapy in recurrent superficial bladder cancer for many years (Guallar-Garrido and Julián 2020). Regular consumption of lactic acid bacteria is considered as preventive measure in bladder cancer (Ohashi et al. 2002). The mode of action is through modulation of both local and systemic immune response and their cytotoxic effects inhibit the carcinogens (Asano et al. 2007). Probiotics enhance the expression of junctional molecules and maintain the gut barrier integrity. Also, they produce IgA and short-chain fatty acids that interfere with the adherence and growth of pathogens (Engevik and Versalovic 2017). Thus, prevent the progression of the cell cycle. *L. plantarum* releases polysaccharides that are known to have anti-tumor activities by decreasing the mRNA expression of MAPK and upregulates PTEN (Asoudeh-Fard et al. 2017). Studies on *L. lactis* strains revealed that, it secretes IL-10, INF-β and expresses antioxidants which reduce the generation of ROS and colonic damage in animal model (De Moreno de Leblanc et al. 2011).

B. longum when administered orally produces 11-amino-acid peptide that alleviates rotovirus induced gastroenteritis (Chenoll et al. 2016). Along with *L. acidophilus*, it reduces the duration of diarrhea in pediatric patients and also inhibits the infection with rotavirus (Lee et al. 2015). LGG is beneficial in infectious diarrhea, ulcerative colitis, antibiotic associated diarrhea, and rotavirus induced diarrhea by stimulation of mucosal IgA and sIgA (Li et al. 2019). Administration of *B. bifidum* G9-1 orally for prophylactic and therapeutic mean alleviates rotavirus induced gastroenteritis by secreting mucosal protective peptides that are effective in managing RV induced gastroenteritis (Kawahara et al. 2017).

11.10.5 Probiotics in Treatment of Anemia

Folic acid is a water soluble B vitamin that is essential for preventing and treating anemia. The biological origin of folic acid pays greater attention than synthetic one. Biological origin is derived from probiotic bacteria such as *L. lactis*, *L. cremoris*, B. *pseudocatenulatum*, *C. famata*, *B. adolescentis*, *C. glabrata*, *C. guilliermondii*, *S. cerevisiae*, *Yarrowia lipolytica*, and *Pichia glucozyma.* These bacterias are used for the treatment instead of folic acid as well as used to enhance the intestinal uptake of folic acids. *P. denitrificans* and *P. shermanii* has been used for the vitamin B12 deficiency. The lactic acid fermented food increases the absorption of iron and is used for treating anemic patients. Moreover, they act via optimizing the pH of the digestive tract and activate the enzyme phytase that aids in absorption. The probiotic bacterial mixture has been used along with food for the treatment of megaloblastic anemia. They increase the colonic fermentation and neutralize the negative impact of antibiotics (Ohtani et al. 2014).

11.11 Treatment and Prevention of Obesity

Obesity is one of the basic risk factors for hypertension, coronary heart disease, and type II diabetes (Liou et al. 2013). The diet, lack of physical activity, age, genes, and developmental stage cause a major impact on obesity (Mekkes et al. 2014). Currently probiotic bacteria have been employing to control and reduce the obesity as a novel approach. Probiotics are capable of reducing the weight gain, obesity, reduce the food intake, and control prolonged satiation. Moreover, they are also used to reduce fat deposition and improve the energy metabolism by enhancing insulin sensitivity (Daniali et al. 2020).

Fecal microbiota transplantation (FMT) is an alternative approach to counterbalance dysbiosis induced by ulcerative colitis and re-establishes the intestinal microbiota that modulates the weight gain. Hence, FMT could be a beneficial tool in ulcerative colitis individuals with obesity (Li et al. 2014). Administration of microbiota from healthy individual to an obese individual alters the composition of microbial flora.

Recent studies proved that, probiotics are able to decrease the serum cholesterol by producing short-chain fatty acid and through bile salt conjugation property (Pereira et al. 2003; Bhat and Bajaj 2019). The hypocholesterolemic activity of probiotic Saccharomyces cerevisiae in a rat model study resulted in significant reduction of low-density lipoprotein, serum total cholesterol, and triglyceride levels (Lay and Min 2010).

11.12 Probiotics as Immunomodulator

Bifidobacterium and *Lactobacillus* act as potent modulator on humoral response, cell mediated responses, and nonspecific immunity (Erickson and Hubbard 2000). Probiotics enhance the secretion of IgA immunoglobulin and thereby modulate the humoral immunity against the invading pathogens. Similar result was observed after consumption of *L. casei* and *L. acidophilus* with yogurt. They cause drastic increase of IgA-producing plasma cells (Fang et al. 2000). Study by Hasan et al. (2019) proved that Heat-killed probiotic *Bacillus* sp. SJ-10 acts as a potent modulator of innate immunity response in olive flounder (Hasan et al. 2019). Moreover, oral administration of probiotics can reduce the occurrence and intensity of viral RTIs via activated cell mediated immune response (Baud et al. 2020). Oral probiotics through Toll-like receptors signaling induce the production of cytokines that activate the macrophage and modulate the intestinal epithelial cells (IECs) and immune cells associated with the lamina propria. Specifically, they trigger the regulatory T cells to release IL-10 and are proven by many studies. Probiotics have immune-modulatory effects in both the humoral, cell mediated immunity and in nonspecific immune response (Galdeano et al. 2019). We illustrated the types of immunity modulated by probiotics in Fig. 11.6.

11.13 Conclusion

Application of microbes has received greater attention for many years. Researchers have focused with aspirations to uncover conclusive evidence about probiotic strains that provide improvements in human health and disease outcomes. The probiotic bacterial species have many beneficial impacts that include anti-inflammatory, anticancer, antimicrobial, antioxidant, and immunomodulatory potential. Thereby, they define the physiological and metabolic functions of the host. Moreover, they act against pathogenic microbes. Their contribution in preventing and treating diabetics, obesity, and cancer is an exciting and rapidly advancing research area. Incorporation of probiotics along with dairy and fermented food products is a simple, cost-effective alternative method to improve human health. Hence, research should focus in evaluating novel strains of human gut microbes and their potential to explore the new direction of probiotics applications in biomedical and clinical research.

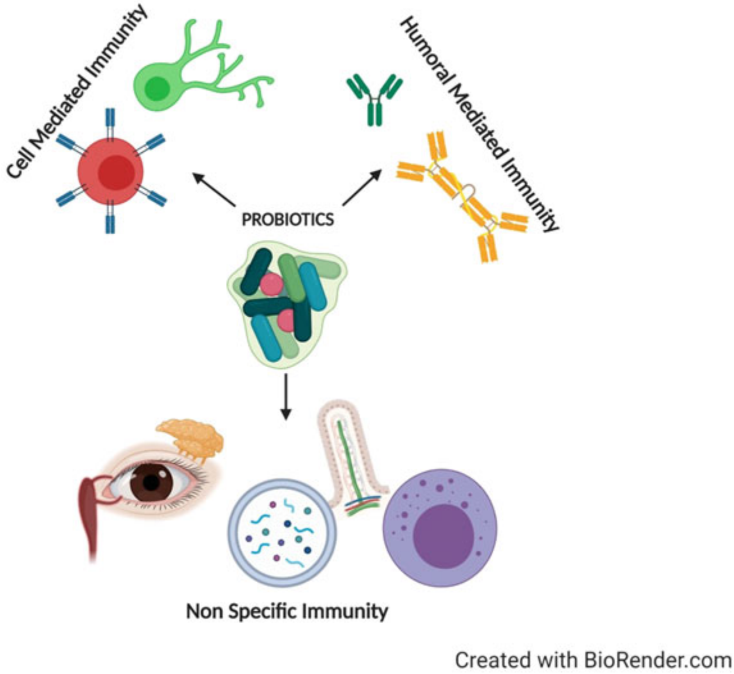

Fig. 11.6 Immunomodulatory potentials of probiotics

References

Abdelahhad B, Wescombe P, Smith L (2020) Evolution of lantibiotic salivaricins: new weapons to fight infectious diseases. Trends Microbiol 28(7):P578–P593. https://doi.org/10.1016/j.tim.2020.03.001

Ahn J, Kim H, Yang KM (2020) An aqueous extract of a Bifidobacterium species induces apoptosis and inhibits invasiveness of non-small cell lung cancer cells. J Microbiol Biotechnol 30 (6):885–892

Ahola AJ, Yli-Knuuttila H, Suomalainen T et al (2002) Shortterm consumption of probiotic-containing cheese and its effect on dental caries risk factors. Arch Oral Biol 47:799–804

Al-Badr A, Al-Shaikh G (2013) Recurrent urinary tract infections management in women: a review. Sultan Qaboos Univ Med J 13(3):359–367

Alkaya B, Laleman I, Keceli S, Ozcelik O, Cenk Haytac M, Teughels W (2017) Clinical effects of probiotics containing Bacillus species on gingivitis: a pilot randomized controlled trial. J Periodontal Res 52:497–504

Amaretti A, di Nunzio M, Pompei A, Raimondi S, Rossi M, Bordoni A (2013) Antioxidant properties of potentially probiotic bacteria: in vitro and in vivo activities. Appl Microbiol Biotechnol 97(2):809–817

Andrews C, McLean MH, Durum SK (2018) Cytokine tuning of intestinal epithelial function. Front Immunol 9:1270. https://doi.org/10.3389/fimmu.2018.01270. PMID: 29922293; PMCID: PMC5996247

Arokiyaraj S, Islam VIH, Bharanidharan R, Raveendar S, Lee J, Kim DH et al (2014) Antibacterial, anti-inflammatory and probiotic potential of Enterococcus hirae isolated from the rumen of Bos primigenius. World J Microbiol Biotechnol 30(7):2111–2118

Aroutcheva A, Gariti D, Simon M, Shott S, Faro J, Simoes JA, Gurguis A, Faro S (2001) Defense factors of vaginal lactobacilli. Am J Obstet Gynecol 185(2):375–379

Asano S, Suzuki K, Iijima K, Motoyama Y, Kuriyama H, Kitagawa Y (2007) Effects of morphological changes in beer-spoilage lactic acid bacteria on membrane filtration in breweries. J Biosci Bioeng 104(4):334–338

Asoudeh-Fard A, Barzegari A, Dehnad A, Bastani S, Golchin A, Omidi Y (2017) Lactobacillus plantarum induces apoptosis in oral cancer KB cells through upregulation of PTEN and downregulation of MAPK signalling pathways. Bioimpacts 7(3):193–198

Atanasova KR, Yilmaz O (2014) Looking in the Porphyromonas gingivalis cabinet of curiosities: the microbium, the host and cancer association. Mol Oral Microbiol 29(2):55–66

Bahrami B, Child MW, Macfarlane S, Macfarlane GT (2011) Adherence and cytokine induction in Caco-2 cells by bacterial populations from a three stage continuous-culture model of the large intestine. Appl Environ Microbiol 77(9):2934–2942

Banna GL, Torino F, Marletta F et al (2017) Lactobacillus rhamnosus GG: an overview to explore the rationale of its use in cancer. Front Pharmacol 8:603

Baud D, Agri VD, Gibson GR, Reid G, Giannoni E (2020) Using probiotics to flatten the curve of coronavirus disease COVID-2019 pandemic. Front Public Health 8

Becker MR et al (2002) Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol 40:1001–1009

Bengmark S (2001) Pre -, pro- and symbiotics. Curr Opin Clin Nutr Metab Care 4:571–579

Bhat B, Bajaj BK (2019) Hypocholesterolemic potential and bioactivity spectrum of an exopolysaccharide from a probiotic isolate Lactobacillus paracasei M7. Bioact Carbohydr Diet Fibre 19:100191

Black FT, Andersen PL, Ørskov J, Ørskov F, Gaarslev K, Laulund S (1989) Prophylactic efficacy of lactobacilli on traveler's diarrhea. In Travel Med:333–335

Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S (2005) Consumption of yogurt with Bifidobacterium DN-173 010 and its effect on dental caries risk factors. Acta Odontol Scand 63:317–320

Chenoll E, Casinos B, Bataller E, Buesa J, Ramón D, Genovés S, Fábrega J, Rivero Urgell M, Moreno Muñoz JA (2016) Identification of a peptide produced by Bifidobacterium longum CECT 7210 with antirotaviral activity. Front Microbiol 7:655. https://doi.org/10.3389/fmicb.2016.00655. PMID: 27199974; PMCID: PMC4855034

Chuayana EL, Ponce CV, Rivera MRB, Cabrera EC (2003) Antimicrobial activity of probiotics from milk products. Philos J Microbiol Infect Dis 32(2):71–74

Clements A, Young JC, Constantinou N, Frankel G (2012) Infection strategies of enteric pathogenic Escherichia coli. Gut Microbes 3(2):71–87. https://doi.org/10.4161/gmic.19182

Comelli EM, Guggenheim B, Stingele F et al (2002) Selection of dairy bacterial strains as probiotics for oral health. Eur J Oral Sci 110:218–224

Cribby S, Taylor M, Reid G (2008) Vaginal microbiota and the use of probiotics. Interdiscip Perspect Infect Dis 2008:256490

Daniali M, Nikfar S, Abdollahi M (2020) A brief overview on the use of probiotics to treat overweight and obese patients. Expert Rev Endocrinol Metab 15(1):1–4. https://doi.org/10.1080/17446651.2020.1719068

Darveau RP (2009) The oral microbial consortium's interaction with the periodontal innate defense system. DNA Cell Biol 28:389–395

De Moreno de Leblanc A, Del Carmen S, Zurita-Turk M, Santos Rocha C, van de Guchte M, Azevedo V, Miyoshi A, Leblanc JG (2011) Importance of IL-10 modulation by probiotic microorganisms in gastrointestinal inflammatory diseases. ISRN Gastroenterol 2011:892971

Delia P, Sansotta G, Donato V, Frosina P, Messina G, De Renzis C, Famularo G (2007) Use of probiotics for prevention of radiation-induced diarrhea. World J Gastroenterol 13(6):912–915

Desai SJ, Prickril B, Rasooly A (2018) Mechanisms of phytonutrient modulation of cyclooxygenase-2 (COX-2) and inflammation related to cancer. Nutr Cancer 70(3):350–375
Di Pierro F, Colombo M, Giuliani MG et al (2016) Effect of administration of Streptococcus salivarius K12 on the occurrence of streptococcal pharyngo-tonsillitis, scarlet fever and acute otitis media in 3 years old children. Eur Rev Med Pharmacol Sci 20(21):4601–4606
Diosma G, Romanin DE, Rey-Burusco MF, Londero A, Garrote GL (2014) Yeasts from kefir grains: isolation, identification, and probiotic characterization. World J Microbiol Biotechnol 30 (1):43–53
Engevik MA, Versalovic J (2017) Biochemical features of beneficial microbes: foundations for therapeutic microbiology. Microbiol Spectr 5(5). https://doi.org/10.1128/microbiolspec
Erickson KL, Hubbard NE (2000) Probiotic immunomodulation in health and disease. J Nutr 130 (2):403S–409S
Fang H, Elina T, Heikki A, Seppo S (2000) Modulation of humoral immune response through probiotic intake. FEMS Immunol Med Microbiol 29(1):47–52
Galdeano CM, Cazorla SI, Dumit JML, Vélez E, Perdigón G (2019) Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab 74(2):115–124
Gaspar C, Donders GG, Palmeira-de-Oliveira R, Queiroz JA, Tomaz C, Martinez-de-Oliveira J, Palmeira-de-Oliveira A (2018) Bacteriocin production of the probiotic Lactobacillus acidophilus KS400. AMB Express 8(1):153
Ghannoum MA, Jurevic RJ, Mukherjee PK, Cui F, Sikaroodi M, Naqvi A, Gillevet PM (2010) Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog 6(1)
Giannattasio A, Guarino A, Lo Vecchio A (2016) Management of children with prolonged diarrhea. F1000Res 5. F1000 Faculty Rev-206
Gibson GR, Roberfroid MB (1995) Dietary modulation of the 9 human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412
Grimoud J, Durand H, De Souza S, Monsan P, Ouarné F, Theodorou V, Roques C (2010) In vitro screening of probiotics and synbiotics according to anti-inflammatory and anti-proliferative effects. Int J Food Microbiol 144(1):42–50
Guallar-Garrido S, Julián E (2020) Bacillus Calmette-Guérin (BCG) therapy for bladder cancer: an update. Immunotargets Ther 9:1–11. https://doi.org/10.2147/ITT.S202006. PMID: 32104666; PMCID: PMC7025668
Gupta C, Prakash D, Gupta S (2014) Natural useful therapeutic products from microbes. J Microbiol Exp 1(1):30–37
Hajishengallis G, Lambris JD (2011) Microbial manipulation of receptor crosstalk in innate immunity. Nat Rev Immunol 11:187–200
Hamilton-Miller JMT, Gibson GR, Bruck W (2003) Some insights into the derivation and early uses of the word ‘probiotic’. Br J Nutr 90(4):845–845
Hasan MT, Jang WJ, Lee BJ, Kim KW, Hur SW, Lim SG et al (2019) Heat-killed Bacillus sp. SJ-10 probiotic acts as a growth and humoral innate immunity response enhancer in olive flounder (Paralichthys olivaceus). Fish Shellfish Immunol 88:424–431
Hellgren K (2009) Assessment of Krillase chewing gum for the reduction of gingivitis and dental plaque. J Clin Dent 20(3):99–102
Hemarajata P, Versalovic J (2013) Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Ther Adv Gastroenterol 6(1):39–51
Holt SC, Ebersole JL (2005) Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia: the “red complex”, a prototype polybacterial pathogenic consortium in periodontitis. Periodontol 2000 38:72–122
Hu X, Zhang Q, Hua H, Chen F (2016) Changes in the salivary microbiota of oral leukoplakia and oral cancer. Oral Oncol 56:e6–e8
Hughes KR, Harnisch LC, Alcon-Giner C, Mitra S, Wright CJ, Ketskemety J, van Sinderen D, Watson AJ, Hall LJ (2017) Bifidobacterium breve reduces apoptotic epithelial cell shedding in an exopolysaccharide and MyD88-dependent manner. Open Biol 7(1):160155

Hummelen R, Changalucha J, Butamanya NL, Cook A, Habbema JD, Reid G (2010) Lactobacillus rhamnosus GR-1 and L. reuteri RC-14 to prevent or cure bacterial vaginosis among women with HIV. Int J Gynaecol Obstet 111(3):245–248. https://doi.org/10.1016/j.ijgo.2010.07.008

Iniesta M, Herrera D, Montero E et al (2012) Probiotic effects of orally administered Lactobacillus reuteri-containing tablets on the subgingival and salivary microbiota in patients with gingivitis. A randomized clinical trial. J Clin Periodontol 39(8):736–744

Ishikawa H, Aiba Y, Nakanishi M, Oh-Hashi Y, Koga Y (2003) Suppression of periodontal pathogenic bacteria by the administration of Lactobacillus salivarius T12711. J Jap Soc Periodontol 45:105–112

Jones SE, Versalovic J (2009) Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. BMC Microbiol 9:35. https://doi.org/10.1186/1471-2180-9-35

Kang MS, Kim BG, Chung J, Lee HC, Oh JS (2006) Inhibitory effect of Weissella cibaria isolates on the production of volatile sulphur compounds. J Clin Periodontol 33:226–232

Kany S, Vollrath JT, Relja B (2019) Cytokines in inflammatory disease. Int J Mol Sci 20(23):6008. https://doi.org/10.3390/ijms20236008. PMID: 31795299; PMCID: PMC6929211

Karpiński TM, Adamczak A (2018) Anticancer activity of bacterial proteins and peptides. Pharmaceutics 10(2):54

Kawahara T, Makizaki Y, Oikawa Y et al (2017) Oral administration of Bifidobacterium bifidum G9–1 alleviates rotavirus gastroenteritis through regulation of intestinal homeostasis by inducing mucosal protective factors. PLoS One 12(3):e0173979

Keelan JA, Newnham JP (2017) Recent advances in the prevention of preterm birth. F1000Res 6. F1000 Faculty Rev-1139

Kekkonen RA, Lummela N, Karjalainen H, Latvala S, Tynkkynen S, Järvenpää S, Korpela R (2008) Probiotic intervention has strain-specific anti-inflammatory effects in healthy adults. World J Gastroenterol 14(13):2029

Koll-Klais P, Mändar R, Leibur E, Marcotte H, Hammarström L, Mikelsaar M (2005) Oral lactobacilli in chronic periodontitis and periodontal health: species composition and antimicrobial activity. Oral Microbiol Immunol 20(6):354–361

Krasse P, Carlsson B, Dahl C, Paulsson A, Nilsson A, Sinkiewicz G (2006) Decreased gum bleeding and reduced gingivitis by the probiotic Lactobacillus reuteri. Swed Dent J 30:55–60

La Storia A, Di Giuseppe FA, Volpe S, Oliviero V, Villani F, Torrieri E (2020) Physical properties and antimicrobial activity of bioactive film based on whey protein and lactobacillus curvatus 54M16 producer of bacteriocins. Food Hydrocolloids:105959

Lalla RV, Saunders DP, Peterson DE (2014) Chemotherapy or radiation-induced oral mucositis. Dent Clin N Am 58(2):341–349

Lambrecht BN, Hammad H (2014) Allergens and the airway epithelium response: 780 gateway to allergic sensitization. J Allergy Clin Immunol 134(3):499–507

Lay GO, Min TL (2010) Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. Int J Mol Sci 11(6):2499–2522

Lee DK, Park JE, Kim MJ, Seo JG, Lee JH, Ha NJ (2015) Probiotic bacteria, B. longum and L. acidophilus inhibit infection by rotavirus in vitro and decrease the duration of diarrhea in pediatric patients. Clin Res Hepatol Gastroenterol 39(2):237–244

Li Q, Wang C, Tang C et al (2014) Therapeutic modulation and reestablishment of the intestinal microbiota with fecal microbiota transplantation resolves sepsis and diarrhea in a patient. Am J Gastroenterol 109(11):1832–1834. https://doi.org/10.1038/ajg.2014.299

Li J, Butcher J, Mack D et al (2015) Functional impacts of the intestinal microbiome in the pathogenesis of inflammatory bowel disease. Inflamm Bowel Dis 21(1):139–153

Li YT, Xu H, Ye JZ, Wu WR, Shi D, Fang DQ, Liu Y, Li LJ (2019) Efficacy of Lactobacillus rhamnosus GG in treatment of acute pediatric diarrhea: a systematic review with meta-analysis. World J Gastroenterol 25(33):4999–5016

Liou AP, Paziuk M, Luevano JM Jr et al (2013) Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. Sci Transl Med 5(178):178ra41

Ljungh A, Wadstrom T (2006) Lactic acid bacteria as probiotics. Curr Issues Intest Microbiol 7 (2):73–90
Madhivanan P, Alleyn HN, Raphael E, Krupp K, Ravi K, Nebhrajani R, Arun A, Reingold AL, Riley LW, Klausner JD (2015) Identification of culturable vaginal Lactobacillus species among reproductive age women in Mysore, India. J Med Microbiol 64(6):636–641
Martinez FAC, Balciunas EM, Converti A, Cotter PD, de Souza Oliveira RP (2013) Bacteriocin production by Bifidobacterium spp. A review. Biotechnol Adv 31(4):482–488
Mastromarino P, Brigidi P, Macchia S et al (2002) Characterization and selection of vaginal Lactobacillus strains for the preparation of vaginal tablets. J Appl Microbiol 93(5):884–893
Mayanagi G, Kimura M, Nakaya S, Hirata H, Sakamoto M, Benno Y, Shimauchi H (2009) Probiotic effects of orally administered Lactobacillus salivarius WB21-containing tablets on periodontopathic bacteria: a double-blinded, placebo-controlled, randomized clinical trial. J Clin Periodontol 36:506–513
McFarland LV (2015) From yaks to yogurt: the history, development, and current use of probiotics. Clin Infect Dis 60(Suppl 2):S85–S90
Mekkes MC, Weenen TC, Brummer RJ et al (2014) The development of probiotic treatment in obesity: a review. Benef Microbes 5(1):19–28
Mercenier A, Hols P, Roussel Y, Perez-Martinez G, Buesa J, Wilks M et al (2004) Screening and construction of probiotic strains with enhanced protective properties against intestinal disorders. Microb Ecol Health Dis 16(2–3):86–95
Meurman JH (2005) Probiotics. Do they have a role in oral medicine and dentistry? Eur J Oral Sci 113:188–196
Morris PS (2009 Feb) Upper respiratory tract infections (including otitis media). Pediatr Clin N Am 56(1):101–117
Narva M, Halleen J, Väänänen K, Korpela R (2004) Effects of Lactobacillus helveticus fermented milk on bone cells in vitro. Life Sci 75(14):1727–1734
Nase L, Hatakka K, Savilahti E et al (2001) Effect of longterm consumption of a probiotic bacterium, Lactobacillus rhamnosus GG, in milk on dental caries and caries risk in children. Caries Res 35:412–420
Nguyen TL, Park CI, Kim DH (2017) Improved growth rate and disease resistance in olive flounder, Paralichthys olivaceus, by probiotic Lactococcus lactis WFLU12 isolated from wild marine fish. Aquaculture 471:113–120
Oelschlaeger TA (2010) Mechanisms of probiotic actions–a review. Int J Med Microbiol 300 (1):57–62
Ohashi Y, Nakai S, Tsukamoto T et al (2002) Habitual intake of lactic acid bacteria and risk reduction of bladder cancer. Urol Int 68(4):273–280
Ohtani N, Yoshimoto S, Hara E (2014) Obesity and cancer: a gut microbial connection. Cancer Res 74(7):1885–1889
Okumura R, Takeda K (2018) Maintenance of intestinal homeostasis by mucosal barriers. Inflamm Regen 38:5. https://doi.org/10.1186/s41232-018-0063-z
Pangsomboon K, Kaewnopparat S, Pitakpornpreecha T, Srichana T (2006) Antibacterial activity of a bacteriocin from Lactobacillus paracasei HL32 against Porphyromonas gingivalis. Arch Oral Biol 51:784–793
Parvez S, Malik KA, Ah Kang S, Kim HY (2006) Probiotics and their fermented food products are beneficial for health. J Appl Microbiol 100(6):1171–1185
Pereira DI, McCartney AL, Gibson GR (2003) An in vitro study of the probiotic potential of a bile-salt-hydrolyzing Lactobacillus fermentum strain, and determination of its cholesterol-lowering properties. Appl Environ Microbiol 69(8):4743–4752
Persson S, Edlund MB, Claesson R, Carlsson J (1990) The formation of hydrogen-sulfide and methyl mercaptan by oral bacteria. Oral Microbiol Immunol 5:195–201
Petrova MI, Lievens E, Malik S, Imholz N, Lebeer S (2015) Lactobacillus species as biomarkers and agents that can promote various aspects of vaginal health. Front Physiol 6:81

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V et al (2017) Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev

Plaza-Díaz J, Ruiz-Ojeda FJ, Vilchez-Padial LM, Gil A (2017) Evidence of the anti-inflammatory effects of probiotics and synbiotics in intestinal chronic diseases. Nutrients 9(6):555

Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A (2019) Mechanisms of action of probiotics. Adv Nutr 10(Suppl_1):S49–S66

Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B (2001) Oral probiotics can resolve urogenital infections. FEMS Immunol Med Microbiol 30(1):49–52. https://doi.org/10.1111/j.1574-695X.2001.tb01549.x

Reid G, Jana J, Tom Sebulsky M, McCormick JK (2003) Potential uses of probiotics in clinical practice. Clin Microbiol Rev 16(4):658–672

Reid G, Kim SO, Kohler GA (2006) Selecting, testing and understanding probiotic microorganisms. FEMS Immunol Med Microbiol 46:149–157

Riaz Rajoka MS, Zhao H, Lu Y, Lian Z, Li N, Hussain N, Shao D, Jin M et al (2018) Anticancer potential against cervix cancer (HeLa) cell line of probiotic Lactobacillus casei and Lactobacillus paracasei strains isolated from human breast milk. Food Funct 9:2705–2715

Riccia DN, Bizzini F, Perilli MG, Polimeni A, Trinchieri V, Amicosante G, Cifone MG (2007) Antiinflammatory effects of Lactobacillus brevis (CD2) on periodontal disease. Oral Dis 13 (4):376–385

Roberfroid M (2007) Prebiotics: the concept revisited. J Nutr 137:830S–837S

Ronai D, Iglesias-Ussel MD, Fan M, Li Z, Martin A, Scharff MD (2007) Detection of chromatin-associated single-stranded DNA in regions targeted for somatic hypermutation. J Exp Med 204 (1):181–190

Rosa LS, Santos ML, Abreu JP, Balthazar CF, Rocha RS, Silva HL et al (2020) Antiproliferative and apoptotic effects of probiotic whey dairy beverages in human prostate cell lines. Food Res Int 137:109450

Rosenbloom RG, Tinanoff N (1991) Salivary Streptococcus mutans levels in patients before, during and after orthodontic treatment. Am J Orthod Dentofac Orthop 100:35–37

Rossi, F., Busetto, M., & Torriani, S. (2007). Isolation of aminopeptidase N genes of food associated propionibacteria and observation of their transcription in skim milk and acid whey. Antonie van Leeuwenhoek,91(1), 87-96

Sanders ME (2008) Probiotics: definition, sources, selection, and uses. Clin Infect Dis 46:S58–S61

Santagati M, Scillato M, Patane F, Aiello C, Stefani S (2012) Bacteriocin-producing oral streptococci and inhibition of respiratory pathogens. FEMS Immunol Med Microbiol 65:23–31

Schaefer L, Auchtung TA, Hermans KE, Whitehead D, Borhan B, Britton RA (2010) The antimicrobial compound reuterin (3-hydroxypropionaldehyde) induces oxidative stress via interaction with thiol groups. Microbiology 156(6):1589–1599

Schultz M, Sartor RB (2000) Probiotics and inflammatory bowel diseases. Am J Gastroenterol 95 (1):S19–S21

Sharma R, Tobin P, Clarke SJ (2005) Management of chemotherapy-induced nausea, vomiting, oral mucositis, and diarrhoea. Lancet Oncol 6:93–102

Sharma A, Tilak T, Bakhshi S, Raina V, Kumar L, Chaudhary SP, Sahoo RK, Gupta R, Thulkar S (2017) Lactobacillus brevis CD2 lozenges prevent oral mucositis in patients undergoing high dose chemotherapy followed by haematopoietic stem cell transplantation. ESMO Open 1(6): e000138

Sheu BS, Wu JJ, Lo CY, Wu HW, Chen JH, Lin YS, Lin MD (2002) Impact of supplement with Lactobacillus-and Bifidobacterium-containing yogurt on triple therapy for Helicobacter pylori eradication. Aliment Pharmacol Ther 16(9):1669–1675

Shimauchi H, Mayanagi G, Nakaya S, Minamibuchi M, Ito Y, Yamaki K, Hirata H (2008) Improvement of periodontal condition by probiotics with Lactobacillus salivarius WB21: a randomized, double-blind, placebocontrolled study. J Clin Periodontol 35:897–905

Shreiner AB, Kao JY, Young VB (2015) The gut microbiome in health and in disease. Curr Opin Gastroenterol 31(1):69–75

Sichetti M, De Marco S, Pagiotti R, Traina G, Pietrella D (2018) Anti-inflammatory effect of multistrain probiotic formulation (L. rhamnosus, B. lactis, and B. longum). Nutrition 53:95–102
Stamatova I, Kari K, Meurman J (2007) In vitro evaluation of antimicrobial activity of putative probiotic lactobacilli against oral pathogens. Int J probiotics and Prebiotics 2:225
Sullivan Å, Nord CE (2005) Probiotics and gastrointestinal diseases. J Intern Med 257(1):78–92
Tasli L, Mat C, De Simone C, Yazici H (2006) Lactobacilli lozenges in the management of oral ulcers of Behçet's syndrome [published correction appears in Clin Exp Rheumatol. 2007 May–Jun;25(3):507–8]. Clin Exp Rheumatol 24(5 Suppl 42):S83–S86
Taverniti V, Minuzzo M, Arioli S, Junttila I, Hämäläinen S, Turpeinen H, Mora D, Karp M, Pesu M, Guglielmetti S (2012) In vitro functional and immunomodulatory properties of the Lactobacillus helveticus MIMLh5-streptococcus salivarius ST3 association that are relevant to the development of a pharyngeal probiotic product. Appl Environ Microbiol 78(12):4209–4216
Teughels W, Loozen G, Quirynen M (2011) Do probiotics offer opportunities to manipulate the periodontal oral microbiota? J Clin Periodontol 38:159–177
Vanderpool C, Yan F, Polk BD (2008) Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. Inflamm Bowel Dis 14(11):1585–1596
Wade WG (2013) The oral microbiome in health and disease. Pharmacol Res 69(1):137–143
Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D et al (2017) Antioxidant properties of probiotic bacteria. Nutrients 9(5):521
Yazdi MH, Mahdavi M, Setayesh N, Esfandyar M, Shahverdi AR (2013) Selenium nanoparticle-enriched Lactobacillus brevis causes more efficient immune responses in vivo and reduces the liver metastasis in metastatic form of mouse breast cancer. Daru 21(1):33
Ye S, Lowther S, Stambas J (2015) Inhibition of reactive oxygen species production ameliorates inflammation induced by influenza A viruses via upregulation of SOCS1 and SOCS3. J Virol 89 (5):2672–2683
Yoo JI, Shin IS, Jeon JG, Yang YM, Kim JG, Lee DW (2019) The effect of probiotics on halitosis: a systematic review and meta-analysis. Probiotics Antimicrob Proteins 11(1):150–157. https://doi.org/10.1007/s12602-017-9351-1
Young A et al (2003) Inhibition of orally produced volatile sulfur compounds by zinc, chlorhexidine or cetylpyridinium chloride- effect of concentration. Eur J Oral Sci 111(5):400–404
Zarco MF, Vess TJ, Ginsburg GS (2012) The oral microbiome in health and disease and the potential impact on personalized dental medicine. Oral Dis 18(2):109–120
Zheng N, Guo R, Yao Y, Jin M, Cheng Y, Ling Z (2019) Lactobacillus iners is associated with vaginal dysbiosis in healthy pregnant women: a preliminary study. Biomed Res Int 2019:6079734

12 Applications of Microbes in Soil Health Maintenance for Agricultural Applications

Awais Ali Aslam, Maria Shamim, Muhammad Shahid Nazir, Mohammad Ishtaiq, Majid Niaz Akhtar, Zulfiqar Ali, Zaman Tahir, and Mohd. Azmuddin Abdullah

Abstract

Agriculture is integral to the world economy and as a means to feed the world populace. The priorities can be multipronged including to overcome famine and eradicate poverty; for economic diversification, industrialization, and investments; and to ensure sustainable resource utilization and environmental management. The excessive utilization of chemical fertilizers, though managed to improve the yield, also kills the pests, weeds, and microflora, with destructive impact on the natural ecosystem. Plant-associated microbes have great potentials to assist in enhancing the yield and plant resilience against pests and diseases. Genetic technology using microorganisms and their metabolites has been applied to increase the nutrient uptake and productivity and control plant stresses and responses to pests. Microbiological tools could enhance environmental health and promote agricultural sustainability. However, the side effects of microbial residents and contaminants must be addressed. This chapter discusses the functions and contributions of microorganisms in promoting health and fertility

A. A. Aslam · M. Shamim · M. S. Nazir (✉) · M. Ishtaiq
Department of Chemistry, COMSATS University Islamabad (CUI), Islamabad, Pakistan
e-mail: shahid.nazir@cuilahore.edu.pk

M. N. Akhtar
Department of Physics, Muhammad Nawaz Sharif University of Engineering and Technology (MNSUET), Multan, Pakistan

Z. Ali · Z. Tahir
Department of Chemical Engineering, COMSATS University Islamabad (CUI), Islamabad, Pakistan

M. A. Abdullah (✉)
Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

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of soil. Different types of microbial sources and strains are highlighted. The use of natural and biological-based fertilizers, pesticides, herbicides, and insecticides in agriculture is elaborated. The importance of microbiome for sustainable agriculture and soil and environmental health is discussed.

Keywords

Agricultural soil · Microbes · Soil health · Biofertilizers · Biopesticides · Bioherbicides · Bioinsecticides · Bioremediation · Microbiome · Sustainable agriculture

Abbreviations

BI	BioDesign Institute
CEB	Center for Environmental Biotechnology
ISR	Induction of Systemic Resistance

12.1 Introduction

Human population is expected to reach nine billion by the year 2050, which may lead to the need to increase the food yield by 70%, from the current productivity. To meet the increasing demand of food supply, the quality of the crop and output must be enhanced, and this is very much dependent on the soil health for agricultural applications. The interactions between plants and the ecosystems where the biodiversity and microbial communities can thrive in symbiosis must be understood. Conservation of soil health ensures steady supply of food (Atapattu and Kodituwakku 2009). Soil health refers to the soil ecosystem and the ability of the soil to adapt to agronomic activities and various environmental conditions and also enhance the crop yield and improve plant health (Kibblewhite et al. 2008; Lal 2016). The fertility of the soil is dependent on the physical, chemical, and biological factors. The physical characteristics of the soil include the texture, structure, and architecture and water retention capability. The chemical conditions of the soil include the salinity, acidity, and alkalinity, while the biological factor constitutes the microbial communities residing in the soil (Johns 2017). Microbes are the most diversified groups of the organisms making up more than half of the biomass on earth (Bar-On et al. 2018).These microbes have significant functions in sustaining the biogeochemical cycles, and the plants have significant contributions to maintain the food chain by utilizing the microbes present in the soil (Curtis and Sloan 2005).

The microbial population includes microalgae, cyanobacteria, fungi, actinomycetes, bacteria, and lichens. These microbiota are present in the biological soil crust (BSC), the uppermost part of the earth, and could play a major role in enhancing agricultural productivity (Manjunath et al. 2016). Photosynthetic carbon is deposited in the plant roots. The root system and the rhizospheric zone are therefore important areas of microbial activities and their interactions with the plants. Microbiota acting as bioinoculants promote plant growth by establishing symbiosis in the root system. Among the beneficial microbiota for plants are plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF) (Singh et al. 2017). Diversified metabolic activities of various microbes contribute towards the provision of major elements such as phosphorus, potassium, and carbon, influencing the soil characteristics and ultimately the crop yield. The diversity and abundance of the microbial resources are therefore important to be conserved. The soil microbes, especially the bacteria and fungi, involve in the recycling of the nutrients and the detoxification and recycling of wastes for soil health and agricultural practices (Singh et al. 2017; Aislabie et al. 2013; De Vero et al. 2019).

12.2 Microbial Sources

12.2.1 Microalgae and Cyanobacteria

Microalgae and cyanobacteria are "beneficial microbes" and important components of the food web, having the ability to grow in extreme environments. Microalgal species such as *Chlorococcum*, *Chlamydomonas*, and *Scenedesmus* produce polysaccharides, while cyanobacteria are known specifically for having nitrogen-fixing capacities (Singh et al. 2011). The importance of cyanobacteria, as illustrated in Fig. 12.1, includes in the recycling of nutrients, decomposition of organic wastes, degradation of toxic chemicals, and as producers of metabolites such as enzymes, hormones, etc. which are essential for soil health and plant growth (Mallavarapu et al. 2000; Renuka et al. 2018).

Excessive farming practices make agricultural lands more vulnerable and are the leading cause of decrease in soil fertility, with 30% of the farmable land undergoes soil degradation. Among all soil microbes, 27% of the total biomass contribution is from microalgae (Abinandan et al. 2019). During climatic changes, green algae and cyanobacteria are responsible for the production of organic content. High organic matter in the soil can be the result of algal cell lysis which releases exopolysaccharides, leading to increased oxidizable carbon in the soil which is the necessary constituent of organic matter. This organic matter is the source of carbon available to plants and also for the growth of soil microorganisms. In order to prevent the leaching of minerals, algae are in competition with higher plants. A few species of *Cyanobacteria* like *Nostoc* colonize root systems of plants which ease the transportation of minerals and metabolites as well (Osman et al. 2010; Li et al. 2010; Svircev et al. 1997).

Cyanobacterial biofilms when used under non-flooded conditions aid in nitrogen fixation and solubilization of phosphate, necessary for plant growth (Prasanna et al. 2014). Cyanobacteria is responsible for the production of oxidizable and soluble carbon along with enhanced paddy yield, in post-harvested soil. Besides carbon residues, cyanobacterial inoculation promotes grain yield, which is responsible for

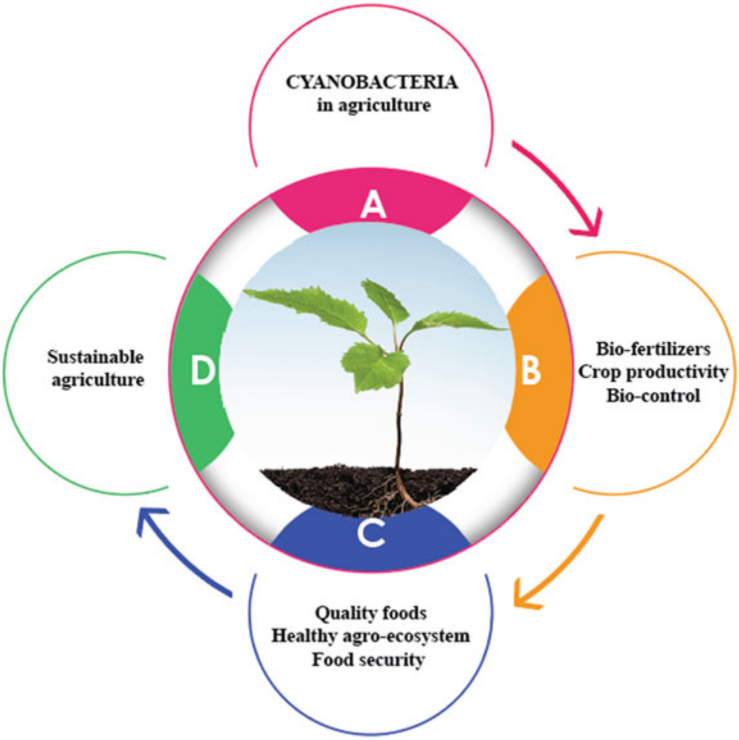

Fig. 12.1 Importance of cyanobacteria in agriculture

plant growth, without utilizing manure. Next to nitrogen is the organic phosphorus present in the upper layer of the soil, which is 20–80% of the total phosphorus (de Mulé et al. 1999; Steffens et al. 2010). Phosphate-solubilizing bacteria convert insoluble phosphate to soluble form, which is readily taken up by the plants. However, soil microalgae incorporate inorganic phosphates and convert it into polyphosphates by making it readily available to plants. Furthermore, cyanobacteria can also produce enzymes that are responsible for the degeneration of inorganic phosphate, making it available to plants. They can also solubilize mineral rock having phosphates in it by producing phthalic acid (Sharma et al. 2013; Whitton et al. 1991).

Cyanobacterial biofilm fertilizers have caught attention owing to the lesser quantity of chemical fertilizers used and also because of lower cost. Cyanobacterial films made of *Anabaena-Trichoderma viride* enhance maize hybrid production by conserving 60 kg per ha and raise accessible N_2 in soil from 20 to 60 kg/hectare (Prasanna et al. 2015). Likewise, biofilms utilizing different species like *Anabaena-Serratia* and *Anabaena-Pseudomonas* result in phosphate activities and acetylene reduction in wheat cultivation. Biofilms increase the content of soil micronutrients like iron (13–46%) and zinc (15–41%) (Adak et al. 2016).

30% of the total land undergoes degradation, and the degraded soils contain saline, alkaline, and acid sulfate which can be improved with the use of fertilizers. These, however, can have harmful effects on soil health. Soil characteristics can be restored with the application of microbes. Combinations of cyanobacterial modifications and natural chemical additives can have big impacts on the soil stability along with improving its water holding capacity (Nkonya et al. 2016; Xiong et al. 2018). Soil health is about maintaining the balance between soil organisms and their surroundings. Soil algae synthesize some compounds which are hydrophobic in nature and may exhibit water repellence characteristics. Algal metabolites which are hydrophobic in nature, help to halt soildegradation by binding the mineral particles (Doerr et al. 2000; Malam Issa et al. 2009).

12.2.2 Fungi

Fungi are among the most significant class of microbes which are beneficial for the growth and productivity of plants and crops (Karun et al. 2018). Useful fungi assist plant development by enhancing solubility of micronutrients (Zn, P,K) and release of plant growth regulators (gibberellins, auxin, ethylene, and cytokinin) and the release of enzymes (gluconase, cellulases, and glycosidase which aid in cell wall lysis) (Ahmed Nouh 2019; Pandya and Saraf 2010). They degrade the soil organic matter and maintain the nutrient and carbon balance. Certain species of fungi are sorbents of harmful metals like Cd, Cu, Hg, Pb, and Zn and entrap these toxic metals into their fruit-bearing bodies (Žifčáková et al. 2016; Baldrian 2003).

Soil fungi, depending on their functions, are categorized into three types: as biological controllers, as regulators of ecosystem, and for the degradation of organic waste matter and bioconversion of compounds (Gardi et al. 2009). Species which act as regulators of ecosystem regulate physiological processes in soil and determine the soil structure formation. Biological controllers maintain the progression of various organisms present in the plants' soil as mycorrhizal fungi regulate uptake of nutrients and enhance plant growth (Bagyaraj and Revanna 2017). Fungal communities influence the growth of plant through mechanisms like mutualism and cyclization effect, and availability of nutrients. Fungi also stabilize organic matter of the soil, necessary for soil health, and play important part in nitrogen fixation, production of hormone, and root pathogen control (Wagg et al. 2014; Hannula and van Veen 2016; Treseder and Lennon 2015; Jayne and Quigley 2014; Baum et al. 2015).

The health of soil is determined by its capability to sustain ecosystem, maintain biological productivity, and improve the well-being of plants and other living organisms (humans and animals). Biodiversity of soil fungal has major role in upgrading the quality of soil and agricultural productivity. Fungi transfer nutrients necessary for plant development through the decomposition of organic matter. They shield the plants against pathogenic microbes which otherwise would affect the soil health. Soil management is therefore essential to ensure future production of food and to minimize soil degradation. Fungal communities are responsible in establishing the plant biodiversity, ecosystem, and productivity (Wagg et al. 2014;

Frac et al. 2015; Abawi and Widmer 2000). Arbuscular mycorrhizal fungi (AMF) are among the useful microbes in soils significant for agricultural purposes. Inoculation with AMF has major contribution towards increasing the crop yield. AMF symbiosis improves root and plant growth, promotes soil architecture, encourages nutrient cyclization, and improves plant resistance to stressful conditions, and enhances uptake of diffusion-limited nutrients like P, Zn and Cu (Smith and Read 2010; Thilagar and Bagyaraj 2013).

Some antagonistic fungi like *Glomus* or *Trichoderma* species are used to fight plant diseases caused by fungal pathogens. *Trichoderma* sp. (*Pythium*, *Phoma*, *Fusarium*, *Alternaria*, *Sclerotinia*, *Botrytis*, etc.) could inhibit over 60% of pathogenic species on plants such as cucumbers, peppers, cabbages, tomatoes, cereals, and ornamentals. Various species of *Trichoderma* such as *T. virens*, *T. atroviride*, *T. asperellum*, *T. harzianum*, and *T. viride* play important role in biological control and are termed as biostimulants for agricultural crops. Other contributions of fungi necessary for plant and soil health are inoculation by microbial association of AMF with PGPR and other microbes important in nitrogen fixation and phosphorus solubilization. AMF and PGPR influence the development of plant and microbial diversity, and soil activity (Dawidziuk et al. 2016; López-Bucio et al. 2015).

Genera *Fusarium*, *Rhizoctonia*, *Phytophthora*, and *Pythium* are the main associations of pathogenic fungi which are present in soil and are of much significance globally as well as on local level. Biodiversity of soil fungi and techniques to enhance the communities of beneficial fungal species are important for soil protection and sustained plant yield (Frąc et al. 2018). For example, *Beauveria bassiana* are naturally-occuring fungi and *Metarhizium anisopliae* are entomopathogenic fungi. The spores originating from these fungi germinate and nourish upon coming into contact with the target insect cuticle and kill the insect by draining its nutrients. The mycelium of *Verticillium lecanii*, an entomopathogenic fungi, releases toxin cyclodepsipeptide, termed as bassianolide, and other toxins (like dipicolinic acid), which poison scale insects, whiteflies, and aphids, leading to their death. *L. lecanii* species are employed in agriculture and horticulture as biological pesticide and control insect pests like whiteflies, aphids, etc. (Singh et al. 2017).

12.2.3 Bacteria

Bacteria, being the most abundant organisms on earth, could easily make up more than 10^{11} (100 billion) cell numbers in one teaspoon of agricultural soil. As an important group of soil microbes, bacteria perform variety of different functions in recycling of nutrients, water dynamics, and disease alleviation. Some bacteria release substances which aid in binding of soil particles and transform them into small aggregates and thereby influence water mobility. These aggregates promote water penetration and water holding capacity of soil. In addition, various bacterial species fight against pathogens in plant roots (Knudsen 2006).

Depending on functions, bacteria fall into four categories. Majority of bacteria are decomposers which convert soil organic matter into other forms beneficial to the organisms in soil. Besides this, they decompose pesticides and contaminants in soil, thereby increasing soil health. Mutualists constitute the second group of bacteria that establish associations with plants. Nitrogen-fixing bacteria are the best among mutualists. Pathogenic bacteria comprise the third group which include the following species: *Zymomonas*, *Erwinia*, and few species from *Agrobacterium*. Lithotrophs, also called chemoautotrophs, are the fourth group which make use of the N, S, Fe, and H, rather than the carbon compounds, and play important part in nitrogen cycling and detoxification of contaminants (Ingham et al. 1985). *Rhizobium* genus involves nitrogen-fixing bacteria through symbiotic associations and includes *Rhizobium leguminosarum*, *Rhizobium tripoli*, *Rhizobium phaseoli*, *Rhizobium lupine*, *Rhizobium meliloti*, and *Rhizobium japonicum* (Young et al. 2006).

PGPR (the term introduced by Joe Kloepper in the 1980s) include *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Pseudomonas putida*. PGPR are responsible for inducing resistance in plants against viral, bacterial, and fungal diseases and other insects, and this mechanism is called Induced Systemic Resistance (ISR). In agriculture and horticulture, *Bacillus polymyxa* is employed as inoculants where the plants are shielded by these biofilms from pathogens. Synergism between bacteria and plant roots changes the physical characteristics of the root hairs (Lavakush et al. 2014; Yegorenkova et al. 2013).

Pseudomonas fluorescens, a non-pathogenic PGPR, enhance plant development, control damage caused by pathogens, and stabilize plant roots. They have tremendous influence on plant development utilizing direct or indirect mechanisms. *Kocuria turfanensis* isolated from rhizospheric soil is capable of solubilizing phosphate and producing indole-3-acetic acid (IAA, a plant hormone important in microbe-plant interactions) (Prasad et al. 2015). *Frateuria aurantia*, a Potassium-mobilizing *Proteobacteria*, has the ability to mobilize usable potash to the plant roots or soil. It can perform its function in any type of soil, more specifically in soil low in potassium content, thereby enhancing the soil health (Johansen et al. 2005).

12.3 Applications of Microbes

12.3.1 Plant Growth Regulators

Plant growth regulators, either synthetic or naturally produced hormones, are important in agriculture to control plant growth and development. These may not be hazardous if utilized as per the recommendation at the right dosage. Microbes residing in the rhizosphere of the plants also have the ability to produce and supply auxin, a regulator of plant growth, as secondary metabolites. The plant morphological changes can be the consequence of the various ratios of plant hormones produced by the rhizosphere bacteria and roots. The production of compounds which possess physiological impacts on the development and growth of plants involves different soil microbes like fungi, bacteria, and algae (Ahemad and Kibret 2014). These include by transforming the plant growth root structure to promote rhizobacteria

(PGPR) and promoting phytohormones like IAA, cytokinins, and gibberellic acid and the synthesis of metabolites such as antimicrobials. There are many PGPR and symbiotic, pathogenic, and free rhizobacterial species which produce auxins in the rhizosphere to induce and increase root formation (Han et al. 2005). Many beneficial fungi are associated with the antagonistic effects on the pathogenic fungi, by synthesizing antibiotics, and involved in the plant defense mechanisms by infecting the spores, hyphae, or sclerotia of pathogenic fungi, thereby taking part in biological control (Mejía et al. 2008). A number of degradable enzymes are produced, e.g., cytinases, gluconates, and proteins, as biological control agents. Many *Trichoderma* strains have colonized various plant roots, thereby importantly improving the development and growth of plant. In *Arabidopsis*, *Trichoderma virens* promotes both biomass and lateral root growth via an auxin-dependent mechanism (Contreras-Cornejo et al. 2009). The synthesis of *Sm1* (small protein 1), an elicitor protein, is normally linked to the promotion of the systemic and local resistance (Živković et al. 2010).

Phosphorus is obtained by the plants from the earth in the form of phosphate. The mobility of this element is very less in the plant unlike other macronutrients. The role of phosphorus-soluble microorganisms (PSMs) is therefore significant in phosphorus-based nutrition, increasing their supply to plants by releasing organic and mineral soil P pools through solvent and mineralization (Kalayu 2019). The mechanisms which are involved in the solubility of phosphorus include by reducing the soil pH through microbial organic acids and mineralization of organic phosphorus by acid phosphatase. Maximum adaptability of phosphorus-soluble bacteria (PSB) is feasible in association with other mycorrhizal fungi or beneficial bacteria (Satyaprakash et al. 2017). Bacteria are found to be more capable than fungi for phosphorus solubility (Sharma et al. 2013). Advantageous microflora, e.g., *Penicillium*, produces an organic acid that diffuses the phosphate in the soil to be easily utilized by the plant roots. In soil bacterial communities, heterozygous species of *Bacillus* and *Pseudomonas*, *Enterobacter*, and endosymbiotic *Rhizobia* have been reported as productive types of phosphate solvents. The latest estimate is that PSB is around 1–50% in common soils, whereas phosphate-soluble fungi make up about 0.1–0.5% of the population (Panhwar et al. 2011).

Potassium (K) is a significant component of plant nutrition which performs numerous biological activities to sustain the quality of plant growth. Potassium is normally found in soil in large amount. The total potassium content on the top surface of the soil is in the range of 3000–1,000,000 kg/ha (Bertsch and Thomas 1985). There are four distinct forms of potassium in water: soluble, interchangeable, non-interchangeable, and structural or mineral soils (Sparks and Huang 1985). The quantity of potassium delivered by the soil depends on the variation in the parameters of soil, e.g., pH, texture, moisture content, soil tiling, oxygen level, and temperature, and topographical and biochemistry (Basak and Biswas 2008). Feldspars are a group of rocks made up of mica, potash, or rock phosphate, where potassium can be extracted through microbial reactions and plants, converting the unavailable K organic acids into available form and secreted during the nutrient cycle (Sessitsch et al. 2013).

12.3.2 Volatile Organic Compounds (VOCs)

Compounds possessing low molecular weight (<300 g/mol) such as alcohol, ketones, aldehydes, and hydrocarbons are among the common VOCs (Choudhary et al. 2008). These may be a signaling response between plants and the microbes, and the VOCs typically exhibit coordinated responses to the numerous stimuli in plants and microorganisms (Ortíz-Castro et al. 2009). VOCs are highly vaporizing under normal conditions, and they enter the atmosphere resulting in an increase in vapor pressures. *Arabidopsis* rhizosphere has been detected with VOC emission, attributable to the biological stressors (Steeghs et al. 2004). Many volatile substances, e.g., alcohol, acids, ketones, aldehydes, terpenes, and esters, are constitutionally produced or specifically induced due to different negative or positive interactions with microorganisms. The excretion of VOCs, e.g., 2,3-butanediol and acetoin, from PGPR strains such as *Bacillus amyloliquefaciens*, *B. subtilis*, and *Enterobacter cloacea*, enhance the development of *Arabidopsis thaliana* significantly with the production of bioactive VOCs (Ryu et al. 2004). Rhizobacterial strains emit VOCs which can behave as signaling molecules to the plant to react with microorganism, and this ultimately triggers the response of plant towards the colonizing microflora. Plant volatiles with lower molecular mass, e.g., green leaf components and terpenes, behave as signaling molecules for various organisms living at different trophic levels (Farmer 2001). It is important to understand the mechanism of VOCs against the pathogens in plants, and the building up of volatile components in the plant-rhizobacteria system and in nature.

12.3.3 Biotic Elicitors

Elicitors are involved in the mechanisms of plant defense (Thakur and Sohal 2013). Elicitor molecules such as methyl jasmonate, salicylic acid, and Nitric oxide (NO), induce the production of secondary metabolites, e.g., phytoalexins, glucosinolates, and alkamides, as stress responses, for example, to microbial pathogens (Yang et al. 1997). Jasmonic acid and methyl esters of jasmonic acid are signalling transducers in the cell suspension cultures of *Rauvolfia canescens* and *Eschscholzia californica* upon treatment with yeast elicitor (Roberts and Shuler 1997). Jasmonic acid elicitor reduces cell growth of *Morinda elliptica* but with enhanced anthraquinones, total carotenoids, vitamin C and E, and lipid peroxidation and hydrogen peroxide levels. With 6 days treatment, glutathione reductase enzymes are elevated, while ascorbate peroxidase level is only half that of control, and catalase is completely reduced (Chong et al. 2005). The molecular basis of signalling exchange between microbial pathogens and the hosts necessitates characterization and purification of specific elicitor-binding proteins which ultimately lead to the activation of defence mechanism.

12.3.4 Bioremediation

Bacteria, archaea, and fungi play an important role in bioremediation to metabolize pollutants. Microorganisms break down and eat complex molecules, convert them into innoxius, natural substances (Kumar et al. 2011), thus ultimately dispose of the pollutants rapidly and reduce the environmental pollution. The organisms employed in the bioremediation process are known as bioremediators and a process in which a fungi is utilized to remediate certain area is called mycoremediation (Rhodes 2014). The fungal mycelium secretes acids and extracellular enzymes that are capable of breaking down the plant fibers including cellulose and lignin. Wood thin fungi are specifically efficient in the decomposition of harmful constituents of petroleum and aromatic pollutants such as chlorinated compounds (Rhodes 2014). Mycofiltration removes water wastes and microorganisms using fungal mycelia to filter the soil. Various REDOX reactions are generally performed by the bioremediators for the oxidation of toxic contaminants. However, this may require the right microbial species to oxidize specific pollutant to achieve effective bioremediation.

During drought, plants regulate physiological responses such as the increase in abscisic acid content, accumulation of specific metabolites, expression of aquaporin, and vacuolar H-pyrophosphatase to maintain cell homeostasis through osmotic adjustment (Gornall et al. 2010). Concentrations of ethylene reach higher levels, which inhibit the plant growth and thereby enhance the root-to-shoot ratio. Therefore, the large-scale root system increases the area of water absorption. There are also accumulations of Reactive Oxygen Species (ROS) that may significantly affect the cell integrity, function, and plant survival. Optimal microbial colonization may involve the endosphere and the rhizosphere where mycorrhizal fungi and plant-growth promoting bacteria (PGPB) can modulate bacterial physiological responses (Vacheron et al. 2013) and thereby help to enhance the plant tolerance under severe environmental conditions. Pot and in vitro experiments have confirmed the ability of endosphere and rhizosphere bacteria to improve tolerance of plant during growth and stress. Microbial vaccines, for instance, increase growth of plant up to 40%, indicating the potential of PGP microorganisms in agriculture (Pérez-Montaño et al. 2014). The role of microorganisms in the adaptation of plants towards drought may depend on the composition of microbiome which varies greatly in a specific ecological state (Marasco et al. 2012), as it also depends on the taxonomic characteristic of the respective plant species.

Adventitious microbes can inhibit the development of phytopathogens by competing for nutrients and space, thus reducing the nutrient availability to the pathogens (Marasco et al. 2012). Disease-resistant soil microflora is typically controlled via hostile microbes that are capable of creating a wide type of antibiotics (Mohseni et al. 2013). *Penicillium*, *Aspergillus*, *Trichoderma*, and the antagonistic actinomycetes are producers of various antibiotics. Many species of *Trichoderma* are strong antagonistic invaders and the antibiotics produced by hostile microorganisms can have biological and biochemical impacts on plant pathogens present in the soil (Rahul et al. 2014).

12.3.5 Biocontrol

Microbial biopesticides and biofertilizers are the latest developments in the field of eco-friendly agriculture (Bhardwaj et al. 2014). Living microorganisms in biofertilizers are applied to the surface of plant, soil, or seeds, to colonize rhizome, and supply primary nutrients to the host (Tanti 2015). Biopesticides are the microorganisms that generate, acquire, and induce systemic resistance against the pathogens, as antibiotics, HCNs, siderophores, or hydrolytic enzymes. Native microorganisms are commonly used for the development of bioinsecticides and biopesticides as well as for pest and disease control to promote plant growth. A bacterium known as *Rhizobium* can also be used as a biofertilizer in agriculture.

Rhizobia is known for its capability to make symbiotic interactions with leguminous plants by colonizing root nodules (Bagali 2012; Wang and Martínez-Romero 2000). Nitrogen is reduced by bacteria to produce ammonia and this can provide for efficient rhizobium strains to the soil, to enhance the soil productivity and improve the growth of plant by improving nutrient availability. *Rhizobium* biofertilizer in legumes could substitute chemical N_2 by 30–35% when *Rhizobium* biofertilizer is applied together with the chemical fertilizers (Mia et al. 2010). Similarly, *Acetobacter*, *Rhizobium*, *Azorhizobium*, *Aspergillus*, *Azospirillum*, *Azotobacter*, *Penicillium*, *Bacillus*, *Pseudomonas*, etc. are also effective in promoting plant growth. However, the scientific synthesis and utilization of microbial formation is significant during the development of agriculture sustainability.

The use of competitive natural rivals to reduce the number of pathogens is known as biological control. Natural rivals include antagonists and competing microbes which destroy or prevent living pathogenic organisms. The biological control agents less harmful, simpler, and less expensive than the chemical pesticides. Bacteria are commonly introduced in the roots and seeds of plants to control different microbial attacks. For example, non-pathogenic *Streptomyces* strains control the crust of the potato caused by the scab (Neeno-Eckwall and Schottel 1999). The different functions of rhizosphere microbes are illustrated in Fig. 12.2. Antagonistic activity of *Streptomyces* is linked to the production of secondary antifungal metabolites and extracellular hydrolytic enzymes. The interaction of *Pseudomonas fluorescens* as a biocontrol against the soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica,* is attributable to the production of 2,4-diacetylphloroglucinol (Cronin et al. 1997).

The management of plant nutrient may involve the microbes enhancing the availability of the macro- and micronutrients in the rhizosphere through the microbial-community consortium. These include associative N_2 fixation, reduced levels of ethylene, and the assembly of phytohormones, siderophores and regulators for development and VOCs emission, thus promoting nutrient uptake and mycorrhizal function (Rana et al. 2012). Direct stimulation involves the synthesis of phytohormones like gibberellin, cytokinin, auxin, and biological nitrogen fixation, such as dissolving minerals, e.g., Fe and P, elevation of enzymes and siderophores, and systemic resistance. *Bacillus*, *Aspergillus*, *Trichoderma*, *Streptomyces*, *Pseudomonas*, and *Beauveria* are known strains as biological control agents for plants. The

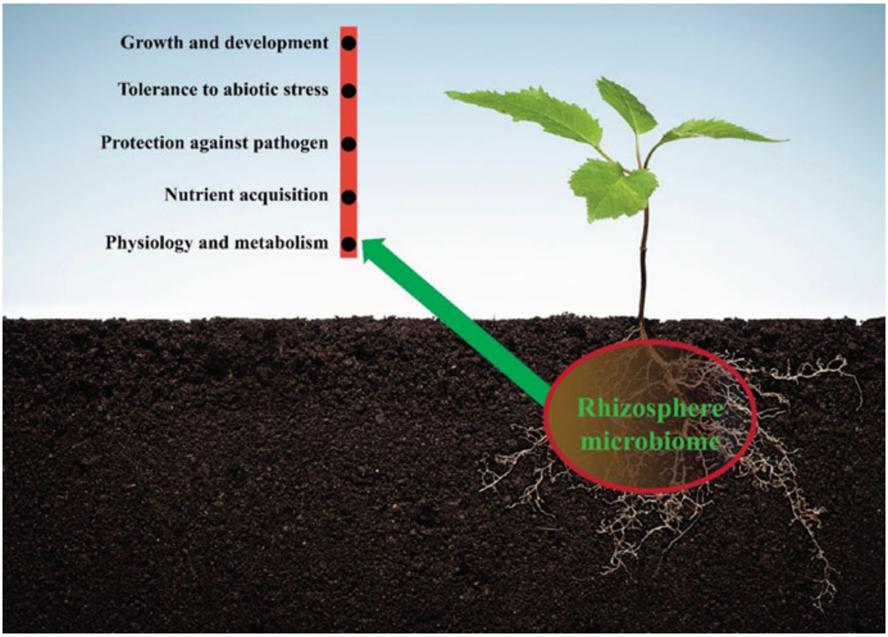

Fig. 12.2 Different functions of rhizosphere microbes in agriculture

mechanisms include their antagonistic activity, immunity, synthesis of elicitor molecules, and environmental stress. Another mechanism for crop control is phytoextraction (Rana et al. 2012). Phytoextraction utilizes minor element accumulation in plants which aggregates contaminants in the respective tissues or cells. Once the pollutants are absorbed, the plants can be removed by cutting. The process of phytoextraction can be developed through soil modification, which increases the accessibility of trace ingredients in the soil. The bacteria associated with the plant facilitate the accessibility of small components in rhizosphere, and this is one of the established defence mechanism and stress responses in the plant-bacterial colonization and interactions (Santhanam et al. 2014) which can be of great assistance in the phytoremediation of soils polluted with trace elements.

12.3.6 Different Types of Microbes

The microbial stimulation of plant growth can be attributed to the ability for biological N2 fixing, production of plant phytohormones e.g., gibberellic acids, indole acetic acid, and cytokinins; and biological control of phytopathogens by antifungal, antibiotic, anti-bacterial or, iron-chelating agents, induction of nutrient uptake, acquired resistance of host, and improved bioavailability of minerals (Verma et al. 2019; Suman et al. 2016a; Kour et al. 2017; Yadav et al. 2016a; Lottmann et al. 2000; Huang et al. 2009). Some of these bacteria also exhibit psychrotolerant characteristic (Verma et al. 2015). The efficiency of crop productions can be improved through the applications of microorganisms in agriculture (Table 12.1).

Utilizing N_2-fixing microorganisms as biofertilizers is among the most effective, eco-friendly, and favorable methods to improve the crop product and growth. The examples of N-fixing bacteria include *Azotobacter*, *Arthrobacter*, *Azospirillum*, *Enterobacter*, *Bacillus*, *Gluconacetobacter*, *Cerattia*, *Pseudomonas*, *Herbaspirillum*, and *Klebsiella* (Table 12.1) (Elbeltagy et al. 2001; Boddey et al. 2003; Wei et al. 2014). The PGPBs are also able to transform insoluble phosphorus into a soluble form (orthophosphate). Rhizospheric B-soluble microorganisms grown in symbiosis with rice, wheat, pulses, and maize could dissolve boron (B), the mineral critical for crop quality and yields, and these include *Azotobacter*, *Burkholderia*, *Arthrobacter*, *Halolamina*, *Enterobacter*, *Pantoea*, *Citrobacter*,

Table 12.1 Plant growth-promoting microbes for agricultural applications

Microbes	Response	Strain	Ref.
Azospirillum brasilense	Affected dry weight	Sp245	Turan et al. (2012)
Azospirillum brasilense	Coleoptiles growth	Sp245	Alvarez et al. (1996)
Azospirillum lipoferum	Alleviate drought stress	AZ1, AZ9, AZ45	Arzanesh et al. (2011)
Aeromonas vaga	Plant growth	BAM-77	Jha et al. (2013)
Aeromonas hydrophila	Plant growth	MAS-765	Ashraf et al. (2004)
Aeromonas vaga	Plant growth	BAM-77	Jha et al. (2013)
Achromobacter xylosoxidans	Plant growth	249	Barra et al. (2016)
Bacillus aryabhattai	Growth and yield	BCZ17	Verma et al. (2016)
Bacillus altitudinis	Growth and yield	BNW15	Verma et al. (2016)
Bacillus endophyticus	Growth and alleviate salinity	BNW9	Verma et al. (2016)
Bacillus amyloliquefaciens	Growth and alleviate salinity	IARI-HHS2–30	Mishra et al. (2011)
Bacillus alcalophilus	Plant growth	BCZ14	Verma et al. (2016)
Bacillus amyloliquefaciens	Growth and alleviate salinity	BNE12	Verma et al. (2016)
Cellulomonas turbata	Growth and yield	AS1	Ozdal et al. (2016)
Klebsiella **sp.**	Plant growth	SBP-8	Rana et al. (2016)
Micrococcus roseus	Growth and yield	SW1	Mahmood et al. (2016)
Paenibacillus xylanexedens	Growth and alleviate salinity	BNW24	Verma et al. (2016)
Planococcus salinarum	Growth and alleviate salinity	BSH13	Verma et al. (2016)
Pseudomonas fluorescens	Growth and alleviate salinity	153	Abbaspoor et al. (2009)
Pseudomonas putida	Plant growth	AKMP7	Ali et al. (2011)
Pseudomonas rhizosphaerae	Growth and alleviate salinity	IARI-DV-26	Verma et al. (2016)

Pseudomonas, and *Azotobacter* (Table 12.1) (Suman et al. 2016a; Gaba et al. 2017; Singh et al. 2016; Yadav et al. 2017a). The applications of phytase and phytospecific microorganisms also have great potentials (Kumar et al. 2013; Singh et al. 2014). The availability of adequate organic P (as phytate) in the soil enhances the importance of phytate-hydrolyzing microorganisms. The utilization of phytase-producing bacterial isolates (*Cellulosimicrobium* sp., *Advenella* sp., *Achromobacter* sp., *Bacillus* sp., and *Tetradios bacterial* sp.) result in enhanced plant growth. This is due to the synthesis of plant growth hormones and siderophores, solubility of P, and inhibition of plant pathogenic fungi (Kumar et al. 2013; Singh et al. 2014). These reduce the utilization of P fertilizers, thereby protecting the environment from P contamination and contributing towards sustainable agriculture. Excessive P could lead to serious environmental pollution in aquatic ecosystem (Kumar et al. 2015). Phytase-generating microbes or those phytases which are neutral furthermore can serve as the diet of aquatic organisms (Huang et al. 2009; Kumar et al. 2014).

These beneficial PGP microorganisms are capable of producing siderophores (iron-chelating substances), antibiotics, chitinases, different pigments having fluorescent properties, and HCN (Yadav et al. 2016a; Lottmann et al. 2000). Siderophore production by microbes inhibits the development of crop pathogens and introduces Fe to the crops. Siderophores have been associated with indirect and direct enhancement of plant growth by PGP microorganisms. Microorganisms with multifunctional PGP properties may be used as environment-friendly biological fertilizers (Verma et al. 2015, 2019; Suman et al. 2016a; Kour et al. 2017).

The salinity of soil is a major issue in a large number of fields, and the high concentration of salt causes soil infertility. Hypersaline soils are present in excess of saline soils and Na^+ – negatively charged clay particles. The growth of plants/crops is hindered by the higher levels of Na salt in soils. The accumulation of salts, e.g., NaCl, $CaCl_2$, and $MgCl_2$, happens constantly by the weather process (the rock is broken to release soluble salts). Beneficial microorganisms are linked to the roots of various plants with the help of root exudates. Epiphytic microorganisms are connected to the phyllosphere component of the plant because of the release of adhesive materials by the plants. Therefore, the interaction of plant microorganisms has been established, and the community of microbes has used elements of exudates as sources of energy (Yadav et al. 2015a, 2017b). Isolated microorganisms from growing crops in the high salinity/salty ecosystems possess the ability to promote plant development. Plant microorganisms that are rhizospheric, endophytic, and epiphytic have assisted in the growth of plant in vitro and in vivo, under osmotic pressure. Direct plant mechanisms through NH_3, HCN, siderophore (iron-sealing compounds), and other metabolites protect the plants from different pathogens and facilitate plant growth under harsh environment (Singh et al. 2016; Verma et al. 2016; Yadav et al. 2015a, 2017c) (Table 12.2).

While halotolerant/halophilic bacteria bacteria may enhance the growth of plant based on increased multifunctional PGP properties, biofertilizers improve germination, length of shoots and roots, biomass, and N_2, for higher yields and increased NPK (nitrogen, phosphorus, potassium) contents, chlorophyll content, and soil protein, and elevate tolerance to salinity (Yadav et al. 2015b, 2017c, d, 2018a;

Kumar et al. 2016, 2017; Verma et al. 2013, 2015; Vazquez et al. 2000; Kaur et al. 2017; Suman et al. 2016b; Yadav 2015).

Table 12.2 Halophilic microbes for agricultural applications under saline environment

Microbes	Response	Strain	Ref.
Aeromonas hydrophila	Growth and alleviate salinity	MAS-765	Ashraf et al. (2004)
Arthrobacter sp.	Salt stress and growth	AS 18	Tiwari et al. (2011)
Azotobacter chroococcum	Alleviated salinity	C5	Rojas-Tapias et al. (2012)
Aeromonas vaga	Plant growth	BAM-77	Jha et al. (2013)
Bacillus insolitus	Growth and alleviate salinity	MAS 17	Ashraf et al. (2004)
Bacillus sp.	Growth and alleviate salinity	MAS 617	Ashraf et al. (2004)
Bacillus licheniformis	Nutrient uptakes	RS656	Siddikee et al. (2011)
Brevibacterium iodinum	Nutrient uptakes	RS16	Siddikee et al. (2011)
Bacillus amyloliquefaciens	Salt tolerance	SN13	Nautiyal et al. (2013)
Bacillus aquimaris	Alleviated salinity	DY-3	Li and Jiang (2017)
Chryseobacterium gleum	Nutrient uptakes	SUK	Bhise et al. (2017)
Enterobacter sp.	Plant growth	12	Barra et al. (2016)
Enterobacter cloacae	Root growth	PD-P6	Yaish et al. (2015)
Kocuria erythromyxa	Alleviated salinity	EY43	Yildirim et al. (2008)
Nitrinicolalacis aponensis	Salt growth stress	SL11	Tiwari et al. (2011)
Pseudomonas putida	Plant growth	TSAU1	Egamberdieva and Kucharova (2009)
Pseudomonas fluorescens	Plant growth	YsS6	Ali et al. (2014)
Paenibacillus xylanexedens	Root growth	PD-R6	Yaish et al. (2015)
Pseudomonas aurantiaca	Growth and alleviate salinity	TSAU22	Egamberdieva and Kucharova (2009)
Pseudomonas extremorientalis	Growth and alleviate salinity	TSAU20	Egamberdieva and Kucharova (2009)
Pseudomonas fluorescens	Plant growth	153	Abbaspoor et al. (2009)
Pseudomonas chlororaphis	Growth and alleviate salinity	TSAU13	Egamberdieva and Kucharova (2009)
Pseudomonas extremorientalis	Growth and alleviate salinity	TSAU6	Egamberdieva and Kucharova (2009)
Planomicrobium okeanokoites	Growth and alleviate salinity	BNE8	Verma et al. (2016)
Xanthomonadales sp.	Plant growth	CSE-34	Piernik et al. (2017)
Zhihengliuela alba	Nutrient uptake	RS111	Siddikee et al. (2011)

Plant microorganisms produce hormones that regulate plant growth, such as cytokinins, IAA (indole acetic acids), and gibberellic acids. The production of IAA is the most abundant and is synthesized by plant-microbial interactions, for example, endophytic, epiphytic, and rhizosphere microorganisms. Gibberellic acids are also common hormones produced by rhizosphere microorganisms, while the synthesis of cytokinins is possible by liposphere/epiphytic microorganisms. The synthesis of growth regulators by various groups of microorganisms gives many benefits to plants, e.g., growth of root, absorption of water, and the uptake of nutrients from soil-to-plant, and enhances stress tolerance, e.g., heat, cold, dryness, as well as salinity (Yadav 2015; Verma et al. 2014; Yadav et al. 2018b; Suman et al. 2015). Microorganisms with the 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity could reduce ethylene levels during high salinity. These include *Bacillus*, *Arthrobacter*, *Bicriteria*, *Methylobacterium*, *Phenazacillin*, *Enterobacter*, *Pantoja*, *Pseudomonas*, *Penicillium*, *Rhizobium*, *Rhizobacteria*, and *Cerattia* (Yadav et al. 2018b; Glick 2020). Plant microorganisms may also involve indirectly in the PGP activities with the production of NH_3, HCN, siderophore (iron-chelating compounds), antimicrobial products, pigments, antibiotics, and hydrolytic enzymes chitinases, (β-1, 3),-glucanase, pectinases, and cellulases (Yadav et al. 2016a, b; Verma et al. 2016). These properties and characteristics play their role in protecting plant crops from different kinds of pathogens of plant, and the use of such microorganisms as biofertilizers may enhance the crop productivity (Verma et al. 2018). The most efficient and excellent microorganisms that increase the growth of plant via direct mechanisms of PGP are *Aeromonas*, *Bacillus*, *Photobacterium*, *Enterobacter*, *Pseudomonas*, *Trichoderma*, and *Xanthomonas*. The utilization of microorganisms as biofertilizers as a substitute to chemical fertilizers improve soil health and promote green agriculture. Rhizospheric microorganisms basically make colonies in the roots and stimulate plant growth under natural and saline environment. Halophilic microorganisms contribute to the development plants via different PGP activities even under salinity (Verma et al. 2013, 2014, 2016; Kumar et al. 2017; Yadav et al. 2018b).

12.4 Healthy Soil and Eco-Friendly Environment

Seed treatments in the form of microbial vaccines transport microbes straight into the rhizosphere of plant, with narrow soil areas surrounding the roots where plants directly interact with the microbes (Philippot et al. 2013). This is an area where intensive microbial activity occurs that depends on the growth of microbes and the availability of nutrients and other molecules, e.g., antibiotics and plant growth regulators. The rhizosphere colonizing species are beneficial microbes that have major role in agriculture with the potential to enhance plant growth through different mechanisms (Babalola et al. 2009).

12.4.1 Biofertilizers

Microorganisms in the soil help to improve productivity of agriculture. The naturally available living organisms are biofertilizers and biopesticides to help the growth of plant and overcome pests, weeds, and diseases. Friendly microbes help plants in the absorption of higher quantity of nutrients through. "Nutrient recycling" and "capture" the energy needed. In return, the waste by-products of the plants serve as food to the microbes. As excessive utilization of chemical fertilizers to meet the demand for agricultural products is one of the major reason for environmental pollution, biological fertilizers are increasingly seen as the antidote. Advantages of biofertilizers are illustrated in Fig. 12.3. Soil bacteria and specific types of fungi known as phosphorus-soluble microorganisms (PSMs) could convert insoluble forms of phosphates into solvable forms of phosphates by releasing organic acids (Meena et al. 2016). The soil pH is decreased by these acids. *Rhizobium*, blue-green algae (BGA), and *Azolla* are considered plant-specific biofertilizers, while *Azospirillum*, *Azotobacter*, Vesicular Arbuscular Mycorrhiza (VAM), and phosphorus soluble bacteria (PSB) are broad-spectrum biofertilizers (Gupta 2004; Teotia et al. 2016).

The major sources of biofertilizers are fungi, bacteria, and cyanobacteria. Other soil bacteria (*Azospirillum* and *Azotobacter*) can fix atmospheric nitrogen, thereby enriching the nitrogen content in the soil through the symbiotic interaction with the plants. *Glomus* is a genus of arbuscular mycorrhizal (AM) fungi. Plants which interact with the VAM exhibit improved nutrient uptake such as the P uptake, tolerance to root-burn pathogens, drought and salinity, and overall improvement in the plant development. Autotrophic microbes, i.e., Cyanobacteria, found in terrestrial and aquatic ecosystems, may retrieve N2, and the blue-green algae help to add

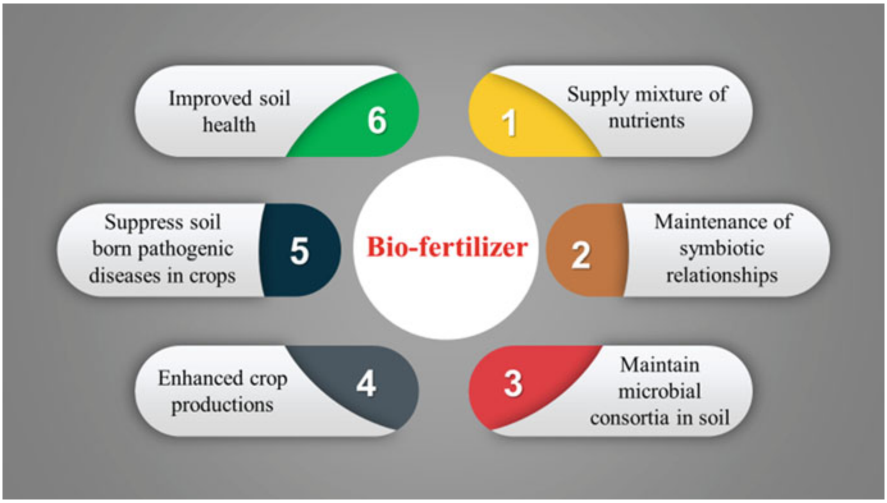

Fig. 12.3 Benefits of biofertilizers in agriculture

organic matter into the soil and enhance its productivity. Phosphate (PO_4^{-3}) and N_2 are significant for the development of plant, and both are easily available from natural resources. PO_4^{-3} has a significant role, directly or indirectly, during plant maturity, for N_2 fixation and for the quality and yield of the crop. A fungus such as *Penicillium bile* produces organic compound with acidic properties, to help in the dissolution of PO_4^{-3} from the soil, which eventually reaches the soil for the absorption by the plant roots. *Rhizobium* which reside in nodules on the roots of the plant are involved in the extraction of N_2 from air and conversion of nitrogen into a usable organic form. Those plants which possess larger populations of friendly bacteria residing in their roots can utilize naturally occurring nitrogen instead of depending on expensive fertilizers. Biofertilizers assist the plants in the utilization of all the nutrients present in the air and soil, and this finally lead to the reduction of the quantity of chemical fertilizers utilized.

12.4.2 Biopesticides

Biopesticides are obtained from natural sources, e.g., plants, animals, bacteria, and certain minerals. These sources can be fungi, e.g., *Bavaria* sp., neem extract, *Bacillus* sp., and pheromones. Baking soda, canola oil, bacteria, fungi, viruses, protozoa, nematodes, and other biologically active, safe substances are all considered as biopesticides, if they are used to control pests in eco-friendly manner. The advantages include for efficient control of pests, plant weeds, and diseases along with environmental and human protection. Biopesticides have found significant application in those areas facing pesticide resistance, and environmental concerns, and in the niche markets aiming to reduce the utilization of chemical pesticides (Mazid et al. 2011). The most commonly known microbes are *Bacillus thuringiensis* (BT), which produce a protein that may kill specific pests or insects in potatoes, cabbage, and other crops. The basic requirement is that the biopesticide only kills the target organisms but not the non-targetted ones or humans. Plant growth-promoting rhizobacteria (PGPR) are functionally diversified bacterial groups that possess higher capability as biopesticides and biofertilizers. They are cost-friendly and eco-friendly substitutes to chemical fertilizers or other synthetic counterparts (Mazid et al. 2011). Some microorganisms which are pathogenic to plants can be genetically-modified to control pests and weeds. The best example is BT which has been successfully utilized as a specific, safe, and effective tool for insect pest control (Roh et al. 2007). BT is effective against Black flies and mosquitoe larvae, but may be harmful to moths and butterfly caterpillars larvae. The target insect species determine whether a certain BT type synthesizes a protein that binds to a gut receptor of larvae or merely by starving the larvae (Kumar et al. 2008).

Microbial pesticides as biological control agents are safe as compared to other conventional synthetic pesticides (Buss and Park-Brown 2002). The formulas (inoculants) of seed coating make use of adventitious organisms to safeguard the seedlings. Biopesticides have a short life span and, unlike synthetic pesticides, do not have harmful effects on animals and ecosystems, as they are super selective with specific targets of the class/type of insect. Traditional pesticide sprays, such as dust, liquid drains, liquid concentrations, wet powders, or granules, are used and the specific feature of each product determines the most effective ways for delivery of agents to the target pests (Nicholson 2007).

The rod-shaped bacteria are the bacterial pathogens of the Bacillus used for pest control and they usually reside in soil. The products with *Bacillus thuringiensis* Kurstaki destroy a variety of kite caterpillars and butterflies. In contrast, *Bacillus papillae* (milky spore disease) destroys the larvae of Japanese beetle, but it shows no response against the annual white grub (*Cyclocephala* mask), which is usually associated with pasture. BT has been the most commonly used microbial pesticide in the United States since the 1960s. BT products are commercially manufactured in huge industrial fermentation tanks. When the bacteria survive and reproduce under optimal conditions, the cells synthesize spores and toxic crystalline protein known as endotoxin. Most existing commercial BT products consist of toxic proteins and spores, but only a few toxin fractions can be cultured (Mueller and Sachs 2015; Singh and Trivedi 2017). Pesticides marketed under the trade names Zapidemic, Doom, Grub Attack, and the common name "milky spore disease" consists of *Bacillus papilla* and *Bacillus lentimorbus*.

The production and utilization of pesticides based on virus is limited. In contrast to BT, the living host insects must produce the insect viruses. Therefore, the product is expensive, time-consuming and less efficient as compared to the already present synthetic chemical pesticides. However, many insect viruses are related to the same species or pests of the forest, such as the gypsy moth, spruce budworm, Douglas-fir tusk moth, and Pine sawdust. They are not attainable commercially, but they are being prepared and utilized by the Forest Services of the United States. Forest pests are specifically better targets to be attacked by viral pathogens as the stability of the forest environment takes an important part in the cycling of pathogen (transmitted from one generation to another). Forest canopy have a significant part in the protection of viral cells from being destroyed by UV radiation. Baculovirus affects pests such as corn bores, flea beetles, potato beetles, and aphids (Berendsen et al. 2012). A special breed is employed as an agent to control the bertha army worm, which attacks flax, canola, and other vegetable crops. Traditional pesticides have no effect on the worm until it reaches a point when there has been extensive damage. Other pest viruses tested for use as pesticides include alfalfa looper, armyworm, soybean looper, imported cabbage, and cabbage looper. However, few of these viruses are manufactured and trialed in the fields and none of them has been recorded or marketed in a commercial manner. Both the cooling moth GV and the Heliothis nuclear polyhedrosis virus (NPV) are simultaneously registered and commercially produced by the US EPA, but these items are no longer attainable.

A large number of insect hosts are naturally infected by protozoan pathogens. Although these pathogens destroy their host insects, they are necessary for their long-term impacts. A significant and general result of the infection of protozoa is the reduction in the number of organisms produced by affected insects. Although pathogens of protozoa possess an important character in the natural population, some pesticides appear to favor development. The species in genera *Nosema* and

Vairimorpha have potentials as insecticides (Weinzierl et al. 1995). The pathogens invade the larvae of the lepidopterans and insects of the Orthoptera (grasshopper and related pests). Protozoan microsporidian is currently available for the manufacturing of registered pesticides. Microbial pesticides offer protection for animals and humans because they are essentially non-toxic and non-pathogenic. Many of the microbial pesticides produce significant effects against narrow range of pest types, and because these pesticides are likely to deactivate rapidly in the environment, consumers should select the pest targets and the formulation having the most efficient and effective application.

12.4.3 Bioherbicides

Weeds are competing with crops for water, sunlight, nutrients, and space, as well as block drainage and irrigation systems, leading to poor quality of crop with deposit of weed seeds in the harvest. Weeds can be controlled by bioherbicides. Bioherbicide utilization, in place of chemical herbicides, lead to an increasingly successful strategies of integrated management (Hoagland 2007). Bioherbicides include phytopathogenic microorganisms or microbial compounds that can be used for the control of weed. Many microorganisms and phytopathogenic bacteria and fungi have bio-herbicidal functionality, and have been described in patents as the agents of weed control. The phytotoxic constituents of many chemical agents as well as other secondary compounds produced by such pathogens may also be poisonous to other mammals. In addition, the translocation, intake, metabolism, and persistence of these phytotoxins and the environmental impacts of increased chemical herbicide applications to other microbial communities are not well-understood. Microbes may contain aggressive genes which may invade the defence genes of weed, thus ultimately leading to death. The advantage of using bioherbicides is that it stays for longer period in the environment during the season of growth. It is cost-effective as compared to synthetic herbicides, so it can decrease the cost of cultivation. In addition, bioherbicide is not dangerous to the environment and does not affect non-target organisms (Singh et al. 2006).

12.4.4 Bioinsecticides

Similar to viruses, fungi sometimes behave as significant agents to control and inhibit the population of insect. Most of the species which create infections in insect are dispersed by the spores of conidia known as conidiophores. The conidia spread from different fungi possess different capability and the germination requires high humidity or free water. Contrary to bacterial spores or virus cells, the conidia of fungal spores originating from the cuticle synthesizes specific structures which can invade and enter the body of the insect. As the fungal infection grows, the toxins kill the infected insects. The advantage is the fungus are not killed by the long-term effects of the parasites (Berendsen et al. 2012). The fungus causes diseases in about fields and none of them has been recorded or marketed in a used as bioinsecticides. Techniques involving fermentation are employed for mass production of fungi. Spores are packed so that they may be spread to areas where the insects can be infected. After plantation, the spores utilize enzymes to enter the insect body. Once injected into the insect, they start to reproduce and ultimately lead to the insect death. Fungal agents have been recommended to have the best potential for chronic pest control. The biological pesticides attack in multiple ways, that the plant resistance to pests may be much increased.

12.5 Microbiome and Sustainable Agriculture

The aim of sustainable agriculture is to achieve high productivity of animals and plants through economical approach, making use of flexible and adaptable technology, with minimum disturbance to the environment. It needs to address the negative impacts of agrochemicals (pesticides, mineral fertilizers) with the applications of symbiotic microbes that facilitate nutrient supply to the livestock and crops, and provide control against biohazards (pests, pathogens) and abiotic stressors (including climate fluctuation and pollution) (Yang et al. 2009). This highlights the significance of microorganisms with respect to sustainable agro-practices and health of environment (Wang et al. 2009). This is attributable to the genetic dependence of the plants on the symbiotic interactions with the surroundings. The potential of plant-microbial symbiosis extends beyond the environmental impacts, as it also involves nitrogen fixation (Franche et al. 2009) and the molecular and ecological processes with multiple pathways for mutual co-evolution and adaptation of the microbes and the plants (Arnold et al. 2010). For the fungi-plant interactions, the host genotype is an important parameter for the spreading of fungal component (mycobionts) and for the development of the specificist-mutualist and specio-genetic continuum interactions (Peay et al. 2010). In the case of leguminous crops, highly active rhizobia strains can be utilized to provide nodulation to support N_2 fixation for sufficient symbiotrophic nitrogen nutrition, using moderate levels of N-fertilizer (Provorov and Tikhonovich 2003). Maximum productivity can be attained by considering the species-specific and genotype-specific types of nutrition (Provorov et al. 1998). The use of beneficial microbes in agro-practices could reduce the use of inorganic fertilizers, water, pesticides and herbicides, without affecting the crop yield (Andrews et al. 2010). Intact tropical forests have been reported to accumulate and recycle higher quantities of N than the temperate forests, attributable to the abundance of N-fixing plants and sustained transport of bioavailable N within the ecosystem (Hedin et al. 2005). The optimal nutrients should lead to efficient formation of the colonies within the host, and the symbiosis can be enhanced according to the specificity of the host (Provorov and Vorobyov 2009). Microbial symbionts or their derivatives represent a promising area for sustainable agricultural technology for plant development and protection. Future prospects of microbial applications include the production of novel multipartite ecto- and endosymbiotic interactions which are based on extensive molecular (metagenomic) and genetic investigation. The basic strategy is to prepare composite inoculants that mimic the microbial communities linked to the natural plants. To balance plant-host metabolism, a combination of P- and N-providing sebum, including endosymbiotic rhizobia + VAM-fungi, appears promising. Some of the issues are related to the opportunistic or common pathogens of humans, which are often present in endophytic communities, including *Klebsiella*, *Escherichia*, *Salmonella*, *Enterobacter*, and *Staphylococcus* species (Shtark et al. 2010; Ryan et al. 2008). Productive handling of symbiotic communities of microbes is possible by utilizing molecular tools based on the pools of microbes that constantly migrate between soil, animal, and plant bodies in agricultural and natural ecosystems (Kupriyanov et al. 2010).

A few bacteria, e.g., agro-bacteria and rhizobia, are employed to deliver seed inoculants to the plants. The importance of microorganisms such as *Azoarcus* sp. to plants is that it serves as grass endophyte (Hurek and Hurek 2003). These types of bacteria mostly support rice crops and they do not harm the environment. After the seeds are sown in the soil, there is a significant role of bacteria in its germination. The bacteria thrive in the seed, which feeds them. Bacteria enhance soil fertility by providing nutrients for plant growth. They assist in food softening in the seeds, which facilitate the plants to grow from the seeds. Bacteria not only play significant part in the early stages of plant development, but also provide protection against pests and tolerance against stressors such as drought (Parke et al. 1983).

12.5.1 Benefits of Mycorrhizal Fungi

Growth of mycorrhizal plants could tolerate adverse conditions such as drought (Parke et al. 1983), soil pathogens, transplantation, poor soil nutrient, and soil contamination (Leyval et al. 1997). Improvement in plant growth and enhanced resistance to unfavorable conditions is often associated with the increased nutrient and water uptake, which is feasible through comprehensive hyphal networks with enhanced root area for assimilation. The impact of mycorrhizal fungi on the plant development, as illustrated in Fig. 12.4, includes enhanced root system growth and improved nutrient/water absorption and utilization. In *Eucalyptus globulus*, the dry weight of the plant is associated positively with the extent of mycorrhiza-colonized root. The benefits of ectomycorrhiza become more apparent in the establishment and development of young transplants in horticulture and forest care (Munro et al. 1999; Scagel and Linderman 1998).

The mycorrhizal symbiosis could improve phosphorus content through a wide range of hypercellular networks. This permits plant root to cross the phosphorus depletion area and reach a stable phosphorus-rich area where the fungus dissolves. Phosphorus, in many cases, can compensate for the effect of mycorrhizal infection on the plant survival under mycorrhizal control. However, increased P content may also lead to reduced mycorrhizal infection. Generally, the beneficial effects of mycorrhiza on the plants disappear as a result of excessive supply of phosphorus. The application of stimulants in conventional agriculture has often overlooked the beneficial symbiotic activity of mycorrhizal fungi (Jacott et al. 2020).

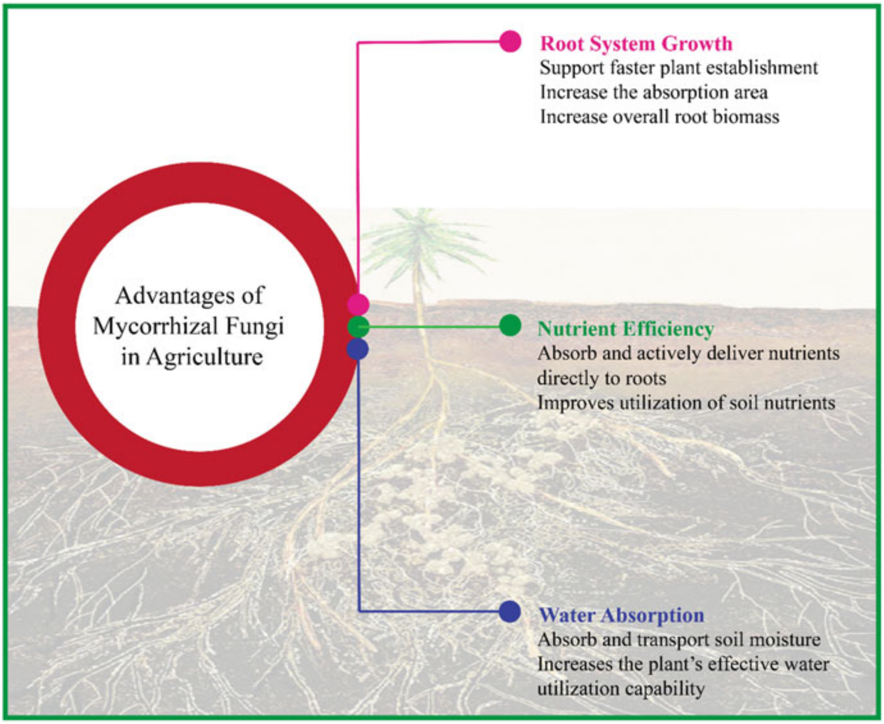

Fig. 12.4 Advantages of mycorrhizal fungi in agriculture

12.5.2 Soil and Environmental Health

Certain communities of microbes influence plant physiology, rhizome, and nutrient soil physiochemical properties directly or indirectly through metabolism. PGPR are the significant constituents of integrated farming, helping in nourishing crops with essential nutrients, and help to address the uptake of atmospheric nitrogen; the soluble and aggregated phosphorus; and the conversion microelements such as Mo, Zn, Cu, etc. into plant constituents. The production of hormones that promote plant development, such as indole acetic acid and gibberellic acid and polysaccharides, helps to improve the soil structure, thereby improving the soil health and increase the crop production. The amount of nutrients like K, Zn, Ca, Fe, Mn, and Cu can be improved by the proton pump ATPase (Mantelin and Touraine 2004). There are many reports on the importance of PGPR in maintaining soil fertility (Singh et al. 2018). PGPR vaccination of seeds has improved the value of accessible phosphorus, populations of microbes, acid and alkaline phosphate, dehydrogenase activity in soil, and high yields from irrigated seeds (Hemashenpagam and Selvaraj 2011).

The problems and solutions for healthy environment through the management of microorganisms can be achieved by combining the understanding in environmental biotechnology with microbial ecology (Damjanovic et al. 2017), to improve the quality of the environment, safety, sustainability, and human health (Umesha et al. 2018). The molecular biology tools based on polymerase chain reaction (PCR) amplification and microbial DNA development can detect the identity and function of individual microbes. The latest technologies on high-throughput genetic and proteomic techniques could identify particular genes along with their metabolic activities. The whole genome of microbes which is once “unusable” can now be reconstituted utilizing current advancements in biology, computing, materials, and engineering. The focus has now shifted to the use of communities of microbes (Demain 2000), for bioremediation of polluted water, sludge, sewage, and sediment; or for soil detoxification; or for extraction of renewable energy from biomass, pathogens, or contaminants, while reducing their hazardous effects.

12.6 Conclusion

The application of commercial fertilizers and synthetic chemicals as pesticides have improved the crop yield, but with equally huge impact on the environment from the polluted and contaminated ecosystems. The growing concern over food safety has led to the development of more eco-friendly techniques, moving away from the toxic synthetic chemicals. Exploiting the links between soil microbial communities and the crops is the right approach to increase food production at low environmental cost while meeting the demand of growing world population. The two main strategies in the management of the soil microbes are based on the development of microbial vaccines or dealing with naturally occurring microbial populations. There has been an increasing interest in the use of biofertilizers, biopesticides, bioherbicides, and bioinsecticides to improve the crop quality and yield. The improvement of plant-microbial symbiotic relationships involve the extent of biocontrol exerted by the microbes, optimal microbial communities, soil modifications, and the types of soil and crops. Microbiological technologies, sustainable approaches, and improvement in regulatory framework could lead the way for emerging microbial-based solutions and new agro-practices with increased productivity.

References

Abawi GS, Widmer TL (2000) Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Appl Soil Ecol 15:37–47. https://doi.org/10.1016/S0929-1393(00)00070-6

Abbaspoor A, Zabihi H, Movafegh S, Hossein M, Akbari, Akbari Asl MH (2009) The efficiency of Plant Growth Promoting Rhizobacteria (PGPR) on yield and yield components of two varieties of wheat in salinity condition. Am-Eurasian J Sustain Agric 3:824–828

Abinandan S, Subashchandrabose SR, Venkateswarlu K, Megharaj M (2019) Soil microalgae and cyanobacteria: the biotechnological potential in the maintenance of soil fertility and health. Crit Rev Biotechnol 39(8):981–998. https://doi.org/10.1080/07388551.2019.1654972

Adak A, Prasanna R, Babu S, Bidyarani N, Verma S, Pal M, Shivay YS, Nain L (2016) Micronutrient enrichment mediated by plant-microbe interactions and rice cultivation practices. J Plant Nutr 39(9):1216–1232. https://doi.org/10.1080/01904167.2016.1148723

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26(1):1–20. https://doi.org/10.1016/j.jksus.2013.05.001

Ahmed Nouh F (2019) Endophytic fungi for sustainable agriculture. Microb Biosyst 4:31–44. https://doi.org/10.21608/MB.2019.38886

Aislabie J, Deslippe J, Dymond J (2013) Soil microbes and their contribution to soil services. In: Ecosystem services in New Zealand: conditions and trends. Manaaki Whenua Press, Lincoln, pp 143–161

Ali SZ, Sandhya V, Grover M, Linga VR, Bandi V (2011) Effect of inoculation with a thermotolerant plant growth promoting Pseudomonas putida strain AKMP7 on growth of wheat (Triticum spp.) under heat stress. J Plant Interact 6(4):239–246. https://doi.org/10.1080/17429145.2010.545147

Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem 80:160–167. https://doi.org/10.1016/j.plaphy.2014.04.003

Alvarez M, Sueldo R, Barassi CJCRC (1996) Effect of Azospirillum on coleoptile growth in wheat seedlings under water stress, pp 101–107

Andrews M, Hodge S, Raven JA (2010) Positive plant microbial interactions. 157(3):317–320. https://doi.org/10.1111/j.1744-7348.2010.00440.x

Arnold AE, Lamit LJ, Gehring CA, Bidartondo MI, Callahan H (2010) Interwoven branches of the plant and fungal trees of life. New Phytol 185(4):874–878

Arzanesh MH, Alikhani HA, Khavazi K, Rahimian HA, Miransari M (2011) Wheat (Triticum aestivum L.) growth enhancement by Azospirillum sp. under drought stress. World J Microbiol Biotechnol 27(2):197–205. https://doi.org/10.1007/s11274-010-0444-1

Ashraf M, Hasnain S, Berge O, Mahmood T (2004) Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol Fertil Soils 40(3):157–162. https://doi.org/10.1007/s00374-004-0766-y

Atapattu SS, Kodituwakku DC (2009) Agriculture in South Asia and its implications on downstream health and sustainability: a review. Agric Water Manag 96(3):361–373. https://doi.org/10.1016/j.agwat.2008.09.028

Babalola OO, Kirby BM, Le Roes-Hill M, Cook AE, Cary SC, Burton SG, Cowan DA (2009) Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. Environ Microbiol 11(3):566–576. https://doi.org/10.1111/j.1462-2920.2008.01809.x

Bagali S (2012) Review: nitrogen fixing microorganisms. Int J Microbiol Res 3:46–52. https://doi.org/10.5829/idosi.ijmr.2012.3.1.61103

Bagyaraj D, Revanna A (2017) Soil biodiversity: role in sustainable horticulture. In: Peter KV (ed) Biodiversity in horticultural crops, vol 5. Daya Publishing House, New Delhi, pp 1–18

Baldrian P (2003) Interactions of heavy metals with white-rot fungi. Enzym Microb Technol 32(1):78–91. https://doi.org/10.1016/S0141-0229(02)00245-4

Bar-On Y, Phillips R, Milo R (2018) The biomass distribution on earth. Proc Natl Acad Sci U S A 115:201711842. https://doi.org/10.1073/pnas.1711842115

Barra PJ, Inostroza NG, Acuña JJ, Mora ML, Crowley DE, Jorquera MA (2016) Formulation of bacterial consortia from avocado (Persea americana mill.) and their effect on growth, biomass and superoxide dismutase activity of wheat seedlings under salt stress. Appl Soil Ecol 102:80–91. https://doi.org/10.1016/j.apsoil.2016.02.014

Basak B, Biswas D (2008) Influence of potassium solubilizing microorganism (Bacillus mucilaginosus) and waste mica on potassium uptake dynamics by Sudan grass (Sorghum vulgare Pers.) grown under two Alfisols. Plant Soil 317:235–255. https://doi.org/10.1007/s11104-008-9805-z

Baum C, El-Tohamy W, Gruda N (2015) Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: a review. Sci Hortic 187:131–141. https://doi.org/10.1016/j.scienta.2015.03.002

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Bertsch PM, Thomas GW (1985) Potassium status of temperate region soils. In: Munson RD (ed) Potassium in agriculture. Wiley Online Library, pp 129–162. https://doi.org/10.2134/1985.potassium.c7

Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Factories 13:66. https://doi.org/10.1186/1475-2859-13-66

Bhise KK, Bhagwat PK, Dandge PB (2017) Synergistic effect of Chryseobacterium gleum sp. SUK with ACC deaminase activity in alleviation of salt stress and plant growth promotion in Triticum aestivum L. 3 Biotech 7(2):105. https://doi.org/10.1007/s13205-017-0739-0

Boddey RM, Urquiaga S, Alves BJR, Reis V (2003) Endophytic nitrogen fixation in sugarcane: present knowledge and future applications. Plant Soil 252(1):139–149. https://doi.org/10.1023/A:1024152126541

Buss EA, Park-Brown SG (2002) Natural products for insect pest management. J UF/IFAS Publication ENY-350 URL: http://edis.ifas.ufl.edu/IN197

Chong TM, Abdullah MA, Fadzillah NM, Lai OM, Lajis NH (2005) Jasmonic acid elicitation of anthraquinones with some associated enzymic and non-enzymic antioxidant responses in Morinda elliptica. Enzym Microb Technol 36:469–477

Choudhary D, Johri B, Prakash A (2008) Volatiles as priming agents that initiate plant growth and defence responses. Curr Sci 94:595–604

Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C, López-Bucio J (2009) Trichoderma virens, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. Plant Physiol 149 (3):1579–1592. https://doi.org/10.1104/pp.108.130369

Cronin D, Moënne-Loccoz Y, Fenton A, Dunne C, Dowling DN, O'Gara F (1997) Ecological interaction of a biocontrol Pseudomonas fluorescens strain producing 2,4-diacetylphloroglucinol with the soft rot potato pathogen Erwinia carotovora subsp. atroseptica. 23(2):95–106. https://doi.org/10.1111/j.1574-6941.1997.tb00394.x

Curtis TP, Sloan WT (2005) Exploring microbial diversity—a vast below. Science 309 (5739):1331–1333. https://doi.org/10.1126/science.1118176

Damjanovic K, Blackall LL, Webster NS, van Oppen MJH (2017) The contribution of microbial biotechnology to mitigating coral reef degradation. 10(5):1236-1243. doi:https://doi.org/10.1111/1751-7915.12769

Dawidziuk A, Popiel D, Kaczmarek J, Strakowska J, Jedryczka M (2016) Optimal Trichoderma strains for control of stem canker of brassicas: molecular basis of biocontrol properties and azole resistance. BioControl 61(6):755–768. https://doi.org/10.1007/s10526-016-9743-2

De Vero L, Boniotti MB, Budroni M, Buzzini P, Cassanelli S, Comunian R, Gullo M, Logrieco AF, Mannazzu I, Musumeci R, Perugini I, Perrone G, Pulvirenti A, Romano P, Turchetti B, Varese GC (2019) Preservation, characterization and exploitation of microbial biodiversity: the perspective of the Italian network of culture collections. Microorganisms 7(12). https://doi.org/10.3390/microorganisms7120685

Demain AL (2000) Microbial biotechnology. Trends Biotechnol 18(1):26–31. https://doi.org/10.1016/S0167-7799(99)01400-6

de Mulé MCZ, de Caire GZ, de Cano MS, Palma RM, Colombo K (1999) Effect of cyanobacterial inoculation and fertilizers on rice seedlings and postharvest soil structure. Commun Soil Sci Plant Anal 30(1–2):97–107. https://doi.org/10.1080/00103629909370187

Doerr SH, Shakesby RA, Walsh RPD (2000) Soil water repellency: its causes, characteristics and hydro-geomorphological significance. Earth Sci Rev 51:33. https://doi.org/10.1016/s0012-8252(00)00011-8

Egamberdieva D, Kucharova Z (2009) Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol Fertil Soils 45(6):563–571. https://doi.org/10.1007/s00374-009-0366-y

Elbeltagy A, Nishioka K, Sato T, Suzuki H, Ye B, Hamada T, Isawa T, Mitsui H, Minamisawa K (2001) Endophytic colonization and in planta nitrogen fixation by a Herbaspirillum sp. isolated from wild rice species. Appl Environ Microbiol 67:5285–5293. https://doi.org/10.1128/AEM.67.11.5285-5293.2001

Farmer EE (2001) Surface-to-air signals. Nature 411(6839):854–856. https://doi.org/10.1038/35081189

Frac M, Jezierska-Tys S, Yaguchi T (2015) Occurrence, detection, and molecular and metabolic characterization of heat-resistant Fungi in soils and plants and their risk to human health. Adv Agron 132:161–204. https://doi.org/10.1016/bs.agron.2015.02.003

Frąc M, Hannula SE, Bełka M, Jędryczka M (2018) Fungal biodiversity and their role in soil. Health 9(707). https://doi.org/10.3389/fmicb.2018.00707

Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. https://doi.org/10.1007/s11104-008-9833-8

Gaba S, Singh RN, Abrol S, Yadav AN, Saxena AK, Kaushik R (2017) Draft genome sequence of Halolamina pelagica CDK2 isolated from natural Salterns from Rann of Kutch, Gujarat, India. Genome Announc 5(6). https://doi.org/10.1128/genomeA.01593-16

Gardi C, Montanarella L, Arrouays D, Bispo A, Lemanceau P, Jolivet C, Mulder C, Ranjard L, Römbke J, Rutgers M, Menta C (2009) Soil biodiversity monitoring in Europe: ongoing activities and challenges 60(5):807–819. https://doi.org/10.1111/j.1365-2389.2009.01177.x

Glick BR (2020) Introduction to plant growth-promoting Bacteria. In: Beneficial plant-bacterial interactions. Springer International Publishing, Cham, pp 1–37. https://doi.org/10.1007/978-3-030-44368-9_1

Gornall J, Betts R, Burke E, Clark R, Camp J, Willett K, Wiltshire A (2010) Implications of climate change for agricultural productivity in the early twenty-first century. 365(1554):2973–2989. https://doi.org/10.1098/rstb.2010.0158

Gupta AK (2004) The complete technology book on biofertilizers and organic farming. National Institute of Industrial Research Press, Delhi, pp 242–253

Han J, Sun L, Dong X, Cai Z, Sun X, Yang H, Wang Y, Song W (2005) Characterization of a novel plant growth-promoting bacteria strain Delftia tsuruhatensis HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Syst Appl Microbiol 28(1):66–76. https://doi.org/10.1016/j.syapm.2004.09.003

Hannula SE, van Veen JA (2016) Primer sets developed for functional genes reveal shifts in functionality of fungal community in soils. Front Microbiol 7:1897–1897. https://doi.org/10.3389/fmicb.2016.01897

Hedin L, Brookshire EN, Menge D, Barron A (2005) The nitrogen paradox in tropical forest ecosystems. Annu Rev Ecol Evol Syst 40:613–635. https://doi.org/10.1146/annurev.ecolsys.37.091305.110246

Hemashenpagam N, Selvaraj TJJoeb (2011) Effect of arbuscular mycorrhizal (AM) fungus and plant growth promoting rhizomicroorganisms (PGPR's) on medicinal plant Solanum viarum seedlings. 32(5):579–583

Hoagland RE (2007) Myrothecium verrucariu fungus: a bioherbicide and strategies to reduce its non-target risks. Allelopathy J 19(1) 179-170-2007 v.2019 no.2001

Huang H, Shao N, Wang Y, Luo H, Yang P, Zhou Z, Zhan Z, Yao B (2009) A novel beta-propeller phytase from Pedobacter nyackensis MJ11 CGMCC 2503 with potential as an aquatic feed additive. Appl Microbiol Biotechnol 83(2):249–259. https://doi.org/10.1007/s00253-008-1835-1

Hurek T, Hurek BR (2003) *Azoarcus* sp. strain BH72 as a model for nitrogen-fixing grass endophytes. J Biotechnol 106:169–178

Ingham RE, Trofymow JA, Ingham ER, Coleman DC (1985) Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth 55(1):119–140. https://doi.org/10.2307/1942528

Jacott CN, Charpentier M, Murray JD, Ridout CJ (2020) Mildew Locus O facilitates colonization by arbuscular mycorrhizal fungi in angiosperms. New Phytol 227(2):343–351. https://doi.org/10.1111/nph.16465

Jayne B, Quigley M (2014) Influence of arbuscular mycorrhiza on growth and reproductive response of plants under water deficit: a meta-analysis. Mycorrhiza 24(2):109–119. https://doi.org/10.1007/s00572-013-0515-x

Jha A, Saxena J, Sharma V (2013) Investigation on phosphate solubilization potential of agricultural soil bacteria as affected by different phosphorus sources, temperature, salt, and pH. Commun Soil Sci Plant Anal 44(16):2443–2458. https://doi.org/10.1080/00103624.2013.803557

Johansen JE, Binnerup SJ, Kroer N, Mølbak L (2005) Luteibacter rhizovicinus gen. nov., sp. nov., a yellow-pigmented gammaproteobacterium isolated from the rhizosphere of barley (Hordeum vulgare L.). Int J Syst Evol Microbiol 55(Pt 6):2285–2291. https://doi.org/10.1099/ijs.0.63497-0

Johns C (2017) Living soils: the role of microorganisms in soil health. Fut Direct Int:1–7

Kalayu G (2019) Phosphate solubilizing microorganisms: promising approach as biofertilizers. Int J Agron 2019:4917256. https://doi.org/10.1155/2019/4917256

Karun N, Sharma B, Sridhar K (2018) Biodiversity of macrofungi in Yenepoya campus, Southwest India. Microb Biosyst 3. https://doi.org/10.21608/mb.2018.12354

Kaur R, Saxena A, Sangwan P, Yadav AN, Kumar V, Dhaliwal H (2017) Production and characterization of a neutral phytase of Penicillium oxalicum EUFR-3 isolated from Himalayan region. Nusantara Biosci 9:68–76. https://doi.org/10.13057/nusbiosci/n090112

Kibblewhite MG, Ritz K, Swift MJ (2008) Soil health in agricultural systems. Philos Trans R Soc Lond Ser B Biol Sci 363(1492):685–701. https://doi.org/10.1098/rstb.2007.2178

Knudsen GR (2006) Bacteria, fungi and soil health. In: Idaho Potato Conference. University of Idaho, Moscow, ID

Kour D, Rana K, Verma P, Yadav A, Kumar V, Singh D (2017) Biofertilizers: eco-friendly technologies and bioresources for sustainable agriculture. In: Proceeding of international conference on innovative research in engineering science and technology

Kumar S, Chandra A, Pandey KC (2008) Bacillus thuringiensis (Bt) transgenic crop: an environment friendly insect-pest management strategy. J Environ Biol 29(5):641–653

Kumar A, Bisht BS, Joshi V, Dhewa TJIJoES (2011) Review on bioremediation of polluted environment: a management tool 1:1079–1093

Kumar V, Singh P, Jorquera MA, Sangwan P, Kumar P, Verma AK, Agrawal S (2013) Isolation of phytase-producing bacteria from Himalayan soils and their effect on growth and phosphorus uptake of Indian mustard (Brassica juncea). World J Microbiol Biotechnol 29(8):1361–1369. https://doi.org/10.1007/s11274-013-1299-z

Kumar V, Sangwan P, Verma AK, Agrawal S (2014) Molecular and biochemical characteristics of recombinant β-propeller phytase from Bacillus licheniformis strain PB-13 with potential application in aquafeed. Appl Biochem Biotechnol 173(2):646–659. https://doi.org/10.1007/s12010-014-0871-9

Kumar V, Singh D, Sangwan P, Gill PK (2015) Management of environmental phosphorus pollution using phytases: current challenges and future prospects. In: Kaushik G (ed) Applied environmental biotechnology: present scenario and future trends. Springer India, New Delhi, pp 97–114. https://doi.org/10.1007/978-81-322-2123-4_7

Kumar V, Yadav AN, Saxena A, Sangwan P, Dhaliwal H (2016) Unravelling rhizospheric diversity and potential of phytase producing microbes. SM J Biol 2:1009

Kumar V, Yadav AN, Verma DP, Sangwan P, Saxena A, Kumar K, Singh B (2017) β-Propeller phytases: diversity, catalytic attributes, current developments and potential biotechnological applications. Int J Biol Macromol 98. https://doi.org/10.1016/j.ijbiomac.2017.01.134

Kupriyanov AA, Semenov AM, Van Bruggen AHC (2010) Transition of entheropathogenic and saprotrophic bacteria in the niche cycle: animals-excrement-soil-plants-animals. Biol Bull 37 (3):263–267. https://doi.org/10.1134/S1062359010030076

Lal R (2016) Soil health and carbon management. Food Energy Secur 5(4):212–222. https://doi.org/10.1002/fes3.96

Lavakush YJ, Verma JP, Jaiswal DK, Kumar A (2014) Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (Oryza sativa). Ecol Eng 62:123–128. https://doi.org/10.1016/j.ecoleng.2013.10.013

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7 (3):139–153. https://doi.org/10.1007/s005720050174

Li HQ, Jiang XW (2017) Inoculation with plant growth-promoting bacteria (PGPB) improves salt tolerance of maize seedling. Russ J Plant Physiol 64(2):235–241. https://doi.org/10.1134/S1021443717020078

Li ZP, Han CW, Han FX (2010) Organic C and N mineralization as affected by dissolved organic matter in paddy soils of subtropical China. Geoderma 157(3):206–213. https://doi.org/10.1016/j.geoderma.2010.04.015

López-Bucio J, Pelagio-Flores R, Herrera-Estrella A (2015) Trichoderma as biostimulant: exploiting the multilevel properties of a plant beneficial fungus. Sci Hortic 196. https://doi.org/10.1016/j.scienta.2015.08.043

Lottmann J, Heuer H, De Vries J, Mahn A, Düring K, Wackernagel W, Smalla K, Berg G (2000) Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. FEMS Microbiol Ecol 33(1):41–49. https://doi.org/10.1111/j.1574-6941.2000.tb00725.x

Mahmood A, Turgay OC, Farooq M, Hayat R (2016) Seed biopriming with plant growth promoting rhizobacteria: a review. FEMS Microbiol Ecol 92(8). https://doi.org/10.1093/femsec/fiw112

Malam Issa O, Défarge C, Trichet J, Valentin C, Rajot JL (2009) Microbiotic soil crusts in the Sahel of Western Niger and their influence on soil porosity and water dynamics. Catena 77(1):48–55. https://doi.org/10.1016/j.catena.2008.12.013

Mallavarapu M, Kantachote D, Singleton I, Naidu R (2000) Effects of long-term contamination of DDT on soil microflora with special reference to soil algae and algal transformation of DDT. Environ Pollut (Barking, Essex : 1987) 109:35–42. https://doi.org/10.1016/S0269-7491(99)00231-6

Manjunath M, Kanchan A, Ranjan K, Venkatachalam S, Prasanna R, Ramakrishnan B, Hossain F, Nain L, Shivay YS, Rai AB, Singh B (2016) Beneficial cyanobacteria and eubacteria synergistically enhance bioavailability of soil nutrients and yield of okra. Heliyon 2(2):e00066. https://doi.org/10.1016/j.heliyon.2016.e00066

Mantelin S, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp Bot 55(394):27–34. https://doi.org/10.1093/jxb/erh010

Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Behairy UA, Sorlini C, Cherif A, Zocchi G, Daffonchio D (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS One 7(10):e48479. https://doi.org/10.1371/journal.pone.0048479

Mazid S, Kalita JC, Rajkhowa RC (2011) A review on the use of biopesticides in insect pest management. Int J Sci Adv Technol 1(7):169–178

Meena V, Bahadur D, Maurya B, Kumar A, Meena R, Meena S, Verma J (2016) Potassium-solubilizing microorganism in evergreen agriculture: an overview. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer India, New Delhi, pp 1–20. https://doi.org/10.1007/978-81-322-2776-2_1

Mejía L, Rojas E, Maynard Z, Bael S, Arnold A, Hebbar P, Samuels G, Robbins N, Herre E (2008) Endophytic fungi as biocontrol agents of Theobroma cacao pathogens. Biol Control 46:4–14. https://doi.org/10.1016/j.biocontrol.2008.01.012

Mia M, Shamsuddin Z, Wahab Z, Marziah M (2010) Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-culture Musa plantlets under nitrogen free hydroponics condition. Aust J Crop Sci 4

Mishra PK, Bisht SC, Ruwari P, Selvakumar G, Joshi GK, Bisht JK, Bhatt JC, Gupta HS (2011) Alleviation of cold stress in inoculated wheat (Triticum aestivum L.) seedlings with psychrotolerant Pseudomonads from NW Himalayas. Arch Microbiol 193(7):497–513. https://doi.org/10.1007/s00203-011-0693-x

Mohseni M, Norouzi H, Hamedi J, Roohi A (2013) Screening of antibacterial producing actinomycetes from sediments of the Caspian Sea. Int J Mol Cell Med 2(2):64–71

Mueller UG, Sachs JL (2015) Engineering microbiomes to improve plant and animal health. Trends Microbiol 23(10):606–617. https://doi.org/10.1016/j.tim.2015.07.009

Munro RC, Wilson J, Jefwa J, Mbuthia KW (1999) A low-cost method of mycorrhizal inoculation improves growth of *Acacia tortilis* seedlings in the nursery. For Ecol Manag 113(1):51–56. https://doi.org/10.1016/S0378-1127(98)00414-9

Nautiyal C, Srivastava S, Chauhan P, Seem K, Mishra A, Sopory S (2013) Plant growth-promoting bacteria Bacillus amyloliquefaciens NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. Plant Physiol Biochem 66C:1–9. https://doi.org/10.1016/j.plaphy.2013.01.020

Neeno-Eckwall EC, Schottel JL (1999) Occurrence of antibiotic resistance in the biological control of potato scab disease. Biol Control 16(2):199–208. https://doi.org/10.1006/bcon.1999.0756

Nicholson GM (2007) Fighting the global pest problem: preface to the special Toxicon issue on insecticidal toxins and their potential for insect pest control. Toxicon 49(4):413–422. https://doi.org/10.1016/j.toxicon.2006.11.028

Nkonya E, Mirzabaev A, Von Braun J (2016) Economics of land degradation and improvement–a global assessment for sustainable development. Springer Nature, Switzerland

Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J (2009) The role of microbial signals in plant growth and development. Plant Signal Behav 4(8):701–712. https://doi.org/10.4161/psb.4.8.9047

Osman M, El-Sheekh M, El-Naggar A, Gheda S (2010) Effect of two species of cyanobacteria as biofertilizers on some metabolic activities, growth, and yield of pea plant. Biol Fertil Soils 46:861–875. https://doi.org/10.1007/s00374-010-0491-7

Ozdal M, Sezen A, Koc K, Algur Ö (2016) Isolation and characterization of plant growth promoting Rhizobacteria (PGPR) and their effects on improving growth of wheat. J Appl Biol Sci 10:41–46

Pandya U, Saraf M (2010) Role of single fungal isolates and consortia as plant growth promoters under saline conditions. Res J Biotechnol 5:5–9

Panhwar QA, Radziah O, Zaharah AR, Sariah M, Razi IM (2011) Role of phosphate solubilizing bacteria on rock phosphate solubility and growth of aerobic rice. J Environ Biol 32(5):607–612

Parke EL, Linderman RG, Black CH (1983) The role of ectomycorrhizas in drought tolerance of douglas-FIR seedlings 95(1):83–95. https://doi.org/10.1111/j.1469-8137.1983.tb03471.x

Peay KG, Bidartondo MI, Elizabeth Arnold A (2010) Not every fungus is everywhere: scaling to the biogeography of fungal–plant interactions across roots, shoots and ecosystems. 185 (4):878–882. https://doi.org/10.1111/j.1469-8137.2009.03158.x

Pérez-Montaño F, Alías-Villegas C, Bellogín RA, del Cerro P, Espuny MR, Jiménez-Guerrero I, López-Baena FJ, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169(5):325–336. https://doi.org/10.1016/j.micres.2013.09.011

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11(11):789–799. https://doi.org/10.1038/nrmicro3109

Piernik A, Hrynkiewicz K, Wojciechowska A, Szymańska S, Lis MI, Muscolo A (2017) Effect of halotolerant endophytic bacteria isolated from Salicornia europaea L. on the growth of fodder beet (Beta vulgaris L.) under salt stress. Arch Agron Soil Sci 63(10):1404–1418. https://doi.org/10.1080/03650340.2017.1286329

Prasad R, Kumar M, Varma A (2015) Role of PGPR in soil fertility and plant health. In: Egamberdieva D, Shrivastava S, Varma A (eds) Plant-Growth-Promoting Rhizobacteria (PGPR) and medicinal plants. Springer International Publishing, Cham, pp 247–260. https://doi.org/10.1007/978-3-319-13401-7_12

Prasanna R, Babu S, Devi N, Kumar A, Sodimalla T, Monga D, Mukherjee A, Kranthi S, Gokte-Narkhedkar N, Adak A, Yadav K, Nain L, Saxena A (2014) Prospecting cyanobacteria-fortified composts as plant growth promoting and biocontrol agents in cotton. Exp Agric 51. https://doi.org/10.1017/S0014479714000143

Prasanna R, Hossain F, Babu S, Devi N, Adak A, Verma S, Shivay Y, Nain L (2015) Prospecting cyanobacterial formulations as plant-growth-promoting agents for maize hybrids. S Afr J Plant Soil 32:1–9. https://doi.org/10.1080/02571862.2015.1025444

Provorov NA, Tikhonovich IA (2003) Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. Genet Resour Crop Evol 50(1):89–99. https://doi.org/10.1023/A:1022957429160

Provorov NA, Vorobyov NI (2009) Host plant as an organizer of microbial evolution in the beneficial symbioses. Phytochem Rev 8(3):519. https://doi.org/10.1007/s11101-009-9140-x

Provorov NA, Saimnazarov UB, Bahromov IU, Pulatova DZ, Kozhemyakov AP, Kurbanov GA (1998) Effect of rhizobia inoculation on the seed (herbage) production of mungbean (Phaseolus aureusRoxb.) grown at Uzbekistan. J Arid Environ 39(4):569–575. https://doi.org/10.1006/jare.1998.0379

Rahul K, Amrita K, Mukesh S (2014) Trichoderma: a most powerful bio-control agent-a review. J Trends Biosci 7(24):4055–4058

Rana A, Saharan B, Nain L, Prasanna R, Shivay YS (2012) Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. Soil Sci Plant Nutr 58(5):573–582. https://doi.org/10.1080/00380768.2012.716750

Rana KL, Kour D, Verma DP, Yadav AN, Kumar V, Dhaliwal H (2016) Diversity and biotechnological applications of endophytic microbes associated with maize (Zea mays L.) growing in Indian Himalayan regions. In: Proceeding of 86th Annual Session of NASI & Symposium on “Science, Technology and Entrepreneurship for Human Welfare in the Himalayan region”, p 80

Renuka N, Guldhe A, Prasanna R, Singh P, Bux F (2018) Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. Biotechnol Adv 36. https://doi.org/10.1016/j.biotechadv.2018.04.004

Rhodes CJ (2014) Mycoremediation (bioremediation with fungi) – growing mushrooms to clean the earth. Chem Spec Bioavailab 26(3):196–198. https://doi.org/10.3184/095422914X14047407349335

Roberts SC, Shuler ML (1997) Large-scale plant cell culture. Curr Opin Biotechnol 8(2):154–159. https://doi.org/10.1016/S0958-1669(97)80094-8

Roh JY, Choi JY, Li MS, Jin BR, Je YH (2007) Bacillus thuringiensis as a specific, safe, and effective tool for insect pest control. J Microbiol Biotechnol 17(4):547–559

Rojas-Tapias D, Moreno-Galván A, Pardo-Díaz S, Obando M, Rivera D, Bonilla R (2012) Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (Zea mays). Appl Soil Ecol 61:264–272. https://doi.org/10.1016/j.apsoil.2012.01.006

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278(1):1–9. https://doi.org/10.1111/j.1574-6968.2007.00918.x

Ryu C-M, Farag MA, Hu C-H, Reddy MS, Kloepper JW, Paré PW (2004) Bacterial volatiles induce systemic resistance in Arabidopsis. Plant Physiol 134(3):1017–1026. https://doi.org/10.1104/pp.103.026583

Santhanam R, Groten K, Meldau DG, Baldwin IT (2014) Analysis of plant-Bacteria interactions in their native habitat: bacterial communities associated with wild tobacco are independent of

endogenous Jasmonic acid levels and developmental stages. PLoS One 9(4):e94710. https://doi.org/10.1371/journal.pone.0094710
Satyaprakash M, Sadhana EUB, Vani S (2017) Phosphorous and phosphate solubilising Bacteria and their role in plant nutrition. Int J Curr Microbiol Appl Sci 6:2133–2144. https://doi.org/10.20546/ijcmas.2017.604.251
Scagel CF, Linderman RG (1998) Influence of ectomycorrhizal fungal inoculation on growth and root IAA concentrations of transplanted conifers. Tree Physiol 18:739–747. https://doi.org/10.1093/treephys/18.11.739
Sessitsch A, Kuffner M, Kidd P, Vangronsveld J, Wenzel WW, Fallmann K, Puschenreiter M (2013) The role of plant-associated bacteria in the mobilization and phytoextraction of trace elements in contaminated soils. Soil Biol Biochem 60(100):182–194. https://doi.org/10.1016/j.soilbio.2013.01.012
Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2(1):587. https://doi.org/10.1186/2193-1801-2-587
Shtark O, Borisov A, Zhukov V, Provorov N, Tikhonovich I (2010) Intimate associations of beneficial soil microbes with host plants. In: Dixon GR, Tilston EL (eds) Soil microbiology and sustainable crop production. Springer Netherlands, Dordrecht, pp 119–196. https://doi.org/10.1007/978-90-481-9479-7_5
Siddikee MA, Glick BR, Chauhan PS, Yim W, Sa T (2011) Enhancement of growth and salt tolerance of red pepper seedlings (Capsicum annuum L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. Plant Physiol Biochem 49(4):427–434. https://doi.org/10.1016/j.plaphy.2011.01.015
Singh BK, Trivedi P (2017) Microbiome and the future for food and nutrient security. Microb Biotechnol 10(1):50–53. https://doi.org/10.1111/1751-7915.12592
Singh HP, Batish DR, Kohli RK (2006) Handbook of sustainable weed management. CRC Press, Boca Raton, FL
Singh DP, Prabha R, Yandigeri MS, Arora DK (2011) Cyanobacteria-mediated phenylpropanoids and phytohormones in rice (Oryza sativa) enhance plant growth and stress tolerance. Antonie Van Leeuwenhoek 100(4):557–568. https://doi.org/10.1007/s10482-011-9611-0
Singh P, Kumar V, Agrawal S (2014) Evaluation of phytase producing bacteria for their plant growth promoting activities. Int J Microbiol 2014:426483. https://doi.org/10.1155/2014/426483
Singh RN, Gaba S, Yadav AN, Gaur P, Gulati S, Kaushik R, Saxena AK (2016) First high quality draft genome sequence of a plant growth promoting and cold active enzyme producing psychrotrophic Arthrobacter agilis strain L77. Stand Genomic Sci 11(1):54. https://doi.org/10.1186/s40793-016-0176-4
Singh S, Singh V, Pal K (2017) Importance of micro organisms in agriculture. Clim Environ Change Impact Chall Solut 1:93–117
Singh R, Ahirwar N, Tiwari J, Pathak J (2018) Review on sources and effect of heavy metal in soil: its bioremediation. Int J Res Appl Nat Soc Sci 2018:1–22
Smith SE, Read DJ (2010) Mycorrhizal symbiosis. Academic Press, Cambridge, MA
Sparks DL, Huang PM (1985) Physical chemistry of soil potassium. In: Munson RD (ed) Potassium in agriculture. Wiley Online Library, pp 201–276. https://doi.org/10.2134/1985.potassium.c9
Steeghs M, Bais HP, de Gouw J, Goldan P, Kuster W, Northway M, Fall R, Vivanco JM (2004) Proton-transfer-reaction mass spectrometry as a new tool for real time analysis of root-secreted volatile organic compounds in Arabidopsis. Plant Physiol 135(1):47–58. https://doi.org/10.1104/pp.104.038703
Steffens D, Leppin T, Luschin-Ebengreuth N, Min Yang Z, Schubert S (2010) Organic soil phosphorus considerably contributes to plant nutrition but is neglected by routine soil-testing methods. J Plant Nutr Soil Sci 173(5):765–771. https://doi.org/10.1002/jpln.201000079

Suman A, Verma DP, Yadav AN, Srinivasamurthy, Singh A, Prasanna R (2015) Development of hydrogel based bio-inoculant formulations and their impact on plant biometric parameters of wheat (Triticum aestivum L.). Microb Ecol 5. https://doi.org/10.20546/ijcmas.2016.503.103

Suman A, Verma P, Yadav AN, Srinivasamurthy R, Singh A, Prasanna R (2016a) Development of hydrogel based bio-inoculant formulations and their impact on plant biometric parameters of wheat (Triticum aestivum L.). Int J Curr Microbiol Appl Sci 5(3):890–901

Suman A, Yadav AN, Verma P (2016b) Endophytic microbes in crops: diversity and beneficial impact for sustainable agriculture. In: Singh DP, Singh HB, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity, Research perspectives, vol 1. Springer India, New Delhi, pp 117–143. https://doi.org/10.1007/978-81-322-2647-5_7

Svircev Z, Tamas I, Nenin P, Drobac A (1997) Co-cultivation of N2-fixing cyanobacteria and some agriculturally important plants in liquid and sand cultures. Appl Soil Ecol 6(3):301–308. https://doi.org/10.1016/S0929-1393(97)00022-X

Tanti A (2015) Emergence in mapping microbial diversity in tea (Camellia sinensis (L.) O. Kuntze) soil of Assam, north-East India: a novel approach. Eur J Biotechnol Biosci 3:20–25

Teotia P, Kumar V, Kumar M, Shrivastava N, Varma A (2016) Rhizosphere microbes: potassium solubilization and crop productivity – present and future aspects. In: Meena VS, Maurya BR, Verma JP, Meena RS (eds) Potassium solubilizing microorganisms for sustainable agriculture. Springer India, New Delhi, pp 315–325. https://doi.org/10.1007/978-81-322-2776-2_22

Thakur M, Sohal BS (2013) Role of elicitors in inducing resistance in plants against pathogen infection: a review. ISRN Biochem 2013:762412–762412. https://doi.org/10.1155/2013/762412

Thilagar G, Bagyaraj D (2013) Influence of different arbuscular mycorrhizal Fungi on growth and yield of chilly. Proc Natl Acad Sci India Section B Biol Sci 85:71–75. https://doi.org/10.1007/s40011-013-0262-y

Tiwari S, Singh P, Tiwari R, Meena KK, Yandigeri M, Singh DP, Arora DK (2011) Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (Triticum aestivum) and chemical diversity in rhizosphere enhance plant growth. Biol Fertil Soils 47(8):907. https://doi.org/10.1007/s00374-011-0598-5

Treseder KK, Lennon JT (2015) Fungal traits that drive ecosystem dynamics on land. Microbiol Mol Biol Rev 79(2):243–262. https://doi.org/10.1128/MMBR.00001-15

Turan M, Gulluce M, Şahin F (2012) Effects of plant-growth-promoting Rhizobacteria on yield, growth, and some physiological characteristics of wheat and barley plants. Commun Soil Sci Plant Anal 43(12):1658–1673. https://doi.org/10.1080/00103624.2012.681739

Umesha SK, Singh PP, Singh R (2018) Chapter 6: Microbial biotechnology and sustainable agriculture. In: Singh RL, Mondal S (eds) Biotechnology for sustainable agriculture. Woodhead Publishing, Cambridge, England, pp 185–205. https://doi.org/10.1016/B978-0-12-812160-3.00006-4

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C (2013) Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci 4:356. https://doi.org/10.3389/fpls.2013.00356

Vazquez P, Holguin G, Puente ME, Lopez-Cortes A, Bashan Y (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol Fertil Soils 30(5):460–468. https://doi.org/10.1007/s003740050024

Verma DP, Yadav AN, Kazy S, Saxena A, Suman A (2013) Elucidating the diversity and plant growth promoting attributes of wheat (Triticum aestivum) associated acidotolerant bacteria from southern hills zone of India. Nat J Life Sci 10:219–227

Verma DP, Yadav AN, Kazy S, Saxena A, Suman A (2014) Evaluating the diversity and phylogeny of plant growth promoting bacteria associated with wheat (Triticum aestivum) growing in central zone of India. Int J Curr Microbiol App Sci 3:432–447

Verma P, Yadav AN, Khannam KS, Panjiar N, Kumar S, Saxena AK, Suman A (2015) Assessment of genetic diversity and plant growth promoting attributes of psychrotolerant bacteria allied with

wheat (Triticum aestivum) from the northern hills zone of India. Ann Microbiol 65 (4):1885–1899. https://doi.org/10.1007/s13213-014-1027-4
Verma P, Yadav AN, Khannam KS, Kumar S, Saxena AK, Suman A (2016) Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (Triticum aestivum L.) rhizosphere from six diverse agro-ecological zones of India. J Basic Microbiol 56 (1):44–58. https://doi.org/10.1002/jobm.201500459
Verma DP, Yadav AN, Kumar V, Khan M, Saxena A (2018) Microbes in termite management: potential role and strategies. In: Khan MA, Ahmad W (eds) Termites and sustainable management: volume 2 - economic losses and management. Springer International Publishing, Cham, pp 197–217. https://doi.org/10.1007/978-3-319-68726-1_9
Verma P, Yadav AN, Khannam KS, Mishra S, Kumar S, Saxena AK, Suman A (2019) Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India. Saudi J Biol Sci 26(7):1882–1895. https://doi.org/10.1016/j.sjbs.2016.01.042
Wagg C, Bender SF, Widmer F, van der Heijden MGA (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proc Natl Acad Sci U S A 111 (14):5266–5270. https://doi.org/10.1073/pnas.1320054111
Wang ET, Martínez-Romero E (2000) Sesbania herbacea–rhizobium huautlense nodulation in flooded soils and comparative characterization of S. herbacea-Nodulating rhizobia in different environments. Microb Ecol 40(1):25–32. https://doi.org/10.1007/s002480000010
Wang HR, Wang MZ, Yu LH (2009) Effects of dietary protein sources on the rumen microorganisms and fermentation of goats. J Anim Vet Adv 8:1392–1401
Wei C-Y, Lin L, Luo L-J, Xing Y-X, Hu C-J, Yang L-T, Li Y-R, An Q (2014) Endophytic nitrogen-fixing Klebsiella variicola strain DX120E promotes sugarcane growth. Biol Fertil Soils 50(4):657–666. https://doi.org/10.1007/s00374-013-0878-3
Weinzierl R, Henn T, Koehler PG, Tucker CL (1995) Microbial Insecticides, University of Florida. http://edis.ifas.ufl.edu (Accessed 12 July 2021)
Whitton BA, Grainger SL, Hawley GR, Simon JW (1991) Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microb Ecol 21(1):85–98. https://doi.org/10.1007/bf02539146
Xiong W, Jousset A, Guo S, Karlsson I, Zhao Q, Wu H, Kowalchuk GA, Shen Q, Li R, Geisen S (2018) Soil protist communities form a dynamic hub in the soil microbiome. ISME J 12 (2):634–638. https://doi.org/10.1038/ismej.2017.171
Yadav AN (2015) Bacterial diversity of cold deserts and mining of genes for low temperature tolerance, PhD Dissertation. IARI New Delhi, India
Yadav AN, Sachan SG, Verma P, Saxena AK (2015a) Prospecting cold deserts of north western Himalayas for microbial diversity and plant growth promoting attributes. J Biosci Bioeng 119 (6):683–693. https://doi.org/10.1016/j.jbiosc.2014.11.006
Yadav AN, Sharma D, Gulati S, Singh S, Dey R, Pal KK, Kaushik R, Saxena AK (2015b) Haloarchaea endowed with phosphorus solubilization attribute implicated in phosphorus cycle. Sci Rep 5(1):12293. https://doi.org/10.1038/srep12293
Yadav AN, Sachan SG, Verma P, Kaushik R, Saxena AK (2016a) Cold active hydrolytic enzymes production by psychrotrophic bacilli isolated from three sub-glacial lakes of NW Indian Himalayas. J Basic Microbiol 56(3):294–307. https://doi.org/10.1002/jobm.201500230
Yadav AN, Ghosh Sachan S, Verma DP, Saxena A (2016b) Bioprospecting of plant growth promoting psychrotrophic bacilli from cold desert of north western Indian Himalayas. Indian J Exp Biol 54:142–150
Yadav AN, Verma P, Singh B, Chauhan V, Suman A, Saxena AK (2017a) Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. J Adv Biotechnol Microbiol 5(5):1–16
Yadav AN, Verma DP, Kour D, Rana KL, Kumar V, Singh B, Chauhan V, Sugitha TCK, Saxena A, Dhaliwal H (2017b) Plant microbiomes and its beneficial multifunctional plant growth

promoting attributes. Int J Environ Sci Nat Resour 3:1–8. https://doi.org/10.19080/IJESNR.2017.03.555601

Yadav AN, Verma P, Singh B, Chauhan V, Suman A, Saxena AKJABM (2017c) Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. Adv Biotechnol Microbiol 5(5):1–16

Yadav AN, Verma P, Sachan S, Saxena AJEME (2017d) Biodiversity and biotechnological applications of psychrotrophic microbes isolated from Indian Himalayan regions 1:48–54

Yadav AN, Verma DP, Kumar V, Sangwan P, Mishra S, Panjiar N, Gupta V, Saxena A (2018a) Biodiversity of the Genus Penicillium in Different Habitats. In: Gupta VK, Rodriguez-Couto S (eds) New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 3–18. https://doi.org/10.1016/B978-0-444-63501-3.00001-6

Yadav AN, Kumar V, Dhaliwal HS, Prasad R, Saxena AK (2018b) Chapter 15: Microbiome in crops: diversity, distribution, and potential role in crop improvement. In: Prasad R, Gill SS, Tuteja N (eds) Crop improvement through microbial biotechnology. Elsevier, Amsterdam, pp 305–332. https://doi.org/10.1016/B978-0-444-63987-5.00015-3

Yaish MW, Antony I, Glick BR (2015) Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (Phoenix dactylifera L.) and their potential role in salinity tolerance. Antonie Van Leeuwenhoek 107(6):1519–1532. https://doi.org/10.1007/s10482-015-0445-z

Yang Y, Shah J, Klessig DF (1997) Signal perception and transduction in plant defense responses. Genes Dev 11(13):1621–1639. https://doi.org/10.1101/gad.11.13.1621

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14(1):1–4. https://doi.org/10.1016/j.tplants.2008.10.004

Yegorenkova I, Tregubova K, Ignatov V (2013) Paenibacillus polymyxa Rhizobacteria and their synthesized exoglycans in interaction with wheat roots: colonization and root hair deformation. Curr Microbiol:66. https://doi.org/10.1007/s00284-012-0297-y

Yildirim E, Turan M, Donmez MF (2008) Mitigation of salt stress in radish (raphanus sativus l.) by plant growth: promoting rhizobacteria. Rom Biotechnol Lett 13:3933–3943

Young JP, Crossman LC, Johnston AW, Thomson NR, Ghazoui ZF, Hull KH, Wexler M, Curson AR, Todd JD, Poole PS, Mauchline TH, East AK, Quail MA, Churcher C, Arrowsmith C, Cherevach I, Chillingworth T, Clarke K, Cronin A, Davis P, Fraser A, Hance Z, Hauser H, Jagels K, Moule S, Mungall K, Norbertczak H, Rabbinowitsch E, Sanders M, Simmonds M, Whitehead S, Parkhill J (2006) The genome of Rhizobium leguminosarum has recognizable core and accessory components. Genome Biol 7(4):R34. https://doi.org/10.1186/gb-2006-7-4-r34

Žifčáková L, Větrovský T, Howe A, Baldrian P (2016) Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. Environ Microbiol 18 (1):288–301. https://doi.org/10.1111/1462-2920.13026

Živković S, Stojanović S, Ivanović Ž, Gavrilović V, Popović T, Balaž J (2010) Screening of antagonistic activity of microorganisms against Colletotrichum acutatum and Colletotrichum gloeosporioides. Arch Biol Sci 62(3):611–623

Co-functional Activity of Microalgae: Biological Wastewater Treatment and Bio-fuel Production

13

V. C. Akubude, E. O. Ajala, and C. Nzediegwu

Abstract

Water pollution raises serious environmental and health issues around the world because of the high demand for water for several human activities ranging from domestic usage to industrial applications. Water treatment and recycling are imperative to meet industrial and agricultural water needs. Existing water treatment facilities are either cost-intensive or create a negative environmental impact. To save cost and ensure environmental sustainability, sustainable and cost-effective water treatment techniques are highly needed. Biological wastewater treatment using microalgae offers notable advantages in terms of cost and environmental sustainability. Microalgae are a class of microbes that use contaminants in wastewater to generate algae biomass via photosynthetic processes. Algae biomass, in turn, serves as a substrate for the production of economic products such as bio-fuel, chemicals, fish feed, and other value-added products. Generation of bio-fuel from microalgae is an attractive research area because of the enormous benefits that can be derived from algae. This work discusses the biological wastewater treatment using algae, algae cultivation systems, conversion routes for algae biomass, comparison of algae harvesting methods, algae bio-refinery and products, and sustainability of algae-based

V. C. Akubude (✉)
Department of Agricultural and Bioresources Engineering, Federal University of Technology, Owerri, Nigeria

E. O. Ajala
Department of Chemical Engineering, University of Ilorin, Ilorin, Kwara State, Nigeria

C. Nzediegwu
Department of Renewable Resources, University of Alberta, Edmonton, AB, Canada

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bio-fuel. Sustainability can be guaranteed via integration of algae bio-refinery strategy which can produce other value-added products alongside bio-fuel.

Keywords

Bio-fuel · Bio-refinery · Microalgae · Sustainability · Wastewater treatment

13.1 Introduction

Fossil fuel, which is an exhaustible resource, supplies about 80% of energy requirements throughout the world (Rangel-Basto et al. 2018). However, its use heightens environmental issues such as release of greenhouse gas (GHG) that contribute to global warming, thereby resulting in harmful impact on human life (Neves et al. 2018). In addition, energy demand has been increasingly affected by the growing human population (expected to reach 9.5 billion by 2050) and industrialization (Maurizio et al. 2017; Aytav and Kocar 2014). To meet up with such increasing energy demand and simultaneously achieve environmental pollution reduction and renewable waste management, alternative and renewable sources of energy are vital (Maurizio et al. 2017; Aytav and Kocar 2014). Bio-fuel, which is renewable, remains a key solution to the energy problems of humankind, and there are ongoing pursuits to strike a balance between sustainability and cost of using bio-fuel (Olaganathan et al. 2014). Microalgae, which are energetic resources with a multipurpose usage capacity, had been suggested as bio-factories with a significant third-generation bio-fuel (Rangel-Basto et al. 2018). Microalgae are among the classes of microbes (or microorganisms) described as unicellular or multicellular eukaryotes that grow via photosynthesis; they are also known as cyanobacteria or blue-green algae. Microalgae contribute half of global photosynthetic activities (Singh and Saxena 2015). In terms of abundance, there are three main classes of microalgae: diatoms, green algae, and golden algae (Allison 2019). Microalgae are attractive because several products can be derived from their feedstocks. Such products include bio-fuel (e.g., biodiesel, bio-hydrogen, methane, bioethanol), food additives (e.g., pigment astaxanthin, ß-carotene), and pharmaceutical products (Chisti 2007; Hu et al. 2008; Williams and Laurens 2010). Moreover, microalgae are considered excellent feedstocks for bioenergy production because they can easily adapt to harsh environment. Notable benefits of microalgae as a feedstock for bio-fuel production are as follows: (1) the ability to be cultivated on non-fertile land space, (2) high lipid content, (3) fast growth rate, (4) reduced CO_2 mitigation/emissions, and (5) the ability to be cultivated on arid or desert lands. Microalgae-based bio-fuels and bio-product utilizations and their related hitches have been the subject of several literature reviews (Williams and Laurens 2010; Elliott et al. 2012; Greenwell et al. 2010; Wijffels and Barbosa 2010; Pienkos and Darzins 2009). Studies on microalgae use in wastewater treatment especially to remove nutrients and trace metals are also on the rise. For instance, a very recent review (Leong and Chang 2020) discusses several studies on microalgae application in wastewater treatment but with emphasis

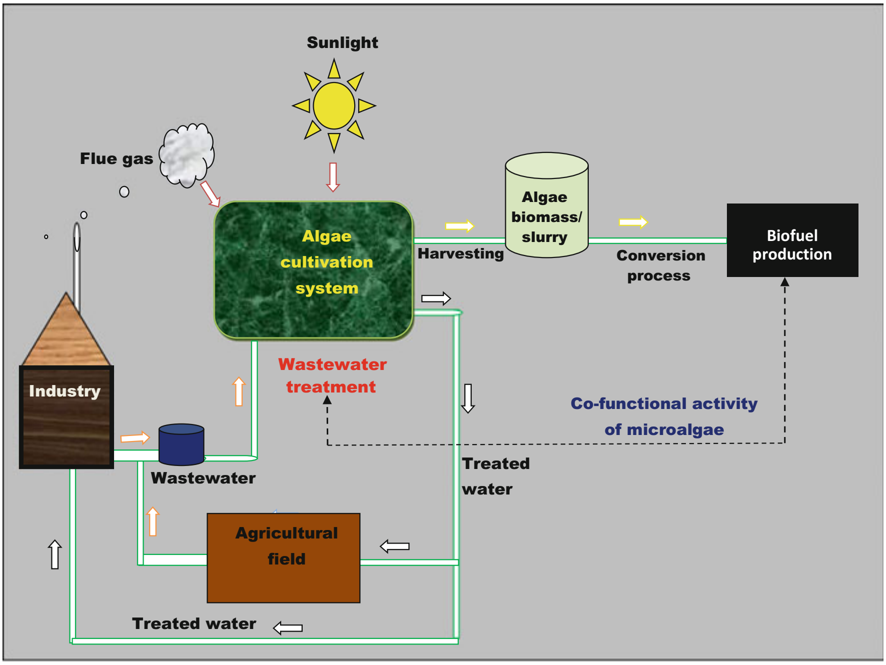

Fig. 13.1 A schematic design of co-functional activity of microalgae in wastewater treatment and bio-fuel production

on trace metal removal. Wastewater is a complex biological system capable of sustaining microalgae growth through a series of relationships, which depend partly on the wastewater composition (e.g., nutrients, heavy metals). Such relationships could simultaneously remove nutrients, immobilize heavy metals, influence microalgae biomass yield, and potentially be harnessed for bio-fuel production. However, there is a gap in the literature that highlights such relationships between wastewater, microalgae, and bio-fuel production. To fill this gap, this paper discusses the co-functional activity of microalgae in biological wastewater treatment and bio-fuel production as summarized in Fig. 13.1.

13.2 Wastewater Treatment Using Microalgae

Wastewater treatment using microalgae can be traced back to the mid-twentieth century in studies proposed by Oswald et al. (1953) and Oswald and Gotaas (1955). Since then, the use of microalgae in wastewater treatment has gained a heightened attention in the literature (Abdel-Raouf et al. 2012; Delgadillo-Mirquez et al. 2016; Li et al. 2020; Lim et al. 2010; Nguyen et al. 2019; Wilde and Benemann 1993; Znad et al. 2018). Of the several microalgae used in wastewater treatment, *Chlorella vulgaris* can be regarded as the most studied because it is easy to cultivate and can tolerate harsh conditions such as metal toxicity (Lim et al. 2010). This section discusses wastewater treatment using microalgae under three subsections, wastewater composition, nutrient, and heavy metal removal.

13.2.1 Wastewater Composition

Wastewater can be described as a complex mix of water with a low to high concentration of non-toxic, less toxic, or high toxic substances such as dyes, dissolved organic compounds, grease, lipids, nutrients, pathogens, pesticide residues, pharmaceutical and personal care products, suspended solids, and trace metals whose proportion or presence depends on the wastewater source (e.g., agriculture, domestic, industry, mining) (Abdel-Raouf et al. 2012; Lim et al. 2010). Because of the several substances present in wastewater, its quality is generally assessed using parameters/indicators such as dissolved oxygen, biological oxygen demand, chemical oxygen demand (COD), pH, salinity, total suspended solids, and turbidity (Delgadillo-Mirquez et al. 2016; Nguyen et al. 2019; Ge and Champagne 2016). In a study by Wilde and Benemann (1993), total nutrient removal efficiency was reported in terms of COD, whereas in other studies nutrient removal efficiency was directly reported as phosphates and nitrates. Wastewaters, especially from agricultural fields, where inorganic fertilizers are applied, are likely enriched with nutrients such as nitrates and phosphates. Discharge of such wastewaters into surface water bodies is regarded as the major cause of surface water pollution through eutrophication, which ultimately results in algal blooms (Abdel-Raouf et al. 2012). Fortunately, microalgae depend on nutrients such as nitrate and phosphate for their growth and development. Such dependence of microalgae on nutrients has been deployed by water treatment experts to remove contaminants (e.g., nutrients) in wastewater. In addition, through mechanisms such as biosorption and bioconversion, other organic and inorganic contaminants (e.g., dyes and heavy metals, respectively) have been removed from wastewater using living or dead microalgae biomass (e.g., *Chlorella vulgaris*) (Lim et al. 2010).

13.2.2 Nutrient Removal

Nutrients in wastewater are mainly a consequence of water discharge/runoff from agricultural fields where inorganic fertilizers are used above recommended dose. The major nutrients of environmental and health concerns are the nitrogen (nitrate, $NO_3^{(-)}$-N; nitrite, $NO_2^{(-)}$-N, ammonium $NH_4^{(+)}$-N) and phosphorus (phosphate, $PO_4^{(3-)}$-P) based. When such nutrients get into surface water bodies, they enhance the growth and development of algae and other unwanted aquatic plants (e.g., microphyte), resulting in eutrophication (Abdel-Raouf et al. 2012). Eutrophication can create hypoxic or anoxic conditions which are detrimental to aquatic ecosystems (Le Moal et al. 2019). To reduce the effects of nutrients in water bodies, several advanced techniques (e.g., chemical precipitation, ozonation, reverse osmosis) have been deployed. Biological treatment using microalgae is by far the most cost-effective and environmental-friendly alternative (Abdel-Raouf et al. 2012). Many studies on the removal of N and P from wastewater using biological treatment with different types of microalgae have been successful in the recent years as shown in Table 13.1.

From such studies (Table 13.1), it is assumed that nutrient removal in wastewater by microalgae maintains a "supplier-consumer" relationship where the wastewater supplies the nutrients to be consumed or metabolized by the microalgae. Such a relationship is possible because microalgae rely on the nutrients in wastewater to grow and develop. Removal efficiencies for P and N in reported wastewaters are 69.3–100% and 38.9–100%, respectively. Such removal efficiencies by a given microalgae in wastewater differ greatly depending on several factors such as substrate type and pH, nutrient type, nutrient initial concentrations, and environmental factors. The influence of pH on nutrient removal in biological wastewater treatment using microalgae is widely reported with pH of the wastewater ranging from 7.5 to 11.5 (Delgadillo-Mirquez et al. 2016; Prandini et al. 2016). During microalgae cultivation, the pH of culture media/wastewater increases as microalgae use up CO_2 from the water medium and/or as organic matter in the wastewater degrades to basic metabolites (Delgadillo-Mirquez et al. 2016; Nguyen et al. 2019). Changes in pH, stimulated by microalgae growth, are crucial in nutrient removal through precipitation, assimilation, and biosorption. Other nutrient removal mechanisms are assimilation by microalgae cells and volatilization (Table 13.1). For a given microalgae, as shown in Table 13.1, removal efficiency of P is always higher as compared to that of N probably because of the additional mechanism (precipitation) for P removal. Under similar experimental conditions, removal efficiency of total P in primary wastewater, secondary wastewater, and petroleum effluent was 100%, while those of total N and micronutrients (e.g., calcium (Ca), magnesium (Mg)) in the same wastewaters differed significantly, partly, attributable to different concentrations of total organic carbon which affected the growth and performance of *Chlorella vulgaris* (Znad et al. 2018). Nutrient removal may be inhibited at certain concentrations of organic carbon in certain wastewaters by inhibiting the growth of microalgae. For petroleum effluent, such inhibitory organic carbon concentration was reported as 109–121 mg L^{-1} for *Chlorella vulgaris* (Znad et al. 2018). Initial nutrient concentrations would partly affect nutrient removal efficiency by microalgae. This was demonstrated for the micronutrient Ca, with an initial concentration of 23 mg L^{-1} in primary wastewater and 27 mg L^{-1} in secondary wastewater (Znad et al. 2018). The concentrations of Ca in the corresponding wastewaters were reduced by 100% and 66% using *Chlorella vulgaris*. Optimum temperature for nutrient removal by microalgae in wastewater has been reported as 15–25 °C (Delgadillo-Mirquez et al. 2016). At low temperatures of 5 °C and below, microalgae may become ineffective for nutrient removal in wastewater due to the inactivation of biological activities. Such an ineffectiveness of microalgae could have a negative implication in biological wastewater treatment and biomass production in both continental and subarctic regions with severe winter periods.

Table 13.1 Nutrient removal in wastewater using microalgae

Microalgae	Additives	Substrate	Nutrient type	Removal efficiency	Mechanism	References
Coelastrella spp.	Zn (0–8 mg L^{-1})	Swine wastewater; pH 8.0–9.6	NH_3-N, total phosphorus (TP)	38.9–62.3% for NH_3-N based on Zn concentration; 69.3–77.6% for TP	NH_3-N was assimilated by microalgae cell through absorption, and NH_3 was volatilized; TP was assimilated	Oswald and Gotaas (1955)
Chlorella vulgaris	None	Primary wastewater (Pw), secondary wastewater (Sw), and petroleum effluent (Pe)	Total nitrogen (TN) and TP	After 13 days, 100% for TN and 80% for TP in Pw; 83% for TN and 100% for TP in Sw; 78% for TN and 100% for TP in Pe	Not mentioned	Lim et al. (2010)
Chlorella vulgaris	Bacteria as a bio-flocculant	Seafood wastewater	As chemical oxygen demand (COD)	As COD: 78.4–88.0%, after 14 days	Not mentioned	Delgadillo-Mirquez et al. (2016)
Native microalgae-bacteria consortium	None	Municipal wastewater	TN and TP	73–83% for TN and 100% for TP	TN was assimilated and stripped; TP was precipitated	Oswald et al. (1953)
Scenedesmus spp.	CO_2 as a biogas	Swine and poultry digestate effluent, pH 7.9	NH_3-N, $PO_4^{(3-)}$-P	96% for NH_3-N and 100% for $PO_4^{(3-)}$-P after 3 days	NH_3-N was assimilated by microalgae	Znad et al. (2018)
Chlorella vulgaris	None	Synthetic wastewater, pH 7.5	$NH_4(+)$-N, TN, $PO_4^{(3-)}$-P	86% for $NH_4(+)$-N, > 84% for TN, and >91% for $PO_4^{(3-)}$-P	$NH_4^{(+)}$-N was assimilated by microalgae, and $PO_4^{(3-)}$-P was precipitated as struvite	

Influence of Additives in Wastewater on Nutrient Removal by Microalgae

Additives such as bio-flocculants, biogas, and metals in wastewater can influence nutrient removal efficiency of microalgae either by affecting the growth and development of the microalgae or by changing the chemical properties (e.g., pH) of the wastewater. Accelerated nutrient removal has been achieved by coupling biogas (e.g., CO_2) to a microalgae-based water treatment (Prandini et al. 2016). Biogas such as CO_2 enhances microalgae photosynthesis by inducing carboxylation which represses the oxygenase activity of rubisco (Prandini et al. 2016). More recently, a bacterium (e.g., *E. coli*) has been used as flocculants to enhance nutrient removal in wastewater by stimulating microalgae growth (Nguyen et al. 2019). Metal additives (e.g., Zn) in wastewater can inhibit microalgae removal capacity for nutrients in wastewater, depending on the type of nutrient (Li et al. 2020; Znad et al. 2018). Such additives can affect the wastewater properties (e.g., pH), which then control nutrient absorbability on the microalgae cells. For example, the assimilation and volatilization of NH_3-N by microalgae cells in swine wastewater were influenced negatively in the presence of Zn. The removal efficiency decreased with an increase in Zn concentration. For phosphorus, however, the removal efficiency was only negatively affected when Zn concentration was less than 2 mg L^{-1}. At higher Zn concentration ($>$2 mg L^{-1}), phosphorus removal by microalgae was facilitated. Therefore, the presence of additives in wastewater should be considered when designing biological wastewater treatment facilities with an additional goal to harvest microalgae for energy production.

13.2.3 Heavy Metal Removal

Heavy metals are a class of transition and post-transition metals that are denser than 5 g cm^{-3}. Although heavy metals can be found naturally in the environment, the main concerns arise from anthropogenic sources such as discharge from agricultural fields and industries (e.g., battery, mining, textile, oil refineries). Heavy metal ubiquitous presence in the environment, including wastewater, has long been declared a global problem (Wilde and Benemann 1993), because they are recalcitrant, non-biodegradable, and environmentally toxic, depending on many factors including the chemical species, dose, and exposure route (Tchounwou et al. 2012). In addition, heavy metals (e.g., cadmium, Cd) are commonly classified as endocrine disruptors and carcinogens (Wilde and Benemann 1993). When in the environment (e.g., soil, water), heavy metals can be taken up by crops and/or move into water bodies. Heavy metals such as thallium (Tl) have been detected in surface water (Xu et al. 2019), and others such as lead (Pb) and Cd have been detected in edible crops (e.g., potatoes) (Nzediegwu et al. 2020), all at concentrations higher than the USEPA (United States Environmental Protection Agency)-recommended dose (Nzediegwu et al. 2020). Thallium in wastewater, as an example, has been associated with Tl poisoning in several countries (Xu et al. 2019). Several approaches to remove heavy metals in wastewater have been widely studied (Abdullah et al. 2019; Fu and Wang 2011). Of the studied approaches (e.g., adsorption, chemical precipitation, ion exchange), bioremoval could be the most cost-effective and environmentally sustainable.

Bioremoval of heavy metals using microalgae provides additional benefits because microalgae biomass can further be utilized for value-added products. Several studies have been implemented in recent years to demonstrate the effectiveness of microalgae in removing heavy metal from wastewater (Birungi and Chirwa 2015; Jaafari and Yaghmaeian 2019). Similar to other sorbent materials, heavy metal removal by microalgae is commonly expressed using parameters from mechanistic models such as Langmuir and Freundlich models or using removal percent commonly estimated as the percent ratio of equilibrium and initial concentrations. Microalgae can be very effective in heavy metal removal in wastewater depending on several factors such as metal type, solution pH, and initial metal concentrations. Microalgae are likely to perform better when heavy metal concentrations in wastewater are less than 250 mg L^{-1} (Birungi and Chirwa 2015; Jaafari and Yaghmaeian 2019). At initial concentrations of 50 to 150 mg L^{-1}, Tl was completely removed from aqueous solution by *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Scenedesmus acuminutus*. However, when Tl concentration was raised to 250 and 500 mg L^{-1}, the removal efficiency was reduced. The sorption capacity (830–1000 mg g^{-1}) of the three microalgae (*Chlamydomonas reinhardtii*, *Chlorella vulgaris*, *Scenedesmus acuminutus*) for Tl removal in the aqueous solution surpassed those of other bio-based sorbents (e.g., activated coal (59.7 mg g^{-1}) and sawdust (13.18 mg g^{-1})). Although optimum pH values for heavy metal removal in wastewater vary from metal to metal, at pH < 4, heavy metal removal by microalgae can be very low due partly to protonation. The optimal pH for Tl removal by microalgae was reported as 5–6 (Birungi and Chirwa 2015). At such pH, more active sites become available on the microalgae surface due to deprotonation. Heavy metal removal capacity would generally increase with an increase in microalgae dose and contact time. The removal of chromium (Cr), cobalt (Co), cadmium (Cd), and iron (Fe) by *Chlorella coloniales* in a synthetic wastewater was studied by Jaafari and Yaghmaeian (2019), and the corresponding removal percents were 33.8–94.8%, 30.5–96.5%, 29.6–92.4%, and 29.1–97.2%, respectively. The more the dose of *Chlorella coloniales*, the higher the removal percentage for all the heavy metals due to enlarged active sites on the microalgae cells. The several mechanisms associated with heavy metal removal by microalgae are well documented in a recent review on bioremediation of heavy metals using microalgae (Leong and Chang 2020).

13.3 Microalgae Cultivation and Harvesting

There are several cultivation pathways for microalgae growth, each having its merits and demerits. Basically, the two classes of cultivation systems are open ponds and photobioreactors as shown in Fig. 13.2. Other cultivation systems such as hybrid and attached growth systems also exist (DOE (U.S. Department of Energy) 2016).

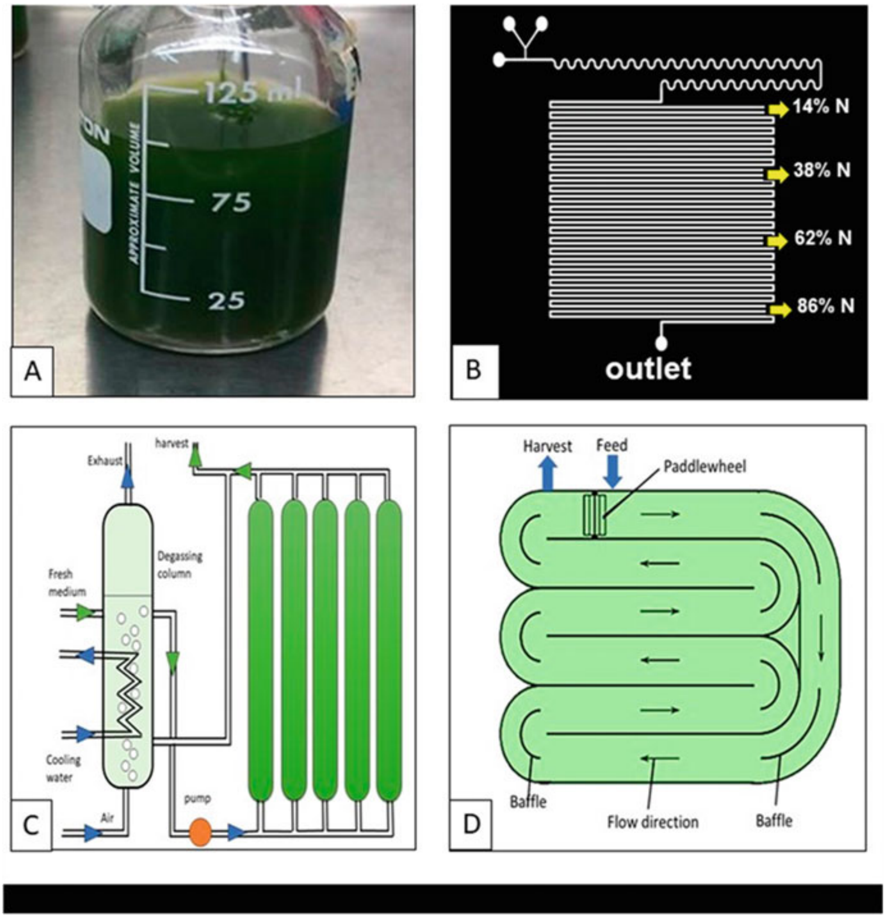

Fig. 13.2 Culture system for microalgae. (**a**) Conventional method. (**b**) Lab-on-a-chip method. (**c**) Photobioreactor. (**d**) Open pond (Sheikh et al. 2017)

13.3.1 Open Ponds

Open ponds, also known as raceways, are made up of independent close-loop recirculation channels in which paddle wheel-generated flow is guided around bends by baffles placed in the flow channels (Greenwell et al. 2010; Sheehan et al. 1998). Open ponds have been used widely for the low-cost production of microalgae because they are easy to construct, simple to operate, and cost-effective (Sheehan et al. 1998; Ugwu et al. 2008). Although they are prone to contaminants (e.g., trace metals and nutrients) because of their exposure to the atmosphere, they give high microalgae biomass yield when not invaded by contaminants. It may be challenging to scale up open ponds for industrial production of microalgae (Rajkumar et al. 2014) because of the wide array of known and yet to be characterized parasites associated with microalgae. Such parasites may pose a significant biological challenge that would affect the growth and development of microalgae and hamper their commercialization (Rajkumar et al. 2014).

13.3.2 Closed System (Photobioreactor PBRs)

Closed system, also known as photobioreactors (PBRs), involves the growing of microalgae cells under a controlled environment where light is supplied artificially from LEDs (light-emitting diodes) or directly by the sun (Laura and Todd 2014). It is made up of a culture vessel and light delivery, gas exchange, and harvesting systems (Greenwell et al. 2010). Closed system produces microalgae cells with a better quality and higher yield relative to the open system. This is because the closed system is restrained from atmospheric contaminants which are very likely in the open system. Different configurations of the closed system, such as vertical tubular PBRs (Masojidek et al. 2009), horizontal tubular PBRs (Masojidek et al. 2009), and flat-plate PBRs (Carvalho et al. 2006), have been implemented. Because of the lighting requirement, a closed system may be difficult to implement in certain climates where reduced sunshine days are associated with winter periods.

13.3.3 Hybrid System

Hybrid system, which is a combination of open ponds and PBRs, is implemented to overcome some of the limitations in closed and open pond systems. It yields excellent biomass productivity with a high nutrient removal. Hybrid system is therefore suitable for large microalgae culture and commercialization (Zijffers et al. 2008; Rawat et al. 2013; Sheikh et al. 2017). In hybrid system, microalgae can be cultured using either in vitro or lab-on-a-chip method (Schenk et al. 2008).

13.3.4 Harvesting Techniques

Microalgae harvesting involves the separation of microalgae cells from water without causing a significant change in the water quality. Different techniques, such as centrifugation, precipitation, sonication, flotation, filtration, and flocculation, have been utilized in microalgae harvesting (Marwa et al. 2019; Brennan and Owende 2010; Grima et al. 2003; Lee et al. 2013; Divakaran and Pillai 2002; Giovannoni et al. 1990; Bosma et al. 2003; Gröschl 1998; Muñoz and Guieysse 2006). Among such techniques, filtration is highly efficient and most suitable for harvesting microalgae (Judge and Earnshaw 2003; Sharma et al. 2013). Effective harvesting has also been achieved by a combination of these techniques. For instance, combining flotation and flocculation gave a better harvesting efficiency than their single application (Judge and Earnshaw 2003; Sharma et al. 2013).

13.4 Bio-refinery

Bio-refinery involves the co-production of a spectrum of high-value bio-based and energy products (Milledge and Heaven 2013; Taylor 2008; Olguin 2012) via the integration of several processes for the complete utilization of algal biomass. Algal biomass is compatible with the integrated bio-refinery vision of producing diverse forms of bio-fuel and other essential co-products (Gonzalez-Delgado and Kafarov 2011). Microalgae bio-refinery (as shown in Fig. 13.3) aims to develop sustainable production technologies of bioenergy and by-products (Davis et al. 2012; Gonzalez et al. 2015). Some of the bio-refinery products are summarized below:

1. ***Bio-alcohol***: Bioethanol production is mainly a consequence of biomass fermentation, and studies have shown that bio-alcohol such as bioethanol, biobutanol, and biomethanol can be generated from microalgae. To date, there are limited studies on algae fermentation despite their potential to serve as a substrate for bioethanol production. Microalgae such as *Chlorococum* sp. has been used as a fermentable substance for bioethanol production under different fermentation conditions. Because it has a simpler system, bioethanol production via fermentation has lower energy consumption as compared to biodiesel production. In addition, side products (e.g., CO_2) obtained in the fermentation process can be recycled as a carbon source for algae growth which could co-functionally facilitate nutrient and trace metal removal in wastewater. Such a recycling of CO_2 can reduce GHG emissions and ameliorate the effects of global warming (Greenwell et al. 2010; Raheem et al. 2018).

   Biobutanol is a bio-fuel that can be obtained from algae biomass via thermochemical conversions such as hydrothermal carbonization, torrefaction (Raheem et al. 2018; Yaşar 2018), and acetone-butanol-ethanol (ABE) fermentation process. The type of biobutanol (in terms of isomer such as *n*-butanol and isobutanol) generated is dependent on the production method used (Robert and Patterson

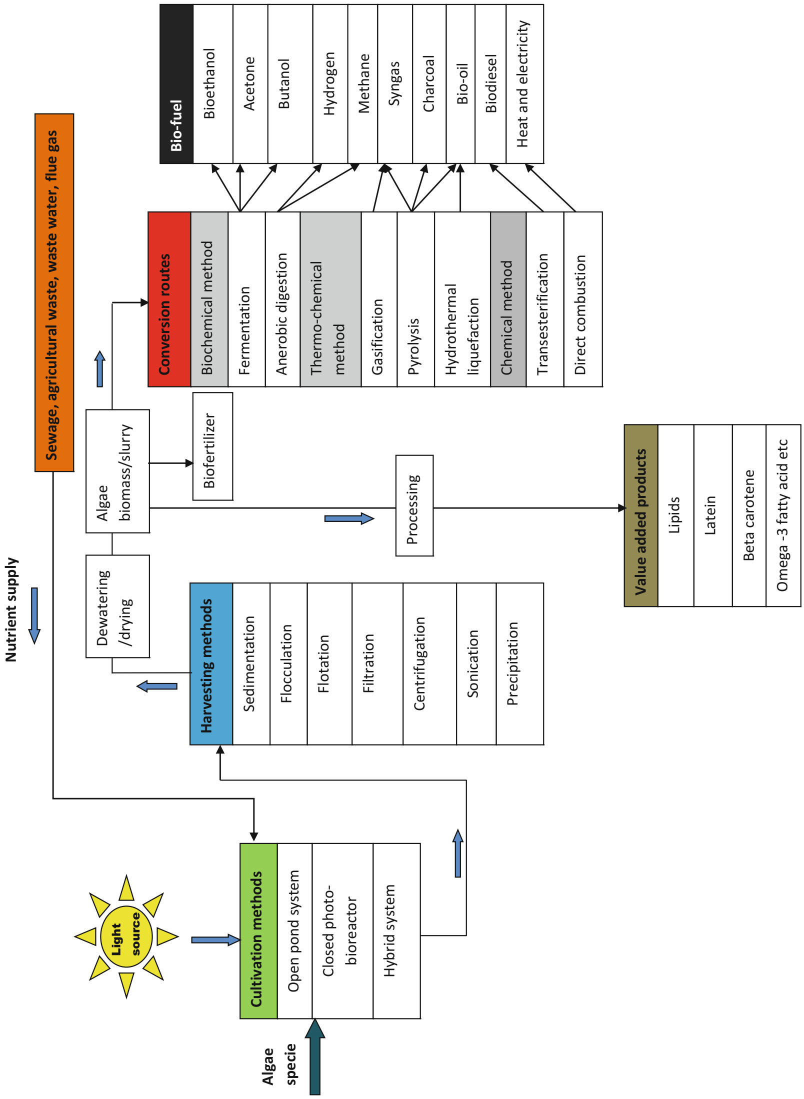

Fig. 13.3 A summary of algae bio-refinery

2004). Studies have shown that biobutanol has similar properties as gasoline (BP and DuPont 2007). In bio-refineries, biobutanol production is more attractive than bioethanol production because butanol has a higher energy density; it also has the properties of both a fuel and an oxygenate, which makes it possible to blend with gasoline in spark-ignition engines (Hagiwara et al. 2015).

2. ***Biodiesel***: Biodiesel is a fatty acid methyl ester derived from bio-based substrates such as vegetable oil, animal fat, and microalgae biomass (Ramadhas et al. 2004). As a liquid fuel produced from biomass resources, biodiesel can be a replacement for fossil-based diesel. Different production techniques applied for biodiesel production include microemulsion (Zabeti et al. 2009), transesterification (Boehman 2005), direct/blend (Keskin et al. 2008; Akansha et al. 2018), and pyrolysis (Greenwell et al. 2010; Wu et al. 1999). There are several microalgae strains with high oil yield, but diatom and green algae offer more prospects for biodiesel production (Allison 2019) because of their advantageous characteristics such as rapid doubling of biomass in matters of hours, easy control of growth process, pervasive presence, and gainful use of biomass (Wang and Seibert 2017).
3. ***Biogas***: Biogas consists of majorly methane and CO_2 and can be used for syngas (i.e., a mixture of hydrogen and carbon monoxide) production via gasification. In recent years, several studies have utilized microalgae as a substrate for biogas production mainly via anaerobic process or pyrolysis (Zamalloa et al. 2012; Inglesby and Fisher 2012; Nguyen et al. 2015; Alghurabie et al. 2013; Buxy et al. 2013; Molina Grima et al. 2003). Anaerobic digestion is an advanced technology where dewatering, which is usually energy-intensive, is not necessary. Hence, wet algae biomass can be used to generate biogas via biochemical processes. The chemical attribute of the microalgae influences the total biogas yield (Varol and Ugurlu 2016). In addition, using microalgae as substrate in biogas production creates an avenue for power generation from wastewater, and the gas generated can be used, on site, as a source of electrical power or heat to offset the cost of biomass processing and extraction (Ward et al. 2014).

4. ***Other value-added products***: *D*espite several research efforts and strategies to generate energy from microalgae biomass, its commercialization is still challenging mainly because of low yield and refining cost. To overcome these challenges, other value-added products, such as food, food additives, health food, animal feed, colorants, omega 3-fatty acid, beta carotene, cosmetic products, and antioxidants, have been produced from microalgae alongside the energy products (Reith 2004; Akubude et al. 2019; Dickinson et al. 2016).

13.5 Bio-fuel Production Using Microalgae

Microalgae have a great potential for bio-fuel production as compared to other crops because it has a high yield of oil, starch, and biomass that is sufficient to produce enough fuel that can meet global demand (Medipally et al. 2015). Potential microalgae strains for bio-fuel generation include *Chlamydomonas reinhardtii*, cyanobacterial mats, *Saccharina japonica*, phytoplanktons, *Symbiodinium* sp., *Nannochloropsis* sp., *Phaeodactylum tricornutum*, *Ostreococcus tauri*, *Botryococcus braunii*, *Chlorella vulgaris*, *Spirulina platensis*, *Chlorella* sp., *Chlorococum* sp., and *Spirogyra* sp. (Hossain et al. 2019). The microalgae usually multiply their biomass within a day with the oil content exceeding 80% by weight of dry biomass (Jegan et al. 2014). However, extraction efficiency and conversion of microalgae oil to bio-energy are the major drawbacks to efficiently utilize microalgae oil to produce bio-energy. To successfully accomplish this, efforts are made to optimize harvesting, extraction, and other processes that would lead to high recovery from the biomass to produce bio-fuels.

Effective extraction of lipids from microalgae is essential to significantly reduce the cost of bio-energy production especially with low-lipid content microalgae. Lipid extraction from microalgae is relatively difficult as compared to extraction of oil from terrestrial crops. This is due to the presence of thick and robust cell wall structure in microalgae; such a cell wall prevents the release of intracellular lipids (Taher et al. 2011). Therefore, for extraction efficiency, microalgae cells have to be disrupted to extents that can ease the lipid recovery. Diverse techniques (such as homogenizer, bed mill, ultrasound, autoclaving, freezing, and osmotic shock) can be employed to disrupt the cell membranes. Homogenizers and bed mills are the most preferred of the methods as they lower the cost of extraction within minimal extraction time (Ali et al. 2014). Thereafter, conversion of oil, starch, or biomass to bio-fuel is another important stage to optimally utilize microalgae for bio-energy production.

Other factors that determine the choice of conversion process to obtain bio-fuels from microalgae include class and amount of biomass feedstock, required form of energy, cost-effectiveness, and precise design requirements. These factors and the expected result of the bio-products are to be justified before deciding on a particular conversion approach of microalgae to bio-fuels (Peng et al. 2019). Conversion to various bio-fuels can be achieved through different production routes and through various technologies which include biochemical/biological conversion (fermentation), thermochemical conversion, chemical reaction (transesterification), and direct combustion (power generation) (Hallenbeck et al. 2016). Recent studies have also shown that the application of nanotechnology in terms of nano-catalyst and nano-additives enhances the overall productivity of microalgae in bio-fuel production (Hossain et al. 2019).

13.5.1 Thermochemical Conversion

Harvested microalgae residues (after some extraction process to remove lipid) and/or the entire microalgae biomass can be converted to bio-fuel through thermochemical processes. These processes optimize the utilization of microalgae for bio-fuel production as no part of the biomass is wasted, thus increasing total energy recovery. Such an optimization of biomass justifies microalgae as a sufficient biomass to overcome global energy crisis (Chen et al. 2015). Thus, thermochemical conversion is an option to process low-lipid microalgae or post-extraction residues of high-lipid microalgae to different fuel molecules or precursors (Milano et al. 2016). To convert lipids, starch, and the whole microalgae biomass into various bio-fuels, thermochemical conversion utilizes several routes, such as gasification, pyrolysis, and hydrothermal liquefaction (HTL) (Greenwell et al. 2010). These routes require no special cultivation conditions for maximum lipid yield because every component of the microalgae is converted into bio-fuels (Chen et al. 2015).

13.5.2 Biochemical Conversion/Fermentation

Fermentation of microalgae biomass utilizes microorganisms and/or enzymes to break down microalgae into liquid fuels such as ethanol, acetone, and butanol. Microalgae biomass has a high starch content that is usually employed in ethanol production. In the biomass conversion process, enzymatic or chemical (acid or base) hydrolysis of the starch to monomeric sugars is followed by yeast fermentation of the monomeric sugars (Suali and Sarbatly 2012). The hydrolysis releases simple sugars from the starch to allow for the biomass fermentation. Several enzymes participate in the biomass fermentation process. Among these enzymes is the pectinase enzyme group which has a high potential to excrete fermentable sugars present in microalgae. Therefore, it is important to select an appropriate enzyme for microalgae/biomass hydrolysis to bioethanol. Irrespective of the enzymes utilized, microalgae fermentation/bioethanol production requires four major stages (Chiaramonti et al. 2015) as shown in Fig. 13.4:

1. Glycolysis: The formation of two molecules of pyruvate ($CHCOCOO-$), water, and hydrogen ions (H^+) as by-products of glucose breakdown.
2. Production of acetaldehyde, CO_2, and H^+ from pyruvate, catalyzed by the enzyme pyruvate decarboxylase.
3. Conversion of acetaldehyde into ethanol anion, aided by coenzyme NADH.
4. Protonation of ethanol anion by hydrogen ions to form bioethanol.

The bioethanol produced is purified by a distillation process to eliminate impurities and water incorporated during the fermentation process as shown in Fig. 13.4.

To obtain a high bioethanol yield, it is important to select microalgae with high starch content. In addition, to ensure sustainability and reduce environmental impact, such microalgae should possess co-functional capabilities for wastewater treatment. Studies show that *C. vulgaris* possesses such capabilities since it can produce about 65% bioethanol (Greenwell et al. 2010) and effectively remove nutrients and heavy metals from wastewater (Table 13.1).

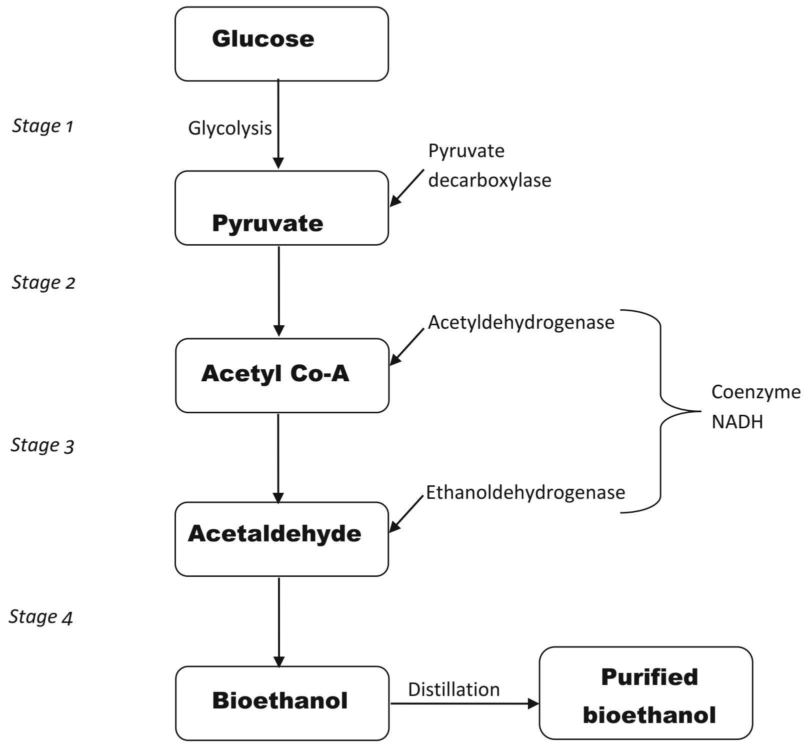

Fig. 13.4 Four major stages of bioethanol production

13.5.3 Chemical Reaction/Transesterification

Transesterification is the most widely used method to convert microalgae biomass into biodiesel; other methods are pyrolysis, blending, direct use, and microemulsions (Medipally et al. 2015). Transesterification is the reaction of triglyceride or lipid extracted from biomass (microalgae) with a mono-alcohol in the presence of a catalyst (e.g., acid, alkali, or enzymes) to produce biodiesel and glycerol (Suali and Sarbatly 2012). To produce biodiesel from microalgae, there are two transesterification routes (direct or single-stage and conventional or two-stage). Each of these routes involves the reaction of triglyceride and methanol in the presence of a catalyst to produce fatty acid methyl esters (FAME) (biodiesel) and glycerol (Hallenbeck et al. 2016). The single-stage route simultaneously carries out the extraction of lipids and transesterification in one-pot synthesis, while the two-stage route requires mechanical or chemical methods to extract the lipids of dried microalgae biomass prior to transesterification reaction and purification steps (Suali and Sarbatly 2012). Irrespective of the route, biodiesel can be more suitable to power compression ignition engines as compared to fossil-based diesel, because biodiesel is a mixture of FAME (Goyal et al. 2008).

In recent years, there is high research interest in the transesterification of microalgae to biodiesel because of the sustainable nature of microalgae biomass. However, the use of microalgae as feedstock for biodiesel production may be limited due to the high processing cost and other challenges (e.g., high enzyme) associated with microalgae conversion and processing (Medipally et al. 2015). In addition, transesterification can be facilitated depending on the choice of a catalyst. For example, in the transesterification of two microalgae species (*Spirogyra* and *Oedogonium*), alkaline-based catalysts resulted in a 90% biodiesel yield (Suali and Sarbatly 2012). However, caution should be taken as undesirable by-products such as soap can be produced via this transesterification route when algae with increased non-esterified fatty acid lipids is used. Another drawback with transesterification is the high moisture content of certain microalgae species. Such high moisture contents in microalgae can hydrolyze triglycerides to diglycerides. Therefore, to overcome the aforementioned drawbacks, microalgae biomass should be preprocessed through drying, lipid extraction, and purification (Suali and Sarbatly 2012). Meanwhile, several research studies had been conducted to model various unit operations in biodiesel production from microalgae with the aim of optimizing the process (Medipally et al. 2015). Intense effort is therefore required to produce biodiesel from microalgae in a sustainable manner to meet global energy demand.

13.5.4 Direct Combustion

Direct combustion is the burning of microalgae biomass in the presence of air either in a furnace, boiler, or steam turbine. The main aim of direct burning is to convert chemical energy into heat or electricity. For direct combustion to be successful, the water content of the microalgae biomass should be less than 50% of its dry weight (Demirbas 2001). Such moisture reduction requirements limit the use of direct combustion to generate electricity from microalgae (Suali and Sarbatly 2012). In addition, the use of direct combustion produces solid inorganic residues or ash, both of which can cause corrosion or fouling of industrial boilers (Milledge et al. 2014; Arora et al. 2019). Because of these shortcomings, co-firing of algae biomass, as compared to direct combustion, is a preferable and more efficient way to generate electricity from microalgae biomass.

13.6 Sustainability of Energy from Microalgae

Microalgae cultivation, also referred to as algal culture, is usually practiced in water bodies, and therefore it does not compete with arable land space. Algal culture can be carried out on saline water or seawater which makes it advantageous over fuel crops which require freshwater for their cultivation. The possibility to use saline water for algal culture gives room for large areas to be potentially utilized. The utilization of wastewater has the potential of decreasing both the operational cost and sustainability challenges associated with bio-fuel production (FAO 2009).

In algal culture, nutrients are supplied to the microalgae system via chemical fertilizers, manures, or organic fertilizers. From a sustainability point of view, the use of chemical fertilizers in the microalgae system is not viable due to high energy input for nitrate and phosphorus production, both of which need to be mined. The use of manure may be viable based on cost but also has its own challenge which is the risk of introducing contaminants such as pathogens and viruses into the microalgae culture system. However, the use of organic fertilizers is more viable as compared to the other two options. This is because the use of organic fertilizer reduces GHG emissions and eliminates chemical fertilizer use with its associated challenges.

Microalgae cultivation and biomass processing (e.g., direct combustion) generate combustion gas which contains GHGs such as CO_2, NO_X, and often SO_2. Such GHGs (e.g., CO_2) can serve as a nutrient source for microalgae cell development. Using GHGs in algal culture system reduces their effect on the environment. However, to avoid contaminating the culture system, gas should be purified (e.g., by stripping or adsorption with microalgae-based adsorbents) before utilizing it within the cultivation system (Mitra and Melis 2008).

Furthermore, microalgae species have been modified for high biomass productivity via the use of modern biotechnological tools. Such modifications include:

1. Decrease in chlorophyll antenna size resulting in more productive use of high light intensity (Dong et al. 2016).
2. Triggering of lipid production (Sheehan et al. 1998).

Genetically modified algae strains seem attractive theoretically but may be infeasible due to safety reasons.

13.7 Conclusions

Sustainable and cost-effective algae-based bio-fuel production depends mainly on the utilization of microalgae which require nutrients such as nitrate and phosphate to generate high-quality biomass. Such nutrients are commonly detected in wastewaters which also contain other contaminants such as heavy metals. Therefore, using wastewater in microalgae cultivation offers economic and environmental benefits. These benefits highlight the co-functionality of microalgae in wastewater treatment for agricultural and industrial use and biomass generation for bio-fuel production through an integrated system. However, additives in wastewater can influence microalgae growth and should be considered when designing such biological wastewater treatment facilities with a co-functional ability to produce quality microalgae biomass for the production of bio-fuel and other value-added products. In addition, at concentrations above 150 mg L^{-1}, trace metals in wastewater can impede microalgae performance in biological wastewater treatment. Therefore, establishing a concentration benchmark (for multi-metals) tolerant for the growth, development, and performance of microalgae is recommended in developing an integrated system for both contaminant removal and microalgae biomass production.

Microalgae-based bio-fuel is a potential substitute for fossil fuel due to the several advantages it offers, but it is still faced with the issue of commercialization because of the high cost of production and high energy required for its processing. There is a need to strategize ways of reducing production cost by improving the production process such as production methods and algae biology. In addition, integrating the co-functional attributes of microalgae such as wastewater treatment, bio-fuel generation, bioremediation, food additive production, and pharmaceutical products in a system known as bio-refinery strategy would alleviate the high operational cost associated with the commercialization process. However, to implement such a bio-refinery strategy, more research on cost analysis is recommended. Meanwhile, such bio-refinery strategy has the potential to reduce operating cost because microalgae biomass would effectively be converted into bio-fuels and other value-added products in a manner that would offset the operating costs (Chiaramonti et al. 2015). Therefore, bio-refinery would facilitate the industrialization and commercialization of microalgae bio-products such as bio-fuels.

References

Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM (2012) Microalgae and wastewater treatment. Saudi J Biol Sci 19(3):257–275. https://doi.org/10.1016/j.sjbs.2012.04.005

Abdullah N, Yusof N, Lau WJ, Jaafar J, Ismail AF (2019) Recent trends of heavy metal removal from water/wastewater by membrane technologies. J Ind Eng Chem 76:17–38. https://doi.org/10.1016/j.jiec.2019.03.029

Akansha M, Arzoo A, Jyoti D, Arindam K, Vinay S (2018) Microreactor technology for biodiesel production: a review. Biomass Convers Biorefin 8(4)

Akubude VC, Nwaigwe KN, Dintwa E (2019) Production of biodiesel from microalgae via nanocatalyzed transesterification process: a review. Mater Sci Energy Technol 2:216–225

Alghurabie IK, Hasan BO, Jackson B, Kosminski A, Ashman PJ (2013) Fluidized bed gasification of Kingston coal and marine microalgae in a spouted bed reactor. Chem Eng Res Des 91:1614–1624

Ali N, Ting Z, Khan YH, Athar MA, Ahmad V, Idrees M (2014) Making biofuels from microalgae – a review of technologies. J Food Sci Technol 1(2):7–14

Allison V (2019) Biodiesel from algae oil. https://microbewiki.kenyon.edu/index.php/biodiesel_from_algae_oil

Arora N, Laurens LML, Sweeney N, Pruthia V, Poluria KM, Pienkos PT (2019) Elucidating the unique physiological responses of halotolerant Scenedesmus sp. cultivated in sea water for biofuel production. Algal Res 37:260–268

Aytav E, Kocar G (2014) Biodiesel from the perspective of Turkey: past, present and future. Renew Sust Energ Rev 25:335–350

Birungi ZS, Chirwa EMN (2015) The adsorption potential and recovery of thallium using green micro-algae from eutrophic water sources. J Hazard Mater 299:67–77. https://doi.org/10.1016/j.jhazmat.2015.06.011

Boehman AL (2005) Biodiesel production and processing: foreword. Fuel Process Technol 86(10):1057–1058

Bosma R, van Spronsen WA, Tramper J, Wijels RH (2003) Ultrasound, a new separation technique to harvest microalgae. J Appl Phycol 15:143–153

BP & DuPont (2007) BP–DuPont biofuels fact sheet. Available online at www.bp.com/liveassets/bp_internet/globalbp/STAGING/global_assets/downloads/B/Bio_bp_dupont_fact_sheet_jun06.pdf

Brennan L, Owende P (2010) Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev 14(2):557–577

Buxy S, Diltz R, Pullammanappallil P (2013) Biogasification of marine algae Nannochloropsis Oculata. In: Wicks G, Simon J, Zidan R, Brigmon R, Fischman G, Arepalli S, Norris A, McCluer M (eds) . John Wiley & Sons, Inc., Hoboken, NJ, USA

Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: a review of enclosed system designs and performances. Biotechnol Prog 22:1490–1506

Chen Y, Wu Y, Hua D, Li C, Harold MP, Wang J, Yang M (2015) Thermochemical conversion of low-lipid microalgae for the production of liquid fuels. RSC Adv:1–28. https://doi.org/10.1039/C4RA13359E

Chiaramonti D, Prussi M, Buffi M, Casini D, Maria A (2015) Thermochemical conversion of microalgae: challenges and opportunities. Energy Procedia 75:819–826

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306

Davis R, Fishman D, Frank ED, Wigmosta MS, Aden A, Coleman AM, Pienkos PT, Skaggs RJ, Venteris ER, Wang MQ (2012) Renewable diesel from algal lipids: an integrated baseline for cost, emissions, and resource potential from a harmonized model. Argonne National Laboratory, National Renewable Energy Laboratory, and Pacific Northwest National Laboratory. U.S. Department of Energy, USA. ANL/ESD/12-4; NREL/TP-5100-55431; PNNL-21437. http://www.nrel.gov/docs/fy12osti/55431.pdf

Delgadillo-Mirquez L, Lopes F, Taidi B, Pareau D (2016) Nitrogen and phosphate removal from wastewater with a mixed microalgae and bacteria culture. Biotechnol Rep 11:18–26. https://doi.org/10.1016/j.btre.2016.04.003

Demirbas A (2001) Biomass resource facilities and biomass conversion processing for fuels and chemicals. Energy Convers Manag 42:1357–1378

Dickinson S, Mientus M, Frey D (2016) A review of biodiesel production from microalgae A review of biodiesel production from microalgae. Clean Techn Environ Policy 19(3):637–668. https://doi.org/10.1007/s10098-016-1309-6

Divakaran R, Pillai VN (2002) Flocculation of river silt using chitosan. Water Res 36:2414–2418

DOE (U.S. Department of Energy) (2016) National Algal Biofuels Technology Review. U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office, USA

Dong T, Knoshaug EP, Davis R, Laurens LML, Van Wychen S, Pienkos PT, Nagle N (2016) Combined algal processing: a novel integrated biorefinery process to produce algal biofuels and bioproducts. Algal Res 19:316–323. https://doi.org/10.1016/j.algal.2015.12.021

Elliott LG, Feehan C, LML L, Pienkos PT, Darzins A, Posewitz MC (2012) Establishment of a bioenergy-focused microalgal culture collection. Algal Res 1:102–113

FAO (2009) Algae-based biofuels: a review of challenges and opportunities for developing countries. FAO, Rome, Italy

Fu F, Wang Q (2011) Removal of heavy metal ions from wastewaters: a review. J Environ Manag 92(3):407–418. https://doi.org/10.1016/j.jenvman.2010.11.011

Ge S, Champagne P (2016) Nutrient removal, microalgal biomass growth, harvesting and lipid yield in response to centrate wastewater loadings. Water Res 88:604–612. https://doi.org/10.1016/j.watres.2015.10.054

Giovannoni SJ, DeLong EF, Schmidt TM, Pace NR (1990) Tangential flow filtration and preliminary phylogenetic analysis of marine picoplankton. Appl Environ Microbiol 56:2572–2575

Gonzalez LE, Díaz GC, Aranda DAG, Cruz YR, Fortes MM (2015) Biodiesel production based in microalgae: a biorefinery approach. Nat Sci 7:358

Gonzalez-Delgado AD, Kafarov V (2011) Microalgae based biorefinery: issues to consider. A review. CT F Cienc Tecnol Futuro 4:5–21

Goyal HB, Seal D, Saxena RC (2008) Bio-fuels from thermochemical conversion of renewable resources: a review. Renew Sust Energ Rev 12:504–517
Greenwell HC, Laurens LML, Shields RJ, Lovitt RW, Flynn KJ (2010) Placing microalgae on the biofuels priority list: a review of the technological challenges. J R Soc Interface 7:703–726
Grima EM, Belarbi EH, Fernández FA, Medina AR, Chisti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20:491–515
Gröschl M (1998) Ultrasonic separation of suspended particles—Part I: Fundamentals. Acta Acust United Acust 84:432–447
Hagiwara S, Nabetani H, Nakajima M (2015) Non-catalytic alcoholysis process for production of biodiesel fuel by using bubble column reactor. J Phys Conf Ser 596:012017
Hallenbeck PC, Grogger M, Mraz M, Veverka D (2016) Solar biofuels production with microalgae. Appl Energy 179:136–145. https://doi.org/10.1016/j.apenergy.2016.06.024
Hossain N, Mahlia TMI, Saidur R (2019) Latest development in microalgae-biofuel production with nano-additives. Biotechnol Biofuel 12:125. https://doi.org/10.1186/s13068-019-1456-0
Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639
Inglesby AE, Fisher AC (2012) Enhanced methane yields from anaerobic digestion of Arthrospira maxima biomass in an advanced flow-through reactor with an integrated recirculation loop microbial fuel cell. Energy Environ Sci 5:7996–8006
Jaafari J, Yaghmaeian K (2019) Optimization of heavy metal biosorption onto freshwater algae (Chlorella coloniales) using response surface methodology (RSM). Chemosphere 217:447–455. https://doi.org/10.1016/j.chemosphere.2018.10.205
Jegan S, Jegathese P, Farid M (2014) Microalgae as a renewable source of energy: a niche opportunity. 2014(ii)
Judge D, Earnshaw D (2003) The European Parliament. Palgrave, Basingstoke, UK
Keskin A, Metin G, Duran A, Kadir A (2008) Using of cotton oil soapstock biodiesel–diesel fuel blends as an alternative diesel fuel. Renew Energy 33(4):553–557
Laura TC, Todd WL (2014) Parasites in algae mass culture. Front Microbiol 5:258–278
Le Moal M, Gascuel-Odoux C, Ménesguen A, Souchon Y, Étrillard C, Levain A, Moatar F, Pannard A, Souchu P, Lefebvre A, Pinay G (2019) Eutrophication: a new wine in an old bottle? Sci Total Environ 651:1–11. https://doi.org/10.1016/j.scitotenv.2018.09.139
Lee DH, Bae CY, Han JI, Park JK (2013) In situ analysis of heterogeneity in the lipid content of single green microalgae in alginate hydrogel microcapsules. Anal Chem 85:8749–8756
Leong YK, Chang JS (2020) Bioremediation of heavy metals using microalgae: recent advances and mechanisms. Bioresour Technol 303:122886. https://doi.org/10.1016/j.biortech.2020.122886
Li X, Yang C, Zeng G, Wu S, Lin Y, Zhou Q, Lou W, Du C, Nie L, Zhong Y (2020) Nutrient removal from swine wastewater with growing microalgae at various zinc concentrations. Algal Res 46:101804. https://doi.org/10.1016/j.algal.2020.101804
Lim SL, Chu WL, Phang SM (2010) Use of Chlorella vulgaris for bioremediation of textile wastewater. Bioresour Technol 101(19):7314–7322. https://doi.org/10.1016/j.biortech.2010.04.092
Marwa GS, Noura SD, Mohamed SZ, Hesham MS (2019) Algal biofuels: current status and key challenges. Energies 12:1920
Masojidek J, Sergejevova M, Rottnerova K, Jirka V, Korecko J, Kopecky J, Zat'kova I, Torzillo G, Stys D (2009) A two-stage solar photobioreactor for cultivation of microalgae based on solar concentrators. J Appl Phycol 21:55–63
Maurizio C, Sonia C, Andrea M (2017) Thermal and fluid dynamic analysis within a batch micro-reactor for biodiesel production from waste vegetable oil. Sustainability 9:2308
Medipally SR, Yusoff F, Banerjee S, Shariff M (2015) Microalgae as sustainable renewable energy feedstock for biofuel production 2015

Milano J, Ong HC, Masjuki HH, Chong WT, Lam MK, Loh PK, Vellayan V (2016) Microalgae biofuels as an alternative to fossil fuel for power generation. Renew Sust Energ Rev 58:180–197
Milledge JJ, Heaven S (2013) A review of the harvesting of micro-algae for biofuel production. Rev Environ Sci Biotechnol 12:165
Milledge JJ, Smith B, Dyer PW, Harvey P (2014) Macroalgae-derived biofuel: a review of methods of energy extraction from seaweed biomass. Energies 7:7194–7222
Mitra M, Melis A (2008) Optical properties of microalgae for enhanced biofuels production. Opt Express 16(26):21807–21820
Molina Grima EM, Belarbi EH, Fernandez FGA, Medina AR, Chisti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20 (7–8):491–515
Muñoz R, Guieysse B (2006) Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res 40:2799–2815
Neves A, Silva T, Reis A, Ramalho L, Eusebio A, Marques IP (2018) Anaerobic digestion of pre-treated microalgae biomass. Chem Eng Trans 64:169–174
Nguyen T, Roddick FA, Fan L (2015) Impact of green algae on the measurement of Microcystis aeruginosa populations in lagoon-treated wastewater with an algae online analyser. Environ Technol 36:556–565
Nguyen TDP, Le TVA, Show PL, Nguyen TT, Tran MH, Tran TNT, Lee SY (2019) Bioflocculation formation of microalgae-bacteria in enhancing microalgae harvesting and nutrient removal from wastewater effluent. Bioresour Technol 272:34–39. https://doi.org/10.1016/j.biortech.2018.09.146
Nzediegwu C, Prasher S, Elsayed E, Dhiman J, Mawof A, Patel R (2020) Biochar applied to soil under wastewater irrigation remained environmentally viable for the second season of potato cultivation. J Environ Manag 254:109822. https://doi.org/10.1016/j.jenvman.2019.109822
Olaganathan R, Ko Qui Shen F, Jun Shen L (2014) Potential and technological advancement of biofuels. Int J Adv Sci Tech Res 4(4) Retrieved from https://commons.erau.edu/publication/833
Olguin EJ (2012) Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. Biotechnol Adv 30:1031–1046
Oswald WJ, Gotaas HB (1955) Photosynthesis in sewage treatment. Trans Am Soc Civil Eng 122:73–105. http://content-calpoly.edu.s3.amazonaws.com/ceenve/1/images/57_photosynthesis_in_sewage_treatment.pdf
Oswald WJ, Gotaas HB, Ludwig HF, Lynch V (1953) Algae symbiosis in oxidation ponds: III. Photosynthetic oxygenation. Sewage Ind Waste 25(6):692–705. www.jstor.org/stable/25032197
Peng L, Fu D, Chu H, Wang Z, Qi H (2019) Biofuel production from microalgae: a review. Environ Chem Lett:0123456789. https://doi.org/10.1007/s10311-019-00939-0
Pienkos PT, Darzins A (2009) The promise and challenges of microalgal-derived biofuels. Biofuels Bioprod Biorefin 3:431–440
Prandini JM, da Silva MLB, Mezzari MP, Pirolli M, Michelon W, Soares HM (2016) Enhancement of nutrient removal from swine wastewater digestate coupled to biogas purification by microalgae Scenedesmus spp. Bioresour Technol 202:67–75. https://doi.org/10.1016/j.biortech.2015.11.082
Raheem A, Prinsen P, Vuppaladadiyam AK, Zhao M, Luque R (2018) A review on sustainable microalgae based biofuel and bioenergy production: recent developments. J Clean Prod 181:42–59
Rajkumar K, Yaakob Z, Takriff MS (2014) Algal biofuel production. Bioresources 9(1):1603–1633
Ramadhas AS, Jayaraj S, Muraleedharan C (2004) Use of vegetable oils as I.C. engine fuels—a review. Renew Energy 29(5):727–242
Rangel-Basto Y, Garcia-Ochoa I, Suárez-Gelvez JH, Zuorro A, Barajas-Solano AF, Urbina-Suarez NA (2018) The effect of temperature and enzyme concentration in the transesterification process of synthetic microalgae oil. Chem Eng Trans 64:331–336

Rawat I, Kumar RR, Mutanda T, Bux F (2013) Biodiesel from microalgae: a critical evaluation from laboratory to large scale production. Appl Energy 103:444–467
Reith JH (2004) Duurzame co-productie van fijnchemicaliënenenergieuit micro-algen : openbaareindrapport E.E.T. project K99005/398510-1010. Petten, Energieonderzoek Centrum Nederland
Robert LG, Patterson TJ (2004) Encyclopedia of toxicology, 3rd edn. Elsevier, Amsterdam, pp 469–475
Schenk PM, Thomas-Hall SR, Stephens E, Marx UC, Mussgnug JH, Posten C (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenergy Res 1:20–43
Sharma KK, Garg S, Li Y, Malekizadeh A, Schenk PM (2013) Critical analysis of current microalgae dewatering techniques. Biofuels 4:397
Sheehan J, Dunahay T, Benemann J, Roessler P (1998) A look back at the US Department of Energy's aquatic species program—biodiesel from algae. Report NREL/TP-580–24190. National Renewable Energy Laboratory, Golden, CO
Sheikh AR, Saad Aldin MA, Mohammad MH, Hugo L (2017) Biological CO_2 fixation with production of microalgae in waste water: a review. Renew Sust Energ Rev 76:379–390
Singh J, Saxena RC (2015) An introduction to microalgae: diversity and significance. In: Kim S-k (ed) Handbook of marine microalgae. Academic Press, Cambridge, pp 11–24
Suali E, Sarbatly R (2012) Conversion of microalgae to biofuel. Renew Sust Energ Rev 16 (6):4316–4342. https://doi.org/10.1016/j.rser.2012.03.047
Taher H, Al-zuhair S, Al-marzouqi AH, Haik Y, Farid MM (2011) A review of enzymatic transesterification of microalgal oil-based biodiesel using supercritical technology 2011. https://doi.org/10.4061/2011/468292
Taylor G (2008) Biofuels and the biorefinery concept. Energy Policy 36:4406–4409
Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. EXS 101:133–164. NIH Public Access. https://doi.org/10.1007/978-3-7643-8340-4_6
Ugwu CU, Aoyagi H, Uchiyama H (2008) Photobioreactors for mass cultivation of algae. Bioresour Technol 99:4021–4028
Varol A, Ugurlu A (2016) Biogas production from microalgae (spirulina platensis) in a two stage anaerobic system. Waste Biomass Valorization 7:193–200
Wang J, Seibert M (2017) Prospects for commercial production of diatoms. Biotechnol Biofuels 10:16. https://doi.org/10.1186/s13068-017-0699-y
Ward AJ, Levis DM, Green FB (2014) Anaerobic digestion of algae biomass: a review. Algal Res 5:204–214
Wijffels RH, Barbosa MJ (2010) An outlook on microalgal biofuels. Science 329(80):796–799
Wilde EW, Benemann JR (1993) Bioremoval of heavy metals by the use of microalgae. Biotechnol Adv 11(4):781–812. https://doi.org/10.1016/0734-9750(93)90003-6
Williams PJB, Laurens MLL (2010) Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy Environ Sci 3:554–590
Wu Q, Shiraiwa Y, Takeda H, Sheng G, Fu J (1999) Liquid-saturated hydrocarbons resulting from pyrolysis of the marine coccolithophores Emiliania huxleyi and Gephyrocapsa oceanica. Mar Biotechnol 1:346–352
Xu H, Luo Y, Wang P, Zhu J, Yang Z, Liu Z (2019) Removal of thallium in water/wastewater: a review. water research 165:114981. https://doi.org/10.1016/j.watres.2019.114981
Yaşar F (2018) Evaluation and advantages of algae as an energy source. JOTCSA 5(3):1309–1318

Zabeti M, Wan MAWD, Aroua MK (2009) Activity of solid catalysts for biodiesel production: a review. Fuel Process Technol 90(6):770–777

Zamalloa C, Boon N, Verstraete W (2012) Anaerobic digestibility of Scenedesmus obliquus and Phaeodactylumtricornutum under mesophilic and thermophilic conditions. Appl Energy 92:733–738

Zijffers JWF, Janssen M, Tramper J, Wijffels RH (2008) Design process of an area-efficient photobioreactor. Mar Biotechnol 10:404–415. https://doi.org/10.1007/s10126-007-9077-2

Znad H, Al Ketife AMD, Judd S, AlMomani F, Vuthaluru HB (2018) Bioremediation and nutrient removal from wastewater by Chlorella vulgaris. Ecol Eng 110:1–7. https://doi.org/10.1016/j.ecoleng.2017.10.008

Microalgae Application in Chemicals, Enzymes, and Bioactive Molecules

14

Paola Lasta, Patricia Arrojo da Silva, Patricia Acosta Caetano, Pricila Nass Pinheiro, Leila Queiroz Zepka, and Eduardo Jacob-Lopes

Abstract

Microalgae feature the ability to develop in different ecosystems, consequently because they are photosynthetic microorganisms with a simple structure. Recently, the interest production of microalgae-based products has increased, due to the integrity of these natural microorganisms in the production of fatty acids, lipids, carbohydrates, pigments, proteins, vitamins, antioxidants, enzymes, and bioactive molecules. It is crucial to study cultivation systems, species, and environmental factors, as they may have strong mastery over the cultivation of microalgae. Microalgae require cheap substrates, such as sunlight, temperature, and carbon dioxide, being used as affordable and effective biocatalysts to obtain products with high added value and commercial applicability (nutraceuticals, pharmaceuticals, biofuels, cosmetics, and functional foods, among others). Therefore, this chapter reports on the mechanisms of formation, production, and application of these components from microalgae (chemicals, enzymes, and bioactive molecules), in addition to providing a description of microalgae-based products, improving the application of microalgal biomass in several segments.

Keywords

Microalgae · Microalgae-based products · Chemicals · Enzymes · Bioactive molecules · Industrial applications

P. Lasta · P. A. da Silva · P. A. Caetano · P. N. Pinheiro · L. Q. Zepka · E. Jacob-Lopes (✉)
Department of Food Science and Technology, Federal University of Santa Maria (UFSM), Santa Maria, RS, Brazil

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14.1 Introduction

Microalgae are considered photosynthetic microorganisms being able to grow in marine or freshwater systems with applications in industrial units (Pignolet et al. 2013). The classification of microalgae includes prokaryotic and eukaryotic microalgae (Borowitzka 2013). According to Gimpel et al. (2015), there are 40,000–70,000 species of microalgae referring to 9 classes, with species not yet discovered or classified.

As photosynthetic microorganisms, microalgae are considered valuable sources for many applications, through biomass, production of various compounds, and environmental applications. Commercial exploitation by these microorganisms has increased due to the need for reliable, efficient, and economical processes (Fernandes et al. 2015).

Microalgae are used to obtain compounds with high added value, requiring only sunlight, temperature, and carbon dioxide (CO_2), for their superior growth (Vilchez et al. 1997). In addition, numerous strains of microalgae produce compounds such as lipids being possibly converted into biodiesel, and microalgae biomass is characterized by having valuable compounds, such as carbohydrates, fatty acids, pigments, proteins, vitamins, and antioxidants, favoring the transformation of these compounds into refined products for various segments (Nur and Buma 2019; Koller et al. 2014).

However, some factors influence the behavior of microalgae, such as high cost of installation and operation, difficulty in controlling culture conditions, contaminating microorganisms, unstable light supply, and local climate (Yen et al. 2013). Therefore, the classes of microalgae and their adaptation changes in climatic factors, in particular light and temperature, must be studied to obtain a successful, economical, and sustainable process (Bhalamurugan et al. 2018).

The industry is focused on expanding products for human nutrition, animal feed, aquaculture food, cosmetic products, pigments, biofertilizers, medicines, and biofuels. Notably, microalgae are producers of many important biochemicals that have not yet been discovered (Rizwan et al. 2018).

Therefore, this chapter addresses an overview of the mechanisms of formation, production, and applications of these components based on microalgae (chemicals, enzymes, and bioactive molecules). In addition, it provides a description of the microalgae-based products generated and their application in various commercial segments.

14.2 Microalgae-Based Products

14.2.1 Chemical Products

Several species of microalgae are considered promising candidates for obtaining useful materials, such as biofuels and chemicals; from this perspective, there is a great demand for more natural and sustainable products (Maeda et al. 2018).

Microalgae are microorganisms capable of accumulating macromolecules, such as proteins, lipids, and carbohydrates, through the capture of solar energy, CO_2, and nutrients. Besides, they are widely used in contemporary nutraceutical foods, through their ability to synthesize aggregate products such as pigments (carotenoids), essential and non-essential amino acids, sugar, enzymes, fatty acids, essential vitamins, and minerals for human consumption (Matos 2017).

These chemical compounds of high added value can be extracted from different microalgae species, being used as bulk commodities in several industrial sectors. In order to obtain chemical products and bioactive compounds, it is essential to cultivate suitable species, together with cultivation systems and ideal conditions, to acquire the desired final product (Mata et al. 2010).

As shown in Fig. 14.1, the productivity of microalgae biomass can be directed to various industrial segments as a source of healthy food, a source of protein for fish farming, a source of animal feed (cattle, swine and poultry), production of cosmetics, medicines and biodiesel (Koller et al. 2012).

The processing of microalgae occurs in three stages: cultivation, harvesting, and extraction. However, the cultivation mode and the choice of species are of paramount importance to define the desired final product (Rizwan et al. 2018). Today, outdoor cultivation is the most economical and viable system in terms of energy and operating costs (Maeda et al. 2018).

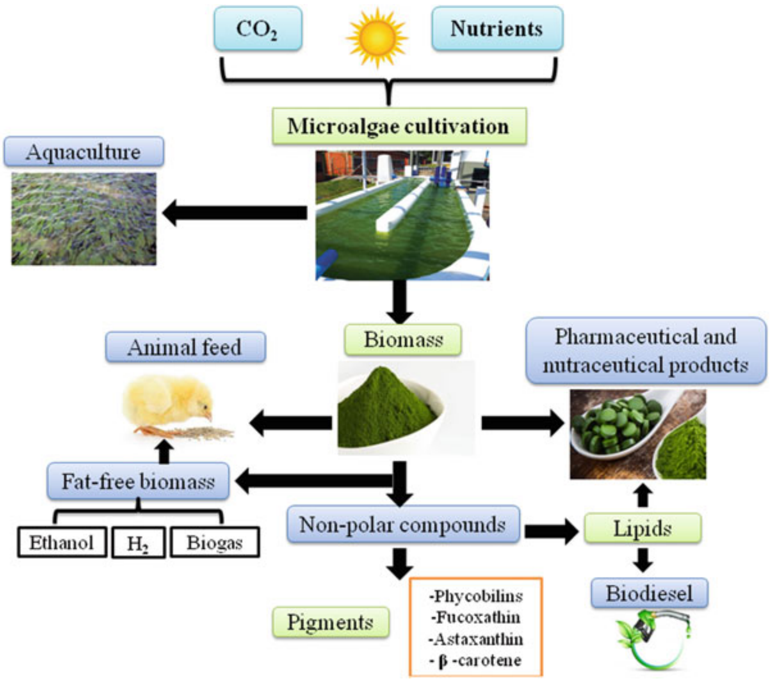

Fig. 14.1 Cultivation of microalgae to generate products with high added value with different industrial segments. (Adapted from Bellou et al. (2014))

The environmental conditions are determining factors for the development of microalgae cultures. In systems exposed to the outdoors, it is imperative to control the parameters, mainly for the generation of biomass (Eriksen 2008). Climatic factors such as carbon dioxide, sunlight, water, temperature, and nutrients are indispensable for the development of microalgae (Chisti 2007). These factors present daily and seasonal variations according to the climatic and geographic location; however, many species behave differently in the face of limiting factors (Bellou et al. 2014).

However, in systems exposed to the outdoors, it is not possible to control the temperature and light intensity, which vary during the day and throughout the year. Therefore, integration technologies and systems engineering are presented, which can be used to optimize the microalgae growth control system and, thus, thrive under ideal conditions (Zhu and Hiltunen 2016).

Notably, when choosing the biomass harvesting method, it is necessary to analyze the profile of the microalgae and their cultivation conditions. So far, the harvesting modes found are flocculation, centrifugation, filtration, sedimentation, and flotation. The capacity of the methods depends on the microalgae strains, including the size, morphology, and composition of the medium used (Japar et al. 2017). After harvesting, the biomass is subjected to the extraction process, obtaining valuable products to produce compounds with high added value (Olguín 2012). In this sense, Fig. 14.2 illustrates several methods of extraction for different chemicals obtained by microalgae.

More specifically, microalgae lipids are divided into storage lipids (triglycerides) and structural lipids (sterols and phospholipids) (Levasseur et al. 2020). However, the increase in lipid production for the generation of biofuels contributes to the sustainability and competitiveness of the microalgae market (Bekirogullari et al. 2017). In this perspective, biodiesel has many benefits, being able to reduce emissions of carbon monoxide, carbon dioxide, and sulfur into the atmosphere. Notably, biodiesel is biodegradable, non-toxic, and own similarities to conventional diesel, such as energy content and chemical and physical properties (Pragya et al. 2013).

Microalgae are fatty acid producers with a high degree of unsaturation and unusual chain lengths, besides to not being found in natural quantities or elsewhere (Hess et al. 2018); examples of fatty acids obtained from microalgae are arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and linolenic acid, being useful to treat diverse disease and as a food source. Several species of microalgae feature the capacity to produce significant amounts of oils and fats, such as omega-3 and omega-6. Currently, DHA is the only microalgae PUFA produced on a commercial scale (Rizwan et al. 2018).

Carbohydrates are divided into sugars (monosaccharides) and polymers (disaccharides, oligosaccharides, and polysaccharides) (Markou et al. 2012). Some strains of microalgae have a high content of carbohydrates (starch and cellulose), being excellent substrates for the generation of bioethanol; the use of carbohydrate to obtain bioethanol becomes advantageous because microalgae proliferate and fix CO_2 at a higher rate compared to other terrestrial plants (Ho et al. 2013).

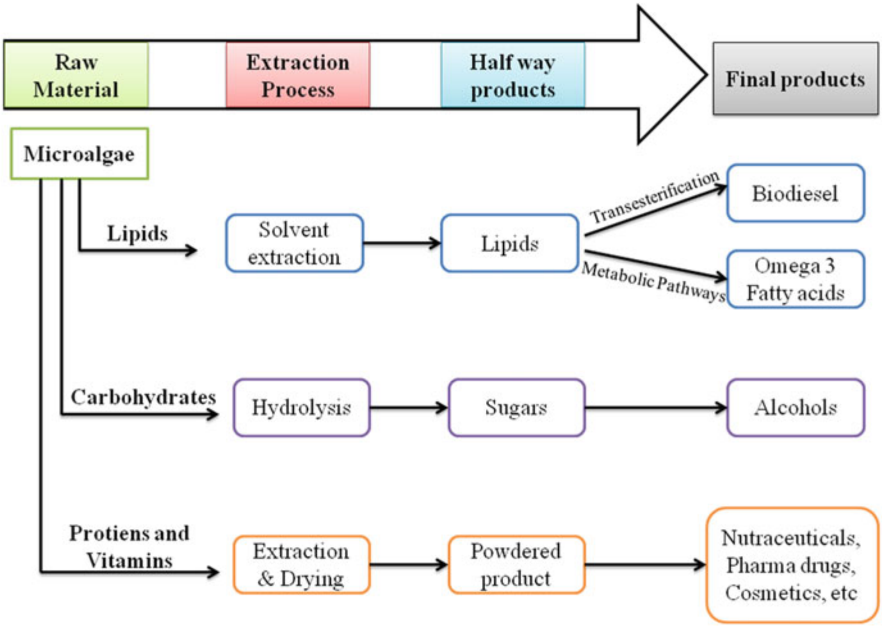

Fig. 14.2 Different chemical products based on microalgae by various extraction processes. (Adapted from Enamala et al. (2018))

Microalgal proteins are similar to food proteins, consequently, due to the excellent profile and compatibility of amino acids, they are used in the pharmaceutical industry to treat some diseases. On the other hand, proteins are defined by their low index of stability and denaturation under acidic and alkaline conditions, making extraction and separation difficult (Markou and Nerantzis 2013; Chew et al. 2017).

Microalgae feature a great capacity to produce several essential vitamins, for example, A, B1, B2, B6, B12, C, E, nicotinate, biotin, folic acid, and pantothenic acid; therefore, the index of microalgal vitamins is of high interest for application in food (Graziani et al. 2013). The number of vitamins is more concentrated in microalgae than in conventional foods (Fabregas and Herrero 1990).

14.2.2 Bioactive Molecules

Bioactive molecules are biologically active substances presenting desirable features in human health. Currently, there is growing interest in the generation of bioactive molecules from natural products through the use of microalgae biomass, driven by a growing body of research demonstrating the beneficial approaches of bioactive molecules to health (Ejike et al. 2017).

The market for bioactive food compounds by microalgae is an opportunity in the segment of bioactive molecules, dominated by synthetic substances and sources of animals and plants (Jacob-Lopes et al. 2019). This composition of biomolecules can be designated as a bioproduct, rich in macro- and micronutrients. Thus, studies show microalgae are an innovation biotechnological applications in industrial sectors related to biofuel, chemical, pharmaceutical, cosmetics, and food (Rodrigues et al. 2015; de Morais et al. 2020). Table 14.1 demonstrates the main bioactive molecules extracted from microalgae (Table 14.1).

These photosynthetic microorganisms can accumulate significant natural bioactive compounds. Among these molecules, natural pigments are the most exciting components produced. Its main classes of phytonutrients are carotenoids, chlorophyll, and phycobiliproteins (Rodrigues et al. 2015). Derivatives of carotenoids can be isolated, and the main include is neoxanthin, violaxanthin, lutein, zeaxanthin, canthaxanthin, mixoxanthophyll, echinenone, (all-*E*)-α-carotene, (all-*E*)-β-carotene, and also its isomeric structures (*Z*). Derived pigments that are produced only by microalgae are echinenone, mixoxanthophyll, and canthaxanthin with antioxidant potential (Nascimento et al. 2020a).

Table 14.1 The main bioactive compounds from microalgae

Bioactive compounds	Microalgae
All-*trans*-β-carotene, all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-canthaxanthin, all-*trans*-myxoxanthophyll, all-*trans*-echinenone, chlorophyll *a,* chlorophyll *b, and* phycobiliproteins	*Phormidium autumnale*
Polysaccharides, phycocyanin, C-phycocyanin, allophycocyanin, phenolic acids, tocopherols (vitamin E), neophytadiene, phytol, PUFAs (*n*-3) fatty acids, oleic acid, linolenic acid, palmitoleic acid, diacylglycerols, terpenoids, alkaloids, and flavonoids	*Spirulina* sp., *S. platensis, S. fusiformis, S. maxima*
All-*trans*-β-carotene, all-*trans*-zeaxanthin, all-*trans*-lutein, *cis*-beta carotene, oleic acid, linolenic acid, palmitic acid, diacylglycerols, and sterols	*Dunaliella salina*
All-*trans*-astaxanthin, all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-canthaxanthin, all-*trans*-β-carotene, and oleic acid	*Haematococcus pluvialis*
Carotenoids, sulfated polysaccharides, sterols, PUFAs (n-3) fatty acids, all-*trans*-canthaxanthin, all-*trans*-astaxanthin, peptide, oleic acid, eicosapentaenoic acid (EPA), all-*trans*-violaxanthin, all-*trans*-lutein, phenolic, terpenoids, alkaloids, phytol, and phenol	*Chlorella* sp., *C. vulgaris*, *C. minutissima*, *C. ellipsoidea*, *C. protothecoides*
Protein (bioactive peptides), all-*trans*-β-carotene, and all-*trans*-lutein	*Scenedesmus obliquus*
Borophycin, cryptophycin, phycocyanin, phenolic, terpenoids, alkaloids, and phycobilins	*Nostoc* sp., *N. muscorum*, *N. humifusum*, *N. linckia*, *N. spongiaeforme*

Adapted from de Morais et al. (2020) and Nascimento et al. (2020b)

The β-carotene is well known to have the highest provitamin A activity (Raposo et al. 2013a). Natural pigments have beneficial health-related properties. Their antioxidant activity balances the harmful effects of free radicals that have been associated with reduced risk of developing several degenerative diseases (da Silva Vaz et al. 2016).

The studies promising pharmacological action bioactivity of chlorophylls compounds is during the photodynamic therapy. There is also, evidences supporting that the role of chlorophyll derivatives can rebalance the gut microbiota (Zepka et al. 2019).

Phycobiliproteins are pigments hydrophilic protein complexes found in microalgae with highly sensitive fluorescent properties that are comprised of C-phycocyanin, phycoerythrin, and allophycocyanin and thus can be used as a detector for specific pharmacological analysis (Levasseur et al., 2020).

Microalgal proteins are sources of alternative nutrition and easy digestibility, acting as antioxidants and antimicrobials, thereby alternative to a healthy diet due to their bioactive peptide, amino acid, fatty acid, and phycobiliprotein content (Zepka et al. 2010). Therefore, when are inserted into a diet, compounds become bio-based decreasing body weight and preventing diet-induced obesity (Patias et al. 2018). The *Spirulina* species, helps in the treatment of many diseases as a result of its exceptional antioxidant, antibacterial, anti-tumor, immunoprotection, and anti-inflammatory properties and also reduces appetite and improves food absorption (Moradi et al. 2019).

The microalgae strain can have a wide range of sterols, from cholesterol to β-sitosterol. These compounds become important due to their antioxidant, anticarcinogenic and anti-inflammatory activity (Fagundes et al. 2020).

Microalgal polysaccharides produce original biopolymers with unique structures and composition to obtain sulfate esters, which are referred to as sulfated polysaccharides (carrageenan, ulvan, and fucoidan), and exhibit various bioactivities, such as antiviral, antioxidant, and anti-inflammatory activities. The production of macromolecules represents high-value products with applications in cosmetics, emulsifiers, food, fabrics, medicines, and stabilizers. Studies are being proposed to use microalgal polysaccharides as a promising prebiotic fiber source (Tang et al. 2020).

But also, according to Lafarga et al. (2020), the microalgae contains a wide spectrum of prophylactic and pharmaceutical phytonutrients including excellent sources of vitamins and minerals. Additionally, there is a lot of attractive biochemical profile that needs better exploited, being the enzymes. Among microorganisms, microalgae become a promising source for future research (Rocha et al. 2018).

14.3 Microalgae Enzymes

The potential application of microalgal biomass extends beyond the bioproducts established to date. There is still great untapped timeliness for utilizing this resource. Indeed, the synthesis of enzymes by microalgae has been recently proposed as a potential niche for the generation of amylases, proteases, lipases, peroxidases, laccases, phytases, and galactosidases (Brasil et al. 2017; Ellatif et al. 2020; Spier et al. 2020).

Amylases belong to a series of glycohydrolase enzymes acting the carbohydrate hydrolysis reaction (Azzopardi et al. 2016; Mohanan and Satyanarayana 2019). Amylases were the first enzymes employed for industrial processes, with large-scale production. Its global market value was estimated at US $ 1.6 billion in 2020, with the largest commercial share of 25%–30% (Mehta and Satyanarayana 2016; Cripwell et al. 2020). Thus, amylases are applied in numerous segments, including the food industry (e.g., in the cheese ripening, baking, chocolate, infant cereal, and brewing and as flavoring), the pharmaceutical industry (high-fructose syrups), the textile and paper industries, and the manufacture of detergents and bioethanol (Brasil et al. 2017; Cripwell et al. 2020; Spier et al. 2020).

Among the enzymes described in microalgae, amylases are the least reported; this is due to their autotrophic metabolism (Patil et al. 2001). However, the species *Chlorella sorokiniana*, *Chlamydomonas reinhardtii*, *Dallina parva*, *Dunaliella tertiolecta*, *Dunaliella marina*, *Klebsormidium* sp., *Oedogonium* sp., *Rhizoclonium* sp., *Rhizoclonium hieroglyphicum*, *Scenedesmus obliquus*, and *Spirogyra* sp. demonstrated amylase activity (Kombrink and Weober 1980; Levi and Gibbs 1984; Patil and Mahajan 2016; Manoj et al. 2018).

Proteases are enzymes that catalyze hydrolytic reactions, resulting in the cleavage of protein molecules into peptides and amino acids and representing the second largest group in market volume. Proteases are extensively exploited in the cleaning, food, and textile manufacturer (Aguilar and Sato 2018; Sharma et al. 2017).

Microalgae studies have shown that protease activity may be related to environmental factors, such as luminosity or nutrient restriction, nitrogen source, and cell apoptosis (Brasil et al. 2017; Spier et al. 2020). Niven (1995) determined the influence of different nutrient sources on the protease activity in *Anabaena variabilis*. In turn, Lockau et al. (1988) and Strohmeier et al. (1994) explored the same microorganisms and their dependence on calcium in the production of protease. Moreover, protease activity has also been observed in *Chlorella vulgaris* and *Arthrospira platensis* (Nanni et al. 2001; Yada et al. 2005; Silva et al. 2017).

Lipases are important biocatalysts due to their capability to hydrolyze triglyceride into fatty acids and glycerol. Accordingly, lipases have attracted commercial attention, falling only behind amylases and proteases in terms of global enzyme sales. The technical features of these enzymes have enabled its introduction in numerous applications in the food, animal feed, pharmaceutical, detergent, paper, cellulose, and bioremediation industries (Brasil et al. 2017; Almeida et al. 2020; Spier et al. 2020).

The lipases investigated in *Botryococcus sudeticus* and *Isochrysis galbana* have promising characteristics for industrial applications, such as substrate specificities, pH endurance (pH 5–11), and temperature resistance (40–70 °C). Furthermore, microalgae species *Arthrospira platensis* and *Nannochloropsis oceanica* also demonstrated the activity of this enzyme (Demir and Teukel 2010; Godet et al.

2012; Savvidou et al. 2016; Yong et al. 2016; Brasil et al. 2017; Hubert et al. 2017; Spier et al. 2020).

Peroxidases are antioxidant enzymes that catalyze the redox reaction for various substrates. Therefore, peroxidases are deliberated a valuable catalyst for several medicinal, industrial, and bioremediation applications (e.g., decolorization of synthetic textile effluents) (Medina et al. 2016). The peroxidases activity was observed in some microalgae strains, as *Coelastrella* sp., *Dunaliella tertiolecta*, *Galdieria sulphuraria*, *Euglena gracilis*, *Phaeodactylum tricornutum*, *Rhizoclonium* sp., *Oedogonium* sp., and *Porphyridium purpureum* (Overbaugh and Fall 1982; Overbaugh and Fall 1985; Murphy et al. 2000; Oesterhelt et al. 2008; Baldev et al. 2013).

The laccase enzyme, act on the oxidation of complex substrates (e.g., phenols and aliphatic or aromatic amines) with the concurrent reduction of a molecule of oxygen and releasing water molecules (Li et al. 2020; Spier et al. 2020). Laccases are widely involved in bioremediation processes of brewing effluents, paper, textile, and pulp (Brasil et al. 2017). Thus, the species *T. aeria* and *C. moewusii* are investigated for the biodegradation of phenolic pollutants in industrial wastewaters (Otto et al. 2015). Moreover, these enzymes have also been described in *Phormidium valderianum*, *Arthrospira platensis*, and *Oscillatoria boryana* (Otto et al. 2010; Afreen et al. 2017; Ellatif et al. 2020).

Phytase enzymes catalyze the hydrolysis of phytate through a series of myo-inositol phosphate intermediate compounds and inorganic phosphate. The phytase own several applications in the industries, mostly in the food manufacturer, where they are used in the elaboration of animal feed, aiming at cost reduction, minimizing the environmental impact, increasing the phosphorus bioavailability, and decreasing the anti-nutrition effect of phytate in monogastric animals (Handa et al. 2020; Sharma et al. 2020).

Due to the commercial appeal of this enzyme, the transgenic microalgae *Chlamydomonas reinhardtii* were studied for the exploration of phytase at a suitable pH and gastrointestinal temperature that can be applied as food supplements. However, other investigated species, such as *C. thermalis Geitler*, *S. bigranulatus Skuja*, and *S. lividus*, also demonstrated phytase activity (Klanbut et al. 2002; Erpel et al. 2016).

Galactosidases are a family of glycoside hydrolase enzymes that further the hydrolysis the glycosidic bonds (Naumoff 2011; Vidya et al. 2020). The enzymes α-galactosidase and β-galactosidase are important glycoside hydrolases with employment in the food, feed, and pharmaceutical industries (Husain 2010; Zhao et al. 2018). The enzyme α-galactosidase aims to hydrolyze the α-galactosyl (α 1–6 linkages) terminal moieties of glycolipids and glycoproteins, whereas, β-galactosidase clive the D-galactosyl (β 1–4 linkages) residues from oligosaccharides or polymers (Spier et al. 2020; Vidya et al. 2020).

In microalgae, α-galactosidase activity was observed in *Poterioochromonas malhamensis* as a metabolic result of external osmotic pressure (Dey and Kauss 1981). On the other hand, the microalgae *C. minutissima*, *D. tertiolecta*, *N. oculata*, *S. obliquus*, and *T. obliquus* demonstrated the formation of β-galactosidase (Davies et al. 1994; Girard et al. 2014; Bentahar et al. 2018; Suwal et al. 2019; Zanette et al. 2019).

Therefore, microalgae can metabolize a wide pool of enzymes, proving how these species are versatile. However, the microalgae are still little explored in comparison to other microorganisms, but numerous enzymes are being investigated and can be applied in various sectors of the industry (Brasil et al. 2017; Spier et al. 2020).

14.4 Industrial Applications of Microalgae

Due to innumerable scientific studies, microalgae have shown great potential as an alternative source for several operations through the bio-refining procedure. Today, microalgae are applied in various industrial sectors, due to their high survival skills in aggressive environments of temperature, pH, light intensity, salinity, and accelerated growth rate (Bhattacharya and Goswami 2020; Tang et al. 2020; Geada et al. 2017). Microalgae are promising for the generation of biodiesel and other products, including feed, nutraceuticals, and food (Giordano and Wang 2018; Rahman 2020).

The world trade in algae biomass is estimated at about US $ 3.8 to 5.4 billion, and approximately 7000 tons of dry algae biomass are manufactured globally (Brasil et al. 2017). In addition, the data indicate that the algae trade is becoming increasingly popular and has the potential to be applied to various branches of the industrial sector afterward (Tang et al. 2020). Today, the United States, Asia, and Oceania control the microalgae generation trade. Despite this, research indicates that Europe is likely to become a significant powerhouse in the field of microalgae bioproducts in the future (Rahman 2020).

Currently, the introduction of synthetic compounds in food, cosmetics, and pharmaceutical products is occurring excessively, becoming emerging issues. Thus, it can cause damage to health, including some allergic reactions and hyperactivity. Therefore, consumers are increasingly demanding and tend to use more natural products, developed from non-toxic resources, hence the emergence of microalgae, as an option for sustainable production and natural sources. Thus, in the market of various sectors of the industry, such as food, beverages, nutritional supplements, and pharmaceutical products, they are implementing bioproducts based on microalgae of the species *Chlorella* sp. and *Spirulina* sp. (Tang et al. 2020). Simultaneously, the species *Dunaliella* and *Arthrospira* (*Spirulina* sp.) also have great potential for numerous commercial uses, as a component for the preparation of various products, not only focusing on the finished product; therefore, the use of microalgae in different sectors of the industry is related to the biomass parameters and structure related to each microalgae (Junior et al. 2020). Thinking in this context, Table 14.2 shows the products and uses of microalgae biomass in different sectors of the industry (Table 14.2).

In fact, microalgae biomass is capable of being used in many industrial sectors. Thus, they are used as a food source, offering a high quality of protein, superior to vegetables. At the same time, microalgae also produce sterols that are used in

Table 14.2 Microalgae products and applications

Microalgae species	Product	Application	References
Arthrospira (*Spirulina*)	Protein, vitamin B_{12}, phycocyanin, carbohydrate	Health food, cosmetics	Chu et al. (2002); Raposo et al. (2013b); Mobin and Alam (2017)
Aphanizomenon flos-aquae	Protein, essential fatty acids, β-carotene	Health food, food supplement	Mobin and Alam (2017)
Phormidium autumnale	Carotenoids, pigments	Food supplement, pharmaceuticals, cosmetics	Rodrigues et al. (2015)
Chlorella zofingiensis	Astaxanthin, colored pigments, biomass, carbohydrate extract	Animal nutrition, health drinks, food supplement	Spolaore et al. (2006)
Chlorella vulgaris	Protein, biomass, carbohydrate extract, ascorbic acid	Health food, food supplement, feeds	Apt and Behrens (1999); Joshi et al. (2018); Mobin and Alam (2017)
Dunaliella salina	Protein, carbohydrate, powders β-carotene, carotenoids, antioxidant	Health food, food supplement, feed	Vonshak (1997); Mobin and Alam (2017); Nascimento et al. (2020a)
Haematococcus pluvialis	Carotenoids, astaxanthin	Health food, food supplement, feed	Nascimento et al. (2020a); Mobin and Alam (2017)
Odontella aurita	Fatty acids, EPA	Pharmaceuticals, cosmetics, anti-inflammatory	Mobin and Alam (2017)
Porphyridium cruentum	Polysaccharides	Pharmaceuticals, cosmetics	Mobin and Alam (2017)
Isochrysis galbana	Fatty acids	Animal nutrition	Lee (1997); Mobin and Alam (2017)
Phaeodactylum tricornutum	Lipids, fatty acids	Nutrition, fuel production	Mobin and Alam (2017)
Lyngbya majuscula	Immune modulators	Pharmaceuticals, nutrition	Mobin and Alam (2017)
Scenedesmus obliquus	Protein, carotenoids	Aquaculture, human nutrition	Mobin and Alam (2017)
Schizochytrium sp.	DHA and EPA	Food, beverage, food supplement	Mobin and Alam (2017)
Crypthecodinium cohnii	DHA	Brain development, infant health, nutrition	Mobin and Alam (2017)
Nannochloropsis oculata	Biomass	Food for larval, juvenile marine fish	Mobin and Alam (2017)
Nannochloropsis sp.	EPA	Food supplement, pharmaceuticals	Mobin and Alam (2017)

Adapted from Rizwan (2018), Mobin and Alam (2017), and Nascimento et al. (2020a) pharmaceutical sectors as medicine for cardiovascular diseases and microalgae extracts used in cosmetics (Rizwan et al. 2018).

About 200 years ago, the Chinese began to implement microalgae as a food source, given the hunger crisis in their country (Geada et al. 2018). Currently, they are used as food in Asian countries, due to their high nutritional value (Chen et al. 2016; Hong et al. 2015; Um and Kim 2009). According to Tang et al. (2020), a commercial product that uses microalgae in its preparation is M&M chocolate, where *Spirulina* sp. biomass is used as a natural dye. In addition, some establishments produce cooking oil using the technique related to microalgae, generating healthier cooking oil. However, despite efforts to implement microalgae as human food, safety regulations and high manufacturing costs make implementation unfeasible. Consequently, it is in the animal feed trade that microalgae biomass is used, because of its nutritional content and health-related advantages. As a result, biomass is generally marketed in dry or wet mode (Geada et al. 2018; Raja et al. 2016).

In the cosmetics area, the company Daniel Jouvance applies microalgae in the production of its products, due to the potential of microalgae to generate compounds that offer essential benefits for the skin (Tang et al. 2020). In addition, extracts derived from *Spirulina* sp. and *Chlorella* sp. are used as compounds in sunscreens. Therefore, it helps to combat sunburn and ultraviolet radiation (Jha et al. 2017).

In the pharmacology sector, representatives who use algae to develop their products include Agri Life SOM, Phytopharma (India) Limited, Piramal Healthcare, Rincon Pharmaceuticals, and Novo Nordisk India Private Ltd, since microalgae synthesize treated substances for the administration of anticancer drugs. Therefore, microalgae use substances of great importance where it is possible to use them for different uses in medical treatments that can be introduced in the development of new drug technologies for the elimination of diseases, specifically in incurable pathologies (Tang et al. 2020).

Through research related to microalgae so far, they demonstrate their development potential in numerous environmental and industrial applications. However, tests are needed to solve some challenges still encountered for microalgae industrialization technologies, such as high installation and operating costs, microbial contamination of the environment, and light and climate conditions, reaching an imbalance. In that regard, researchers must focus on research related to the processing of microalgae, assessing its potential as a raw material with high promising capacity in biotechnological processes, as well as carrying out tests and technological studies on life cycle assessment, thus obtaining results to prove the economic and sustainable viability concerning microalgae-based processing models (Caporgno and Mathys 2018; Rizwan et al. 2018).

14.5 Conclusions and Future Perspectives

The diversity of microalgal products confirms the excellent performance of these microorganisms in the manufacture of various chemical products, enzymes, and bioactive molecules. The components present in microalgae are precious, with a wide range of applicability, such as human and animal nutrition, biofuels, pharmaceuticals, and cosmetics. In order to obtain these compounds, a more detailed study of cultivation conditions, species, and mainly climatic factors is necessary. Compared to other microorganisms, microalgae have benefits in terms of cost-effectiveness, efficiency, and sustainability.

Microalgae should be exploited among the best strains that produce compounds such as pigments, carbohydrates, lipids, fatty acids, proteins, vitamins, antioxidants, and enzymes. However, parameters that interfere with crop growth, such as climatic factors, must be better analyzed so that the number of desired compounds is produced.

Therefore, the commercial-scale generation of microalgae becomes an economical source, encouraging the manufacture of new products developed and commercialized in the next decade. Until the moment, genetic modifications are being studied to increase the production yield of these microorganisms.

In the near future, new research is expected to endeavor to reduce product losses and thus reduce equipment and energy costs. Also, large-scale processing should be further developed, making processes economically viable and environmentally friendly.

References

Afreen S, Shamsi TN, Baig MA et al (2017) Um romance multicopper oxidase (lacase) de cianobactérias: purificação, caracterização com potencial na descoloração de corante antraquinônico. PLoS One 12:1–20. https://doi.org/10.1371/journal.pone.0175144

Aguilar JGS, Sato HH (2018) Microbial proteases: production and application in obtaining protein hydrolysates. Food Res Int 103:253–262. https://doi.org/10.1016/j.foodres.2017.10.044

Almeida JM, Alnoch RC, Souza EM et al (2020) Metagenomics: is it a powerful tool to obtain lipases for application in biocatalysis? BBA-Proteins Proteom 1868(2):140320. https://doi.org/10.1016/j.bbapap.2019.140320

Apt KE, Behrens PW (1999) Commercial developments in microalgal biotechnology. J Phycol 35 (2):215–226. https://doi.org/10.1046/j.1529-8817.1999.3520215.x

Azzopardi E, Lloyd C, Teixeira SR et al (2016) Clinical applications of amylase: novel perspectives. Surgery 160(1):26–37. https://doi.org/10.1016/j.surg.2016.01.005

Baldev E, MubarakAli D, Ilavarasi A et al (2013) Degradation of synthetic dye, rhodamine B to environmentally non-toxic products using microalgae. Colloids Surf B Biointerfaces 105:207–214. https://doi.org/10.1016/j.colsurfb.2013.01.008

Bekirogullari M, Fragkopoulos IS, Pittman JK et al (2017) Production of lipid-based fuels and chemicals from microalgae: an integrated experimental and model-based optimization study. Algal Res 23:78–87. https://doi.org/10.1016/j.algal.2016.12.015

Bellou S, Baeshen MN, Elazzazy AM et al (2014) Microalgal lipids biochemistry and biotechnological perspectives. Biotechnol Adv 32(8):1476–1493. https://doi.org/10.1016/j.biotechadv.2014.10.003

Bentahar J, Doyen A, Beaulieu L et al (2018) Investigation of β-galactosidase production by microalga *Tetradesmus obliquus* in determined growth conditions. J Appl Phycol 31:301–308. https://doi.org/10.1007/s10811-018-1550-y

Bhalamurugan GL, Valerie O, Mark L et al (2018) Valuable bioproducts obtained from microalgal biomass and their commercial applications: a review. Environ Eng Res 23(3):229–241. https://doi.org/10.4491/eer.2017.220

Bhattacharya M, Goswami S (2020) Microalgae-a green multi-product biorefinery for future industrial prospects. Biocatal Agric Biotechnol 101580. https://doi.org/10.1016/j.bcab.2020.101580

Borowitzka MA (2013) High-value products from microalgae-their development and commercialisation. J Appl Phycol 25(3):743–756. https://doi.org/10.1007/s10811-013-9983-9

Brasil BSAF, Siqueira FG, Salum TFC et al (2017) Microalgae and cyanobacteria as enzyme biofactories. Algal Res 25:76–89. https://doi.org/10.1016/j.algal.2017.04.035

Caporgno MP, Mathys A (2018) Trends in microalgae incorporation into innovative food products with potential health benefits. Front Nutr 5:58. https://doi.org/10.3389/fnut.2018.00058

Chen J, Wang Y, Benemann JR et al (2016) Microalgal industry in China: challenges and prospects. J Appl Phycol 28(2):715–725. https://doi.org/10.1007/s10811-015-0720-4

Chew KW, Yap JY, Show PL et al (2017) Microalgae biorefinery: high value products perspectives. Bioresour Technol 229:53–62. https://doi.org/10.1016/j.biortech.2017.01.006

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25(3):294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Chu WL, Phang SM, Miyakawa K et al (2002) Influence of irradiance and inoculum density on the pigmentation of *Spirulina platensis*. Asia Pac J Mol Biol Biotechnol 10(2):109–117

Cripwell R A, Van Zyl WH, Viljoen-Bloom M (2020) Fungal biotechnology: fungal amylases and their applications. Elsevier. doi: https://doi.org/10.1016/b978-0-12-809633-8.21082-0

Davies CM, Apte SC, Peterson SM et al (1994) Plant and algal interference in bacterial β-D-galactosidase and β-D-glucuronidase assays. Appl Environ Microbiol 60:3959–3964. https://doi.org/10.1128/AEM.60.11.3959-3964.1994

Demir BS, Teukel SS (2010) Purification and characterization of lipase from *Spirulina platensis*. J Mol Catal B Enzym 64:123–128. https://doi.org/10.1016/j.molcatb.2009.09.011

Dey PM, Kauss H (1981) α-Galactosidase of *Poterioochromonas malhamensis*. Phytochemistry 20:45–48. https://doi.org/10.1016/0031-9422(81)85216-8

Ejike CE, Collins SA, Balasuriya N et al (2017) Prospects of microalgae proteins in producing peptide-based functional foods for promoting cardiovascular health. Trends Food Sci Tech 59:30–36. https://doi.org/10.1016/j.tifs.2016.10.026

Ellatif SA, El-Sheekh MM, Senousy HH (2020) Role of microalgal ligninolytic enzymes in industrial dye decolorization. Int J Phytoremediation:1–12. https://doi.org/10.1080/15226514.2020.1789842

Enamala MK, Enamala S, Chavali M et al (2018) Production of biofuels from microalgae-a review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. Renew Sust Energ Rev 94:49–68. https://doi.org/10.1016/j.rser.2018.05.012

Eriksen NT (2008) The technology of microalgal culturing. Biotechnol Lett 30(9):1525–1536. https://doi.org/10.1007/s10529-008-9740-3

Erpel F, Restovic F, Arce-Johnson P (2016) Development of phytase-expressing *Chlamydomonas reinhardtii* for monogastric animal nutrition. BMC Biotechnol 16(1):1–7. https://doi.org/10.1186/s12896-016-0258-9

Fabregas J, Herrero C (1990) Vitamin content of four marine microalgae. Potential use as source of vitamins in nutrition. J Ind Microbiol 5(4):259–263. https://doi.org/10.1007/BF01569683

Fagundes MB, Vendruscolo RG, Wagner R (2020) In: Jacob-Lopes E, Maroneze MM, Queiroz MI, Zepka LQ (eds) Sterols from microalgae. Handbook of microalgae-based processes and products. Academic Press. p 573–596. doi:https://doi.org/10.1016/b978-0-12-818536-0.00021-x.

Fernandes BD, Mota A, Teixeira JA et al (2015) Continuous cultivation of photosynthetic microorganisms: approaches, applications and future trends. Biotechnol Adv 33 (6):1228–1245. https://doi.org/10.1016/j.biotechadv.2015.03.004

Geada P, Vasconcelos V, Vicente A et al (2017) In: Rastogi RP, Madamwar D, Pandey A (eds) Microalgal biomass cultivation. Algal green chemistry. Elsevier. p 257-284. doi: 10.1016/B978-0-444-63784-0.00013-8.

Geada P, Rodrigues R, Loureiro L et al (2018) Electrotechnologies applied to microalgal biotechnology-applications, techniques and future trends. Renew Sust Energ Rev 94:656–668. https://doi.org/10.1016/j.rser.2018.06.059

Gimpel JA, Henríquez V, Mayfield SP (2015) In the metabolic engineering of eukaryotic microalgae: potential and challenges come with great diversity. Front Microbiol 6:1376. https://doi.org/10.3389/fmicb.2015.01376

Giordano M, Wang Q (2018) In: Vaz S Jr (ed) Microalgae for industrial purposes, Biomass and green chemistry. Springer, Cham, pp 133–167. https://doi.org/10.1007/978-3-319-66736-2_6

Girard J-M, Roy M-L, Hafsa MB et al (2014) Mixotrophic cultivation of green microalgae *Scenedesmus obliquus* on cheese whey permeate for biodiesel production. Algal Res 5:241–248. https://doi.org/10.1016/j.algal.2014.03.002

Godet S, Herault J, Pencreach G et al (2012) Isolation and analysis of a gene from the marine microalga *Isochrysis galbana* that encodes a lipase-like protein. J Appl Phycol 24:1547–1553. https://doi.org/10.1007/s10811-012-9815-3

Graziani G, Schiavo S, Nicolai MA et al (2013) Microalgae as human food: chemical and nutritional characteristics of the thermo-acidophilic microalga *Galdieria sulphuraria*. Food Funct 4(1):144–152. https://doi.org/10.1039/C2FO30198A

Handa V, Sharma D, Kaur A et al (2020) Biotechnological applications of microbial phytase and phytic acid in food and feed industries. Biocatal Agri Biotechnol 25:101600. https://doi.org/10.1016/j.bcab.2020.101600

Hess SK, Lepetit B, Kroth PG et al (2018) Production of chemicals from microalgae lipids–status and perspectives. Eur J Lipid Sci Tech 120(1):1700152. https://doi.org/10.1002/ejlt.201700152

Ho SH, Huang SW, Chen CY et al (2013) Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. Bioresour Technol 135:191–198. https://doi.org/10.1016/j.biortech.2012.10.015

Hong JW, Jo SW, Yoon HS (2015) Research and development for algae-based technologies in Korea: a review of algae biofuel production. Photosynth Res 123(3):297–303. https://doi.org/10.1007/s11120-014-9974-y

Hubert F, Poisson L, Loiseau C et al (2017) Lipids and lipolytic enzymes of the microalga *Isochrysis galbana*. Oilseeds fats crop lipids 24: D407. Doi: 10.1051 / ocl / 2017023

Husain Q (2010) β galactosidases and their potential applications: a review. Crit Rev Biotechnol 30:41–62. https://doi.org/10.3109/07388550903330497

Jacob-Lopes E, Maroneze MM, Deprá MC et al (2019) Bioactive food compounds from microalgae: an innovative framework on industrial biorefineries. Curr Opin Food Sci 25:1–7. https://doi.org/10.1016/j.cofs.2018.12.003

Japar AS, Takriff MS, Yasin NHM (2017) Harvesting microalgal biomass and lipid extraction for potential biofuel production: a review. J Environ 5(1):555–563. https://doi.org/10.1016/j.jece.2016.12.016

Jha D, Jain V, Sharma B et al (2017) Microalgae based pharmaceuticals and nutraceuticals: an emerging field with immense market potential. Chembioeng Rev 4(4):257–272. https://doi.org/10.1002/cben.201600023

Joshi S, Kumari R, Upasani VN (2018) Applications of algae in cosmetics: an overview. Int J Innov Res Sci Eng Technol 7(2):1269. https://doi.org/10.15680/IJIRSET.2018.0702038

Junior WGM, Gorgich M, Corrêa PS et al (2020) Microalgae for biotechnological applications: cultivation, harvesting and biomass processing. Aquac 735562. https://doi.org/10.1016/j.aquaculture.2020.735562

Klanbut K, Peerapornpisarn Y, Khanongnuch C et al (2002) Phytase from some strains of thermophilic blue-green algae. J Phycol S2:57–60

Koller M, Salerno A, Tuffner P et al (2012) Characteristics and potential of micro algal cultivation strategies: a review. J Clean Prod 37:377–388. https://doi.org/10.1016/j.jclepro.2012.07.044

Koller M, Muhr A, Braunegg G (2014) Microalgae as versatile cellular factories for valued products. Algal Res 6:52–63. https://doi.org/10.1016/j.algal.2014.09.002

Kombrink E, Weober G (1980) Identification and subcellular localization of starch-metabolizing enzymes in the green alga *Dunaliella marina*. Planta 149:130–137. https://doi.org/10.1007/BF00380873

Lafarga T, Fernández-Sevilla JM, González-López C et al (2020) *Spirulina* for the food and functional food industries. Food Res Int 109356. https://doi.org/10.1016/j.foodres.2020.109356

Lee YK (1997) Commercial production of microalgae in the Asia-Pacific rim. J Appl Phycol 9 (5):403–411. https://doi.org/10.1023/A:1007900423275

Levasseur W, Perré P, Pozzobon V (2020) A review of high value-added molecules production by microalgae in light of the classification. Biotechnol Adv 107545. https://doi.org/10.1016/j.biotechadv.2020.107545

Levi C, Gibbs M (1984) Starch degradation in synchronously grown *Chlamydomonas reinhardtii* and characterization of the amylase. Plant Physiol 74:459–463. https://doi.org/10.1104/pp.74.3.459

Li X, Li S, Liang X et al (2020) Applications of oxidases in modification of food molecules and colloidal systems: laccase, peroxidase and tyrosinase. Trends Food Sci Tech 103:78–93. https://doi.org/10.1016/j.tifs.2020.06.014

Lockau W, Massalsky B, Dirmeier A (1988) Purification and partial characterization of a calcium-stimulated protease from the cyanobacterium, *Anabaena variabilis*. Eur J Biochem 172:433–438. https://doi.org/10.1111/j.1432-1033.1988.tb13906.x

Maeda Y, Yoshino T, Matsunaga T et al (2018) Marine microalgae for production of biofuels and chemicals. Curr Opin Biotech 50:111–120. https://doi.org/10.1016/j.copbio.2017.11.018

Manoj BS, Sushma CM, Karosiya A (2018) Western Ghats terrestrial microalgae serve as a source of amylase and antioxidants enzymes. J Pharmacogn Phytochem 7:1555–1560

Markou G, Nerantzis E (2013) Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions. Biotechnol Adv 31(8):1532–1542. https://doi.org/10.1016/j.biotechadv.2013.07.011

Markou G, Angelidaki I, Georgakakis D (2012) Microalgal carbohydrates: an overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels. Appl Microbiol Biot 96(3):631–645. https://doi.org/10.1007/s00253-012-4398-0

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renew Sust Energ Rev 14(1):217–232. https://doi.org/10.1016/j.rser.2009.07.020

Matos ÂP (2017) The impact of microalgae in food science and technology. J Am Oil Chem Soc 94 (11):1333–1350. https://doi.org/10.1007/s11746-017-3050-7

Medina JDC, Woiciechowski AL, Guimarães LRC et al (2016) In: Pandey a, Negi S, Soccol CR (eds) peroxidases. Current developments in biotechnology and bioengineering: production, isolation and purification of industrial products. Elsevier, p. 217-232. doi: https://doi.org/10.1016/B978-0-444-63662-1.00010-5

Mehta D, Satyanarayana T (2016) Bacterial and archaeal α-amylases: diversity and amelioration of the desirable characteristics for industrial applications. Front Microbiol 7:1129. https://doi.org/10.3389/fmicb.2016.01129

Mobin S, Alam F (2017) Some promising microalgal species for commercial applications: a review. Energy Procedia 110:510–517. https://doi.org/10.1016/j.egypro.2017.03.177

Mohanan N, Satyanarayana T (2019) Amylases. In: Schmidt TM (ed) Encyclopedia of microbiology. Fourth, p. 107-126

Moradi S, Ziaei R, Foshati S et al (2019) Effects of *spirulina* supplementation on obesity: a systematic review and meta-analysis of randomized clinical trials. Complement Ther Med 47:102211. https://doi.org/10.1016/j.ctim.2019.102211
de Morais WGJ, Gorgich M, Corrêa PS et al (2020) Microalgae for biotechnological applications: cultivation, harvesting and biomass processing. Aquac 735562. https://doi.org/10.1016/j.aquaculture.2020.735562
Murphy CD, Moore RM, White RL (2000) Peroxidases from marine microalgae. J Appl Phycol 12:507–513. https://doi.org/10.1023/A:1008154231462
Nanni B, Balestreri E, Dainese E et al (2001) Characterization of a specific Phycocyanin hydrolysing protease purified from *Spirulina platensis*. Microbiol Res 156:259–266. https://doi.org/10.1078/0944-5013-00110
Nascimento TC, Cazarin CBB, Maróstica JMR et al (2020a) Microalgae carotenoids intake: influence on cholesterol levels, lipid peroxidation and antioxidant enzymes. Food Res Int 128:108770. https://doi.org/10.1016/j.foodres.2019.108770
Nascimento TC, Nass PP, Fernandes AS et al (2020b) Exploratory data of the microalgae compounds for food purposes. Data Brief 29:105182. https://doi.org/10.1016/j.dib.2020.105182
Naumoff DG (2011) Hierarchical classification of glycoside hydrolases. Biochem Mosc 76:622–635. https://doi.org/10.1134/s0006297911060022
Niven GW (1995) The characterization of two aminopeptidase activities from the cyanobacterium *Anabaena flosaquae*. Biochim Biophys Acta Protein Struct Mol Enzymol 1253:193–198. https://doi.org/10.1016/0167-4838(95)00175-0
Nur MMA, Buma AG (2019) Opportunities and challenges of microalgal cultivation on wastewater, with special focus on palm oil mill effluent and the production of high value compounds. Waste Biomass Valorization 10(8):2079–2097. https://doi.org/10.1007/s12649-018-0256-3
Oesterhelt C, Vogelbein S, Shrestha RP et al (2008) The genome of the thermoacidophilic red microalga *Galdieria sulphuraria* encodes a small family of secreted class III peroxidases that might be involved in cell wall modification. Planta 227:353–362. https://doi.org/10.1007/s00425-007-0622-z
Olguín EJ (2012) Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. Biotechnol Adv 30 (5):1031–1046. https://doi.org/10.1016/j.biotechadv.2012.05.001
Otto B, Schlosser D, Reisser W (2010) First description of a laccase-like enzyme in soil algae. Arch Microbiol 192:759–768. https://doi.org/10.1007/s00018-009-0169-1
Otto B, Beuchel C, Liers C et al (2015) Laccase-like enzyme activities from chlorophycean green algae with potential for bioconversion of phenolic pollutants. FEMS Microbiol Lett 362:1–8. https://doi.org/10.1093/femsle/fnv072
Overbaugh JM, Fall R (1982) Detection of glutathione peroxidases in some microalgae. FEMS Microbiol Lett 13:371–375. https://doi.org/10.1111/j.1574-6968.1982.tb08290.x
Overbaugh JM, Fall R (1985) Characterization of a selenium-independent glutathione peroxidase from *Euglena gracilis*. Plant Physiol 77:437–442. https://doi.org/10.1104/pp.77.2.437
Patias LD, Maroneze MM, Siqueira SF et al (2018) Single-cell protein as a source of biologically active ingredients for the formulation of Antiobesity foods. Alternative and Replacement Foods:317–353. https://doi.org/10.1016/b978-0-12-811446-9.00011-3
Patil K, Mahajan RT (2016) Enzymatic study of fresh water macro and micro algae isolated from Jalgaon, Maharashtra. Int J Pharm Bio Sci 7:207–215. https://doi.org/10.4172/2155-952X.C1.068
Patil KJ, Mahajan RT, Mahajan SR et al (2001) Enzyme profile of fresh water uncultured algae belonging to Bhusawal region, Maharashtra. J Chem biosphere 2(1): 33-28. Doi: https://doi.org/10.20546/ijcrbp.2016.301.013
Pignolet O, Jubeau S, Vaca-Garcia C et al (2013) Highly valuable microalgae: biochemical and topological aspects. J Ind Microbiol Biot 40(8):781–796. https://doi.org/10.1007/s10295-013-1281-7

Pragya N, Pandey KK, Sahoo PK (2013) A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renew Sust Energ Rev 24:159–171. https://doi.org/10.1016/j.rser.2013.03.034

Rahman KM (2020) In: Alam MA, Jing-Liang X, Zhongming W (eds) Food and high value products from microalgae: market opportunities and challenges. Microalgae biotechnology for food, health and high value products. Springer, Singapore, . p 3-27. doi: 10.1007/978-981-15-0169-2_1

Raja R, Hemaiswarya S, Ganesan V et al (2016) Recent developments in therapeutic applications of cyanobacteria. Crit Rev Microbiol 42(3):394–405. https://doi.org/10.3109/1040841X.2014.957640

Raposo MFJ, de Morais RMSC, de Morais AMMB (2013a) Health applications of bioactive compounds from marine microalgae. Life Sci 93(15):479–486. https://doi.org/10.1016/j.lfs.2013.08.002

Raposo MFDJ, De Morais RMSC, Bernardo de Morais AMM (2013b) Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs 11(1):233–252. https://doi.org/10.3390/md11010233

Rizwan M, Mujtaba G, Memon SA et al (2018) Exploring the potential of microalgae for new biotechnology applications and beyond: a review. Renew Sust Energ Rev 92:394–404. https://doi.org/10.1016/j.rser.2018.04.034

Rocha CM, Genisheva Z, Ferreira-Santos P et al (2018) Electric field-based technologies for valorization of bioresources. Bioresour Technol 254:325–339. https://doi.org/10.1016/j.biortech.2018.01.068

Rodrigues DB, Menezes CR, Mercadante AZ et al (2015) Bioactive pigments from microalgae *Phormidium autumnale*. Food Res Int 77:273–279. https://doi.org/10.1016/j.foodres.2015.04.027

Savvidou MG, Sotiroudis TG, Kolisis FN (2016) Cell surface and cellular debris-associated heat-stable lipolytic enzyme activities of the marine alga *Nannochloropsis oceanica*. Biocatal Biotransformation 34:24–32. https://doi.org/10.1080/10242422.2016.1212843

Sharma KM, Kumar R, Panwar S et al (2017) Microbial alkaline proteases: optimization of production parameters and their properties. J Genet Eng Biotechnol 15(1):115–126. https://doi.org/10.1016/j.jgeb.2017.02.001

Sharma A, Ahluwalia O, Tripathi AD et al (2020) Phytases and their pharmaceutical applications: mini-review. Biocatal Agric Biotechnol 23:101439. https://doi.org/10.1016/j.bcab.2019.101439

da Silva Vaz B, Moreira JB, de Morais MG et al (2016) Microalgae as a new source of bioactive compounds in food supplements. Curr Opin Food Sci 7:73–77. https://doi.org/10.1016/j.cofs.2015.12.006

Silva PEC, Souza FASD, Barros RC et al (2017) Enhanced production of fibrinolytic protease from microalgae *Chlorella vulgaris* using glycerol and corn steep liquor as nutrient. Ann Microbiol Res 1: 9-19. Doi: 10.36959/958/564

Spier MR, Peron-schlosser B, Paludo LC et al (2020) Microalgae as enzymes biofactories. In: Jacob-Lopes E, Queiroz MI, Maroneze MM, Zepka LQ (eds) Handbook of microalgae-based processes and products. Academic, New York, pp 687–706. https://doi.org/10.1016/B978-0-12-818536-0.00025-7

Spolaore P, Joannis-Cassan C, Duran E et al (2006) Commercial applications of microalgae. J Biosci Bioeng 101(2):87–96. https://doi.org/10.1263/jbb.101.87

Strohmeier U, Gerdes C, Lockau W (1994) Proteolysis in heterocyst-forming cyanobacteria: characterization of a further enzyme with trypsin-like specificity, and of a prolyl endopeptidase from *Anabaena variabilis*. Z Naturforsch C J Biosci 49:70–78. https://doi.org/10.1515/znc-1994-1-212

Suwal S, Bentahar J, Marciniak A et al (2019) Evidence of the production of galactooligosaccharide from whey permeate by the microalgae *Tetradesmus obliquus*. Algal Res 39:101470. https://doi.org/10.1016/j.algal.2019.101470

Tang DYY, Khoo KS, Chew KW et al (2020) Potential utilization of bioproducts from microalgae for the quality enhancement of natural products. Bioresour Technol 304:122997. https://doi.org/10.1016/j.biortech.2020.122997

Um BH, Kim YS (2009) A chance for Korea to advance algal-biodiesel technology. J Ind Eng Chem 15(1):1–7. https://doi.org/10.1016/j.jiec.2008.08.002

Vidya CH, Kumar BS, Chinmayee CV et al (2020) Purification, characterization and specificity of a new GH family 35 galactosidase from *Aspergillus awamori*. Int J Biol Macromol 156:885–895. https://doi.org/10.1016/j.ijbiomac.2020.04.013

Vilchez C, Garbayo I, Lobato MV et al (1997) Microalgae-mediated chemicals production and wastes removal. Enzyme Microb Technol 20(8):562–572. https://doi.org/10.1016/S0141-0229(96)00208-6

Vonshak A (1997) Microalgal biotechnology: new development in production facilities and products. In 2: Asia-Pacific marine biotechnology conference and 3. Asia-Pacific conference on algal biotechnology, Phuket (Thailand)

Yada E, Nagata H, Noguchi Y et al (2005) An arginine specific protease from *Spirulina platensis*. Mar Biotechnol 7:474–480. https://doi.org/10.1007/s10126-004-4115-9

Yen HW, Hu IC, Chen CY et al (2013) Microalgae-based biorefinery-from biofuels to natural products. Bioresour Technol 135:166–174. https://doi.org/10.1016/j.biortech.2012.10.099

Yong SK, Lim BH, Saleh S et al (2016) Optimisation, purification and characterization of extracellular lipase from *Botryococcus sudeticus* (UTEX 2629). J Mol Catal B Enzym 126:99–105. https://doi.org/10.1016/j.molcatb.2016.02.004

Zanette CM, Mariano AB, Yukawa YS et al (2019) Microalgae mixotrophic cultivation for β-galactosidase production. J Appl Phycol 31:1597. https://doi.org/10.1007/s10811-018-1550-y

Zepka LQ, Jacob-Lopes E, Goldbeck R et al (2010) Nutritional evaluation of single-cell protein produced by *Aphanothece microscopica Nägeli*. Bioresour Technol 101(18):7107–7111. https://doi.org/10.1016/j.biortech.2010.04.001

Zepka LQ, Jacob-Lopes E, Roca M (2019) Catabolism and bioactive properties of chlorophylls. Curr Opin Food Sci 26:94–100. https://doi.org/10.1016/j.cofs.2019.04.004

Zhao R, Zhao R, Tu Y et al (2018) A novel α-galactosidase from the thermophilic probiotic *Bacillus coagulans* with remarkable protease-resistance and high hydrolytic activity. PLoS One 13: e0197067. https://doi.org/10.1371/journal.pone.0197067

Zhu LD, Hiltunen E (2016) Application of livestock waste compost to cultivate microalgae for bioproducts production: a feasible framework. Renew Sust Energ Rev 54:1285–1290. https://doi.org/10.1016/j.rser.2015.10.093

Microbes for the Synthesis of Chitin from Shrimp Shell Wastes

15

Gincy Marina Mathew, Rajeev Kumar Sukumaran, Raveendran Sindhu, Parameswaran Binod, and Ashok Pandey

Abstract

Shrimp meat is consumed globally on a large scale, and their processing releases a large amount of shell waste. The major constituents of shrimp shells are chitin, proteins, calcium carbonate, and lipids. To extract chitin from the shrimp shell, it has to undergo deproteination (DP) to remove the proteins and demineralization (DM) to separate the minerals. Traditionally shrimp shell wastes were dried and directly added as a fertilizer to soil or added in animal feed or dumped in landfills. In recent years, shrimp shell wastes are valorized for producing chitin, chitosan, and other beneficial products like protein hydrolysates, carotenoids, lactic acid, etc. Industries producing chitin are employing chemicals like hydrochloric acid and sodium hydroxide for demineralization and deproteination, respectively, and the residual water is dumped into the water bodies. Considering environmentally friendly approaches, the usage of microorganisms has been tried out for chitin extraction from the shrimp shell. The recent review highlights the production of chitin using microorganisms and mentions other recent greener approaches in chitin production.

Keywords

Chitin · Biofermentation · Deproteination · Demineralization

G. M. Mathew · R. K. Sukumaran · R. Sindhu (✉) · P. Binod
Microbial Processes and Technology Division, CSIR-National Institute for Interdisciplinary Science and Technology (CSIR- NIIST), Trivandrum, India

A. Pandey
Center for Innovation and Translational Research, CSIR- Indian Institute of Toxicology Research (CSIR-IITR), Lucknow, India

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15.1 Introduction

The seafood industry supports the livelihood of 10–12% of the world population (FAO 2020). The proliferation of the different seafood industries across the world has enhanced the problem of waste handling and disposal. The global volume of shellfish food such as prawn, shrimp, crab, lobster, etc. reached 9.3 billion tons according to FAO (2020) reports. Since the shells or exoskeletons of the crustaceans are inedible, a significant portion of the shellfish ends up as waste and finds its way to landfills or water bodies polluting the environment and causing health hazards. Shrimp wastes are alkaline with a pH range of 7.5–8 that supports the growth of putrefying microbes that are hazardous to the environment (Bhaskar et al. 2007).

Due to the massive scale of shellfish landing and its processing, the waste generation is also huge, and the amount is increasing annually. Currently, there is no satisfactory technology for the valorization of these entire shellfish wastes to value-added products. In some Southeast Asian countries like Indonesia, Thailand, and the Philippines, the monetary value of dry shellfish wastes is very low, with prices ranging from 100 to 120 USD per ton. Considering their lack of profitability, the shellfish wastes are not utilized and eventually get disposed in water bodies or land filled causing environmental pollution. In developed countries like Australia and Canada, the shellfish waste disposal is costly, with a processing cost of up to 150 USD per ton. There are several active programs in the developed seafaring nations for valorizing this resource which includes eco-friendly waste management strategies in Canada; production of lime for construction removal of heavy metals and usage as pre-formed baits in fishery, etc. in the UK; conversion to aquaculture feed in Japan; and chitin and chitosan production in the USA and most Scandinavian countries (www.seafish.org). Interestingly, Norway has developed a technology to utilize seafood-processing waste involving enzyme treatment followed by membrane filtration at nano-level to target value-added products (The Marine Products Export Development Authority [MPEDA] 2013). However, a fully integrated process/technology for an effective total shrimp shell waste management is yet to emerge globally.

The shrimp shell composition varies from species, seasonal variation, and geographic locations. The constituents of the shrimp shell wastes include 10–25% chitin, 13–50% protein, 15–70% mineral matter (Babu et al. 2008), and low-fat content (Cira et al. 2002). The major mineral found in the shrimp shell cuticle is calcium carbonate, which helps in strengthening the exoskeleton. Depending on the tons of renewable shrimp shell waste generated annually, the potential value of these wastes is left unexplored. It is necessary to consider a greener prawn shell waste management methodology benefitting the environment and produce value-added products for economic development. The value-added products like proteins generated from the prawn shell waste are used in animal feed for livestock and aquaculture (Evers and Carroll 1998; Sumardiono and Siqhny 2018). Calcium carbonate derived from the prawn shell wastes are in greater demand due to their biological components and superior origin than limestone and marble. Chitin is the most significant component derived from the shellfish wastes with applications in different fields varying from water purification to biomedical applications. The current commercial method for shellfish waste management uses harmful chemicals, creating environmental and economic issues. Utilization of crustacean shell wastes for the extraction of chitin and other bioactive compounds has been studied using different methods including enzymatic approaches (Hayes et al. 2008), microwave irradiation (El Knidri et al. 2016), and ultrasonication (Kjartansson et al. 2006). Strategy for chitin extraction from shrimp wastes includes demineralization (DM), deproteination (DP), and bleaching/depigmentation; and deacetylation can yield chitosan (CHS) which is an even more valuable product finding applications as surgical sutures and wound dressings (Değim et al. 2002). All these processes use acidic and basic solutions under elevated temperature and longer incubation times.

Addition of strong acids and bases for the chitin extraction affects the physiochemical properties of chitin and releases effluent wastewater containing chemicals, requiring further purification. The use of proteolytic bacteria for DP and lactic acid bacteria for DM could curtail the application of concentrated bases and acids. Therefore, biological methods using microbes or microbial enzymes are in demand due to their better reproducibility, lower processing times, easier handling, less solvent and chemical requirements, and lower energy input for producing value-added products (Hayes et al. 2008). Bio-based chitin has distinct properties like biodegradability, non-toxicity, and biocompatibility and is applied in agriculture, medicine, pharmaceutics, environmental waste management, biotechnology, and food processing (Kaur and Dhillon 2015). The protein-rich liquid fractions find applications in human and animal feed (Mizani et al. 2005). Bioprocessing of shrimp wastes for chitin production is reported using lactic acid bacteria and proteolytic bacteria/enzyme for DM and DP as single-stage fermentation (Rao and Stevens 2006), two-stage fermentation (Xu et al. 2008) and cofermentation (Francisco et al. 2015).

15.2 Economic Aspects of Chitin

The main source of raw material for synthesizing chitin is from the waste materials obtained from seafood pre-processing centers deshelling crab, shrimp, prawn, lobster, etc. (Hamdi 2017; Maruthiah and Palavesam 2017). The shrimp wastes are rich in pigments like astaxanthin, β-carotene, and other carotenoids. For several years, chitin is considered as a promising biomaterial due to its characteristic properties and has found applications in many fields like biomedical, engineering, wastewater treatment, cosmetic, food industry, and packaging. Chitin is of great economic significance as it costs 220 dollars per kilo (Jaganathan et al. 2016). The commercial value of chitin and its derivatives is accounted for 100 billion tons per year (Ioelovich 2014). The global research statistics have concluded that the chitin market is expected to rise to 53 million US dollars in 2024 (Global Chitosan Derivatives Market 2019).

15.3 Chitin Structure and their Properties

Chitin is a linear semi-crystalline polymer with high molecular weight comprising N-acetyl glucosamine units bonded by β-glycosidic bonds. They resemble cellulose polysaccharide with the C-2 position of the hydroxyl group replaced by the acetamido group. To be distinguished as a chitin, their degree of acetylation is greater than 50% (Anitha et al. 2014). Chitin is tough, inert, and insoluble in water and other organic solvents. The other characteristics of chitin are its ability to chelate metal ions and form films and polyoxy salts. Chitin is consists of three allomorphs containing α-, β-, and γ-forms. The α-chitin is abundantly found in shrimps, lobsters, and crabs with antiparallel chains with strong intra- and intermolecular bonds. The β-form consists of parallel chains bonded by intrasheet hydrogen bonding, which are of weak bonds, hence unstable, and are mainly found in squid (Ioelovich 2014), whereas γ-chitin is an amalgamation of α- and β-chitin forms comprising parallel and antiparallel chains, e.g., *Ptinus* beetles and *Loligo* squids (Ramirez-Coutino et al. 2006; Casadidio et al. 2019). The characteristics of pure chitin are dependent on their molecular weight, degree of acetylation, purity, and polydispersity index (Kaur and Dhillon 2015). The characteristics like biodegradability, bioactivity, non-toxicity, and biocompatibility have made these marine polymers useful for various versatile applications. Factors like the degree of deacetylation (DD) are used to determine the number of glucosamine units present in a chitin structure. If the degree of deacetylation exceeds 50%, it improves the solubility of chitin, by changing into chitosan. The molecular weight of chitin is based on the emergence of the source, acid and base concentration used in demineralization and deproteination, duration for incubation, and temperature required for the processes (No and Meyers 1995). The average molecular weight of chitin is reported to have a range of 0.4 to 2.5×10^6 (No and Meyers 1995; Ravi Kumar 2000). *Chitin portrays biological properties like antimicrobial, antiulcer, hemostatic, wound healing, fungistatic, antiacid, anticholesterolemic,* etc.*; hence, it can be used for biomedical applications* (Dutta et al. 2004; Zargar et al. 2015; Lim and Hudson 2003; Cheba 2011). Processes involved in synthesizing chitin are (a) demineralization (DM), (b) deproteination (DP), and (c) depigmentation.

15.4 Chemical Methods in the Extraction of Chitin

Traditional methods in chitin extraction from shrimp shells involved the usage of chemicals (Table 15.1 and Fig. 15.1). The usage of a strong alkali like NaOH and acids like HCl affects the ecosystem as the water obtained after processing chitin is highly acidic or basic, which are dumped into the water bodies. The process is expensive as the costs involved in neutralizing the dumped wastes are high.

Table 15.1 Shrimp shell processing with chemicals

	Deproteination			Demineralization			
Shrimp source	NaOH concentration	Temperature (°C)	Incubation (h)	HCl concentration	Temperature (°C)	Incubation (h)	References
Shrimp	1.25 M	100	0.5	1.57 M	20–22	1–3	Moorjani et al. (1975)
Metapenaeus dobsoni	0.125 M	100	0.5	1.25	Room temperature	1	Madhavan and Nair (1974)
Metapenaeus dobsoni	0.75 M	100	–	1.25	Room temperature	1	Madhavan and Nair (1974)
Shrimp	1%	65	1	0.5 M	Room temperature	–	Wu and Bough (1977)
Shrimp	3%	100	1	1 M	Room temperature	0.5	Bough et al. (1978)
Shrimp	4%	100	1	5%	Room temperature	–	Sluyanarayana Rao et al. (1987)
Penaeus monodon	4%	Room temperature	21	4%	Room temperature	2 or 12	Lertsutthiwong et al. (2002)
Litopenaeus vannamei	5%	90	12	4%	Room temperature	4	Ploydee and Chaiyanan (2014)
Shrimp shell wastes	4%	28 ± 2	20	4%, 3%, 2%	28 ± 2	16	Hossain and Iqbal (2014)
Nephrops norvegicus	150 g dm^{-3}	65	3	1 M	Room temperature	2	Beaney et al. (2005)
Seafood wastes comprising shrimps, krill, crab, and lobster	1.25 M	90	2	1 M	Room temperature	1	Kaya et al. (2015)
Shrimp shell waste	1.25	90	2	1 M	Room temperature	1	Pachapur et al. (2016)
Litopenaeus stylirostris	2 M	50	4	1	25	2.5	Díaz-Rojas et al. (2006)

(continued)

Table 15.1 (continued)

	Deproteination			Demineralization			
Shrimp source	NaOH concentration	Temperature (°C)	Incubation (h)	HCl concentration	Temperature (°C)	Incubation (h)	References
Penaeus monodon	1 M	95	0.5	0.25	Room temperature	6	Charoenvuttitham et al. (2006)

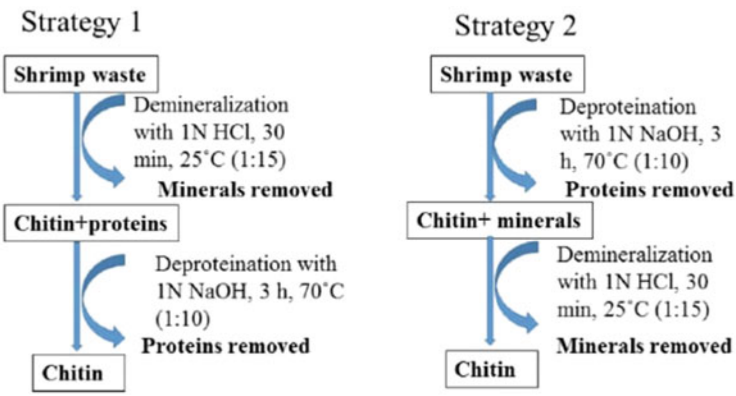

Fig. 15.1 Strategies for chitin production from shrimp wastes by chemical processes. Strategy 1: Demineralization followed by deproteination. Strategy 2: Deproteination followed by demineralization

15.4.1 Chemical Demineralization

The chitin entrapped in the shrimp exoskeleton can be extracted by the removal of the process of demineralization and deproteination. In demineralization, the inorganic minerals like calcium carbonate from the crustacean exoskeleton are removed using inorganic acids, like HCl, HNO_3, and H_2SO_4 (Younes and Rinaudo 2015; Kumar Gadgey and Bahekar 2017), and organic acids like HCOOH and CH_3COOH (Regis et al. 2015). Predominantly, hydrochloric acid is used for higher removal rate of minerals from shell wastes. HCl combines with calcium carbonate ($CaCO_3$) to form calcium chloride ($CaCl_2$) that can be removed by using activated carbon (Fadli et al. 2018) (15.1).

$$CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2 \qquad (15.1)$$

15.4.2 Chemical Deproteination

The next step for the extraction of chitin is deproteination, which involves the removal of proteins. Proteins are removed from the shell wastes using chemicals like NaOH, KOH, $Ca(OH)_2$, $CaHSO_4$, $NaHSO_4$, $NaHCO_3$, Na_3PO4, Na_2CO_3, Na_2S, and K_2CO_3 (Younes and Rinaudo 2015). NaOH is mostly preferred for deproteination. A higher concentration of NaOH at elevated temperature causes deacetylation of chitin to chitosan (40% NaOH incubated at 100–130 °C) (Hülsey 2018).

Deproteination and demineralization can be reversed based on the quality of chitin produced with less incubation time and temperature.

15.4.3 Depigmentation

The process of demineralization and deproteination cannot completely remove the carotenoid pigments like astaxanthin, lutein, β-carotene, and astacene. In order to obtain colorless chitin, the pigments are removed using organic solvents like glacial acetone (Soon et al. 2018) and inorganic solvent like sodium hypochlorite (Srinivasan et al. 2018; Devi and Dhamodharan 2018). Duan et al. (2012) decolorized colored chitin from shrimp wastes with potassium permanganate followed by incubating in oxalic acid (1%). Through the process of decolorization, colorless chitin is obtained which improves their commercial value and utilization for various industrial applications.

15.5 Microbial Action on Shrimp Shells for Chitin Recovery

Shrimp shell waste biofermentation is probably the ideal environmentally friendly method that is cost-effective and sustainable. Although shrimp shells are insoluble and not easily degraded by natural degradation, they contain chitin, a natural polymer resembling cellulose in chemical structure. Chitin and its derivative chitosan have been used widely for commercial applications in agriculture, biomedicine, biotechnology, waste treatment, food industry, etc. Biofermentation of shrimp shell wastes is advantageous over chemical methods. The usage of chemicals release effluents into the soil and water body and are harmful that biological methods using microorganisms. Khanafari et al. (2008) found out that the quality of chitin obtained from the biological methods was better than chemical methods. Chitin with high molecular weight was produced by the deproteination of shrimp shells using proteolytic microorganisms (Bustos and Healy 1994). Shrimp shells are fermented by single-stage fermentation, cofermentation, or two-stage fermentation processes, which involve lactic acid bacteria and non-lactic acid bacteria that assist in demineralization and deproteination (Table 15.2).

15.5.1 Lactic Acid Bacteria

Conventional methods of demineralization used HCl which affected the quality of chitin altering their molecular weight and intrinsic properties (Percot et al. 2003). Lactic acid is used as an alternative instead of HCl for demineralization, and it was found that a) usage of lactic acid was less toxic to the environment due to the release of acid and alkali liquid obtained after chitin processing, b) it was also cost-effective, and c) calcium lactate ($Ca(C_3H_5O_3)_2$) formed by the action of lactic acid ($C_3H_6O_3$) (15.2) with calcium carbonate can be used as anti-icing agents (Mahmoud et al. 2007). Lactic acid is naturally produced by lactic acid-producing bacteria, which is preferred over commercial lactic acid considering their cost (Ghaffar et al. 2014). Lactic acid-fermenting bacteria can be isolated from the shrimp shell itself (Duan et al. 2012). Lactic acid fermentation converts sugars to form lactic acid, which

Table 15.2 Lactic acid and non-lactic acid bacteria in shrimp shell demineralization

Microorganisms	Type of fermentation	Shrimp species	Demineralization (%)	Deproteination (%)	Chitin (%)	Reference
Lactic acid bacteria						
Lactobacillus sp. B2 with sucrose and whey	Single fermentation	*Penaeus* shrimp waste (2 kg)	85	87.6	13.1	Cira et al. (2002)
Lactococcus lactis with 15% glucose	Monocultivation	Shrimp wastes	78.8	66.5	49.4	Aytekin and Elibol (2009)
L. plantarum 541	Single fermentation	Shrimp waste	86			Rao et al. (2000)
L. plantarum 541 with 5% glucose and 2% salt	Single fermentation	Shrimp waste	81.4	59.8	NA	Rao and Stevens (2006)
Lactobacillus paracasei strain A3 with glucose	Single fermentation	*Nephrops norvegicus*	61	77.5	17.5	Zakaria et al. (1998)
L. helveticus cultivated with date juice	Single fermentation	*Parapenaeus longirostris*	44	91	23.6	Adour et al. (2008)
L. Plantarum	Single fermentation	Shrimp waste	87	99	NA	Neves et al. (2017)
P. acidolactici CFR2182 with 15% glucose	Single fermentation	*Penaeus monodon*	72.5 ± 1.5%			Bhaskar et al. (2007)
Pediococcus acidolactici CFR2182 (with 15% glucose)	Single fermentation	*Penaeus monodon* shrimp wastes	76	92	91.67 ± 1.86	Narayan et al. (2010)
Pediococcus sp. *L1/2* with 5% sucrose	Single fermentation	Shrimp shell wastes	83.47	NA	NA	Choorit et al. (2008)
Lactobacillus futsaii LAB06 and *L. plantarum* LAB14 (with 2% sucrose)	Cofermentation	*Litopenaeus vannamei*	88.6	84.8	15	Ximenes et al. (2019)

(continued)

Table 15.2 (continued)

Microorganisms	Type of fermentation	Shrimp species	Demineralization (%)	Deproteination (%)	Chitin (%)	Reference
Lactobacillus strains T1 and L137	Cofermentation	Shrimp wastes	82–83	84.4	NA	Francisco et al. (2015)
First-stage fermentation with native proteolytic shrimp bacteria followed by fermentation with *L. casei* MRS1 in the presence of glucose	Two-stage fermentation	*P. monodon*	99.6	97.4	36	Xu et al. (2008)
Fermentation with bacterial enrichment cultures from ground meat and bio-yoghurt	Pilot-scale fermentation	Pre-purified shrimp shell wastes	85–90	89–91	NA	Bajaj et al. (2015)
Lactobacillus acidophilus FNCC 116 followed by *Bacillus licheniformis* F11.1	Two-stage batch fermentation process	*P. vannamei*	97.19	94.42	NA	Junianto and Setyahadi (2013)
First-stage fermentation with native proteolytic shrimp bacteria followed by fermentation with *L. casei* MRS1 in the presence of glucose	Two-stage fermentation	*C. crangon*	99.7	90.8	46	Xu et al. (2008)
Teredinobacter turnirae followed by demineralization with *Lactococcus lactis* (using 5% glucose)	Successive fermentation	Shrimp wastes	95	95	64.5	Aytekin and Elibol (2009)
Fermentation of *Lactobacillus brevis* first followed by *Rhizopus oligosporus*	Successive fermentation	Shrimp wastes	66.45 +/− 2.14%	96% +/− 0.43%	NA	Aranday-García et al. (2017)
Streptococcus thermophilus, *Lactobacillus acidophilus*, and	Cofermentation	*Penaeus vannamei*	91.3	97.7	4.42 ± 0.60	Duan et al. (2011)

Lactobacillus bulgaricus with 6.5% glucose						
Lactobacillus acidophilus FNCC116 and *Bacillus licheniformis* F11.1	Cofermentation	*Penaeus vannamei*	99.54	96.7	NA	Setyahadi et al. (2014)
Deproteination with *Serratia marcescens* B742 followed by *Lactobacillus plantarum* ATCC 8014	Two-step fermentation	Pulverized *P. vannamei* wastes	93	94.5	18.9 (chitin yield)	Zhang et al. (2012)
Lactobacillus pentosus L7 and *Bacillus thuringiensis* SA	Two-step fermentation	*Litopenaeus vannamei*	98.1 ± 0.3	96.8 ± 0.7	NA	Ploydee and Chaiyanan (2014)
SILALL 4 × 4® silage additive: *Lactobacillus salivarius*, *Enterococcus faecium*, and *Pediococcus acidilactici*	Fermentation with microbial consortia	*Nephrops norvegicus*	99.75	NA	NA	Beaney et al. (2005)
Non-lactic acid bacteria						
Bacillus cereus 8–1	Single fermentation (large-scale fermentation in 12 L)	10% shrimp shell waste	73	78.6	NA	Sorokulova et al. (2009)
Teredinobacter turnirae	Single fermentation without glucose	Shrimp wastes	23.3	77.8	40.1	Aytekin and Elibol (2009)
Bacillus subtilis with jaggery as sugar source	Single fermentation	*Metapenaeus dobsoni*	72	84	93.2 ± 0.6%	Sini et al. (2007)
Bacillus cereus	Single fermentation	3% shrimp waste	95	97.1	NA	Sorokulova et al. (2009)

(continued)

Table 15.2 (continued)

Microorganisms	Type of fermentation	Shrimp species	Demineralization (%)	Deproteination (%)	Chitin (%)	Reference
Exiguobacterium acetylicum	Single fermentation	3% shrimp waste	92	92.8	NA	Sorokulova et al. (2009)
Pseudomonas aeruginosa 2 cultured with 5% glucose	Single fermentation	*Metapenaeus monoceros* shrimp wastes	92	90	19	Ghorbel-Bellaaj et al. (2012b)
Pseudomonas aeruginosa	Single fermentation	*Penaeus merguiensis*	82	92	47	Sedaghat et al. (2017)
Kurthia gibsonii and *Aspergillus* sp.	Two-stage fermentation	*Fenneropenaeus semisulcatus* (1:25 shell to bacterial broth)	NA	NA	16.06	Bahasan et al. (2017)
Kurthia gibsonii and *Aspergillus* sp.	Two-stage fermentation	*Fenneropenaeus indicus* (1:25 shell to bacterial broth)	NA	NA	13.87	Bahasan et al. (2017)
Bacillus licheniformis 21,886 and *Gluconobacter oxydans* DSM-2003	Successive cofermentation	*Litopenaeus vannamei*	93.5	87	90.8	Liu et al. (2014)

reduces the pH of the fermentation broth, reducing the growth of unwanted bacteria (Vandenbergh 1993).

$$2C_3H_6O_3 + CaCO_3 \rightarrow Ca(C_3H_5O_3)_2 + H_2O + CO_2 \qquad (15.2)$$

For lactic acid fermentation using shrimp shells, different parameters have been considered; these are various sugar sources and their optimal concentrations, the concentration of inoculum used, and incubation time to produce lactic acid (Mathew and Nair 2006; Healy et al. 2003; Rao et al. 2000; Bhaskar et al. 2007). Lactic acid fermentation of shrimp wastes is optimized using different parameters like type of lactic acid bacteria used, sugar concentration, incubation time, etc. using response surface methodology (RSM), a statistical method that uses a sequence of designed experiments with different variables to obtain an optimal condition (Bhaskar et al. 2007). The addition of glucose in shrimp shell fermentation leads to the formation of lactic acid that lowers the pH causing demineralization (Khanafari et al. 2008). Different concentrations of glucose were added to test the demineralization efficiency. It was observed that the presence of glucose inhibited the protease activity of non-lactic acid bacteria; hence, other sugar sources were also considered (Aytekin and Elibol 2009). Some of the commonly used sugar sources that were added along with shrimp waste to enhance lactic acid production included sucrose (Cira et al. 2002), molasses (Fagbenro 1996; Evers and Carroll 1998), date juice (Khorrami et al. 2011), cassava starch (Francisco et al. 2015), fruit peels, etc. (Tan et al. 2020).

LAB can undergo single fermentation or cofermentation for shrimp shell degradation. Shrimp shells were fermented with *Lactobacillus plantarum* 541 resulting in a demineralization value of 90% (Rao et al. 2000). Natural curd containing lactic acid bacteria (LAB) was used for shrimp biofermentation having a demineralization value of 69% and deproteination of 89% (Prameela et al. 2010). Pacheco et al. (2011) isolated *Lactobacillus* strain B2 from the shellfish waste, and through fermentation, it resulted in 92% demineralization and 94% deproteination, respectively. Lactic acid bacteria can be combined with other non-lactic acid-producing bacteria that aid in protease activity causing deproteination. Some LAB organisms can carry both demineralization and deproteination and hence be used as a single strain for the biofermentation of shrimp shells. Chitin was obtained using *Lactobacillus plantarum* from fresh shrimp shell wastes by batch fermentations adjusting the pH, incubation time, and inoculum to obtain a deproteination of 99% and demineralization of 87% (Neves et al. 2017). The chitin produced by biological fermentation was observed to be 40% better than the chemical produced chitin. Lactic acid bacteria are used for deproteination of shrimp shells (Woods 1998).

Lactic acid bacteria were co-cultured with other lactic acid bacteria/non-lactic acid bacteria to enhance the demineralization and deproteination efficiency in shrimp shells. Co-culturing of *Lactobacillus* isolates T1 and L137 in the presence of sugar sources like glucose and cassava starch led to DM efficiency of 82–83% and deproteination value of 84.4% (Francisco et al. 2015). Evers and Carroll (1998) co-cultured *Lactobacillus plantarum* and *Enterococcus faecium* for shrimp shell biofermentation using dry molasses. Ploydee and Chaiyanan (2014) co-cultured

Lactobacillus pentosus and *Bacillus thuringiensis* for shrimp shell processing resulting in calcium carbonate removal efficiency of 98.1 ± 0.3% with a protein removal efficiency of 96.8 ± 0.7% (w/w). Junianto and Setyahadi (2013) demonstrated three different strategies for the pretreatment of shrimp shells using *Lactobacillus acidophilus* FNCC 116 and *Bacillus licheniformis* F11.1 by two-stage fermentation processes. 99.6% of minerals were removed when 100% of the medium was replaced by fresh media after 24 h of incubation with *Lactobacillus acidophilus* FNCC 116. 95.37% of protein was removed after subsequent fermentation and 100% media removal and replaced with fresh media after 24 h. Co-culturing of *L. plantarum* subsp. *plantarum* ATCC14917 and *B. subtilis* subsp. *subtilis* ATCC 6051 in the presence of fruit peels enhanced the shrimp biofermentation to produce good-quality chitin (Tan et al. 2020). Zhang et al. (2012) demonstrated two-stage fermentation of shrimp shells using *Lactobacillus plantarum* and *Serratia marcescens.* For the deproteination, *S. marcescens* was cultured with the shrimp shells at 30 °C for 4 days. The solid mass obtained after drying was further demineralized at 37 °C for 2 days. Their deproteination efficiency was 93% and demineralization 94.5% resulting in a chitin yield of 18.9% (Zhang et al. 2012). Similarly, heterofermenting *Lactobacillus brevis* was cultured with *Rhizopus oligosporus* for the biological shrimp shell processing (Aranday-García et al. 2017). In this study, *L. brevis* was cultured first followed by *R. oligosporus* to yield 66.45 ± 2.14% demineralization and 96 ± 0.43% of deproteination efficiency. Aytekin and Elibol (2009) studied the fermentative action of *Lactococcus lactis* and *Teredinobacter turnirae* on shrimp shell wastes for demineralization and deproteination. From their studies, co-culturing of *Lactococcus lactis* and *Teredinobacter turnirae* showed the best results, especially when proteolytic *T. turnirae* was cultured first followed by the demineralization with *L. lactis* displaying a DP and a DM value of 95%.

15.5.2 Non-lactic Acid Bacteria

Non-lactic acid bacteria produce proteases responsible for the deproteination process. The non-lactic acid bacteria produce protein hydrolysates, which help in the growth of lactic acid bacteria that help in demineralization. The proteolytic activities of the microorganisms are responsible for the deproteination of the shrimp shells (Table 15.3). Wang and Chio (1998) observed that the deproteination efficiency of *Pseudomonas aeruginosa* K-187 grown with shrimp and crab shell wastes was 82%. Shimahara et al. (1984) used *P. maltophilia* LC 102 for the protein removal of shrimp shells of *Penaeus japonicus* supplemented with EDTA. Paul et al. (2015) deproteinized the shrimp shells of *P. monodon* with *Paenibacillus woosongensis* TKB2 containing NaCl and chicken feather leading to 80% deproteination efficiency.

Bacillus species were used in shrimp shell deproteination. The proteolytic activities of six *Bacillus* species namely, *B. amyloliquefaciens*, *B. subtilis* A26, *Bacillus pumilus* A1, *B. licheniformis* RP1, and *B. cereus* SV1 strain, were studied

Table 15.3 Microorganisms involved in deproteination (that produce proteases)

Microorganisms	Shrimp species	Proteolytic activity	Deproteination (%)	Reference
Teredinobacter turnirae	Shrimp wastes	1139 l g/mL h	77.8	Aytekin and Elibol (2009)
Serratia marcescens	Shrimp waste	0.043 U/mL	90	Damodarasamy et al. (2012)
Paenibacillus woosongensis TKB2 with NaCl and chicken feather	*Penaeus monodon*	1.57 mg/mL of 71.4 U/mL	80	Paul et al. (2015)
Brevibacillus parabrevis TKU046	Cooked tiger shrimp shell	NA	96.44 ± 0.72	Doan et al. (2019a)
Rhizopus oligosporus	Shrimp waste	NA	96 ± 0.43	Aranday-García et al. (2017)
B. subtilis	Shrimp waste	137.5 U/mL	74	Pachapur et al. (2016)
B. Licheniformis	Shrimp wastes	178.7 U/mL	84	Pachapur et al. (2016)
Pseudomonas aeruginosa	*Penaeus merguiensis*	NA	92	Sedaghat et al. (2017)
Bacillus mojavensis A21	*Metapenaeus monoceros*	7.75 U/mg	88 ± 5%	Younes et al. (2012)
Pseudomonas aeruginosa K-187	Shrimp shell waste	21.2 U/mL	78	Oh et al. (2000)
Bacillus cereus SV1 (without adding glucose)	*Metapenaeus monoceros*	1152 ± 53 U/mL	95	Ghorbel-Bellaaj et al. (2012a)
Bacillus subtilis A26 (without adding glucose)	*Metapenaeus monoceros*	193 ± 90 U/mL	79.9	Ghorbel-Bellaaj et al. (2012a)
Paenibacillus sp. TKU047	0.5% shrimp head powder	2.98 U/mL	NA	Doan et al. (2019b)

for deproteination (Ghorbel-Bellaaj et al. 2012a). The deproteination of shrimp shells enzymatically was optimized by Box-Behnken design using *Bacillus mojavensis* A21 crude protease resulting in 88% deproteination (Younes et al. 2012). A chitinase-free extracellular protease was isolated from *Brevibacillus parabrevis* TKU046 which was used for the deproteination study against shrimp shell wastes (Doan et al. 2019a). It was observed that maximum deproteination of 96.44 ± 0.72% was observed on cooked tiger shrimp shell by liquid fermentation.

In a single reactor, the concurrent production of chitin was initiated by adding shrimp shell with *Aspergillus niger*. The proteases produced from *A. niger* caused deproteination releasing protein hydrolysates that were of low pH. Lower pH of the supernatant facilitated the demineralization process aiding in chitin separation (Teng et al. 2001). Cofermentation of non-lactic acid-producing microorganisms also helped in shrimp shell degradation. Successive cofermentation of proteolytic

B. licheniformis and *Gluconobacter oxydans* produced a DP efficiency of 87% followed by a DM value of 93.5%, and the chitin content was 90.8%.

15.6 Other Green Methods for Chitin Synthesis

Biological fermentation can be combined with other greener approaches to extract chitin. Some methods are ionic liquid extraction, the usage of protease enzymes for deproteination, micro-irradiation, and ultrasonication before or after the demineralization and deproteination in shrimp shell biofermentation (Qin et al. 2010; Mao et al. 2017; Suryawanshi et al. 2020; El Knidri et al. 2016). Extraction of chitin using ionic liquids is a one-pot method using ionic liquids (ILs) like hydroxyl ammonium acetate that has low inflammability, low vapor pressure, and highly soluble nature (Shamshina et al. 2016). Apart from using ionic liquids in chitin extraction, deep eutectic solvents (DESs) are preferred over ionic liquids in chitin extraction for their better solubility and economical and simple extraction process. In a two-step chitin extraction process, shrimp shells were pretreated first using citric acid leading to a DM value of 98% followed by the addition of DESs with the microwave irradiation causing deproteination with an efficiency of above 88% (Zhao et al. 2019). High-quality chitin (DESs-chitin) was produced in this method and matched the standards of chemically produced chitin. Huang et al. (2018a, b) devised a chitin extraction method from shrimp shells with Natural Deep Eutectic Solvent (NADES) along with microwave irradiation. Demineralization was attained by the adding malic acid, which removed 99% calcium chloride. The deproteination efficiency was dependent on the microwave radiation, the incubation time, and the shrimp shell-to-NADES ratio. Maximum deproteination efficiency was obtained at 93.8% with a shrimp shell-to-NADES ratio of 1:20 and microwave irradiation for 9 min. The chitin obtained through this process had a high crystallinity index of 71%. Devi and Dhamodharan (2018) developed a green and facile process to obtain chitin nanofibers from prawn shell wastes. The prawn shells were pretreated in hot glycerol (at 200 °C, for 4 min) that caused deproteination leading to the release of low molecular weight water-soluble proteins. The deproteinated shells were demineralized using citric acid forming calcium citrate salt and chitin of high crystallinity index (80.9%). From this process, the glycerol could be reused by using charcoal. Ultrasonication is another method for enhancing the pretreatment processes involved in deproteination and demineralization (Suryawanshi et al. 2019). In an ultrasonication-assisted method, a mild concentration of HCl (0.6 M HCl) and NaOH (O.6 M NaOH) was employed for demineralization and deproteination of shellfish wastes (Suryawanshi et al. 2020). Through ultrasonication, microbubbles are generated leading to an increase in the reaction rate with temperatures of 5000 K and 1000 atmospheric pressure.

For the deproteination of shrimp shells, commercial enzymes like pepsin, papain, bluefin trypsin, Alcalase®, and protease are used. Shrimp shell wastes of *Penaeus indicus* were demineralized with 1.75 N glacial acetic acid and papain (1:100 papain to shrimp shells) incubated at 72 h room temperature to obtain a deproteination value of 73.1%, and the degree of acetylation (DA) of the chitin produced was 19.37% (Gopalakannan et al. 2000). Pepsin enzyme was incubated with white shrimp shells for 16 h at 40 °C, and it resulted in 92% deproteination efficiency (Duong and Nghia 2014).

Hongkulsup et al. (2016) used commercial protease enzyme from *Streptomyces griseus* for deproteination of *L. vannamei* shells and effectively removed 91.1% proteins, and the chitin produced had a DA of 90.83% with a crystallinity index of 82.56%, with lactic acid as the demineralization agent. Another enzyme like Alcalase® was used in the removal of proteins from shrimp heads to recover chitin (Valdez-Peña et al. 2010). Hence, commercial proteolytic enzymes can be used in shrimp shell degradation to obtain chitin, but are expensive compared to using proteolytic microorganisms.

15.7 Functional Aspects of Chitin

Due to the insoluble nature of chitin, chitin is deacetylated to chitosan, which has pleiotropic applications in the field of agriculture, food, waste management, and biomedical sectors (Table 15.4). In the wastewater management, green chitin nanoadsorbents were developed for the removal of carmine dyes (Meshkat et al. 2019). Adsorption of anionic dyes was initiated using a chitin biopolymer (Longhinotti et al. 1998). Chitin derivatives are used in heavy metal removal of lead (Zhou et al. 2005), chromium (Baran et al. 2007), cadmium (Benguella and Benaissa 2002), copper, and arsenic (Kartal and Imamura 2005). Biological denitrification and sulfate reduction in groundwater were initiated using crab shell chitin (CS-20) (Robinson-Lora and Brennan 2009). Chitin is also used for coagulating and flocculating activated sludge (Kurita 2006).

In the biomedical application, chitin fabrics (non-woven) and chitin threads are used in the development of artificial skin and sutures for wound dressing because of their biocompatibility and degradability (Nishimura 2001). The mechanical strength of pure chitin sutures can be improved by incorporating graphene oxide with chitin monofilament (Zhang et al. 2019).

In the field of agriculture, chitin is used for developing resistance against plant diseases and develops elicitor activity in fruits and vegetables (Parada et al. 2018; Pusztahelyi 2018). Nanochitin, derived from shrimp shells, is used to improve the quality and quantity of winter wheat: multi-spike wheat and large spike wheat, respectively (Xue et al. 2018). To improve soil fertility, chitin can be used as a fertilizer due to their rich nitrogen content (Malerba and Cerana 2019).

In the food sector, chitin derivatives are utilized as a food preservative (Hu and Gänzle 2019). They are also used as thickener mixed with vegetable oil for developing bio-lubricants (Sánchez et al. 2011). As a stabilizer/emulsifier, chitin is used in food, cosmetics, and biomedical applications (Casadidio et al. 2019; İlyasoğlu et al. 2018). Lipophilized chitin as chitin fatty esters (chitin laurate, chitin palmate, chitin stearate, chitin octanoate) is used for developing novel stabilizers with oil in water emulsions (İlyasoğlu et al. 2018). Chitin materials are replacing petroleum-based

Table 15.4 Functional aspects of chitin

Areas	Functions	References
Agriculture	Used as coatings in seeds, vegetables, and fruits; mixed as an anti-nematode agent along with fertilizer; soil improvement; as elicitors to enhance plant immunity against pests	Malerba and Cerana (2019), Shamshina et al. (2019), Parada et al. (2018), Sahu et al. (2017)
Aquaculture	Act as protective coating for raw shrimp and shellfish spat (juvenile stage) in the hatcheries; shrimp canning; formulated fish feed	Abdel-Ghany and Salem (2020)
Animal husbandry	Poultry feed	Khempaka et al. (2006)
Food and nutrition	Emulsifiers; stabilizers; thickeners; dietary fiber in tempeh; antioxidants; in food packaging	Harkin et al. (2019), Elhussieny et al. (2020)
Biomedicine	Wound dressings and sutures; anticoagulants; gene therapy; as scaffolds for drug delivery; in tissue engineering; regenerative medicine	Değim et al. (2002), Zhang et al. (2019), Anitha et al. (2014)
Cosmetics	Moisturizers; thickening agents; skin smoothener; anti-static agents; oral healthcare	Aranaz et al. (2018)
Biotechnology	Support material for immobilization and encapsulation of enzymes and cells.	Verma et al. (2020)
Nanotechnology	Development of chitin nanocrystals, chitin nanofibers, and composite materials	Salaberria et al. (2015), Aranday-García et al. (2019)
Waste management	Adsorbents for the removal of dyes, heavy metals, and petroleum derivatives	Akkaya et al. (2009), Meshkat et al. (2019), Anastopoulos et al. (2017), Jaafarzadeh et al. (2015), Barros et al. (2014)

packaging materials as they are eco-friendly and biodegradable (Srinivasa and Tharanathan 2007). Chitin-based packaging materials, in the form of antimicrobial films and composite materials, are used in preserving fruits and vegetables after post-harvest to maintain their freshness and enhance the shelf life (Srinivasa and Tharanathan 2007; Suryawanshi et al. 2019). In paper finishing, hydroxyl methyl chitin is added to improve the wet strength characteristics of paper (Allan et al. 1980; Song et al. 2018). For cosmeceutical applications, chitin was used as a skin conditioner, moisturizer, emollient, and surfactant, shows antimicrobial activity against skin acne, was used as an ingredient in hair care products, and in oral health-care acts as a carrier for herbal extracts in toothpaste, mouthwash, and chewing gums (Aranaz et al. 2018). In the field of nanotechnology, chitin nanoparticles developed from shrimp wastes of *P. semisulcatus* are used in developing iron/chitin nanocomposite with aqueous leaf extract of *Corchorus olitorius* that were analyzed for their antimicrobial activity and heavy metal and dye adsorption (Gomaa 2018). In the textile industry, chitin can be used to prevent the wear and tear of fabrics while weaving and can be used to improve properties like water resistance and antimicrobial resistance to the fabric (Hahn et al. 2019). Chitin is used in textile dyeing as anti-wrinkle, anti-static, and anti-bacterial finishing by blending chitosan with cotton, silk, wool, etc., thus enhancing the value of the fabric and utilizing the natural polymers (Huang et al. 2018a, b). Hence, chitin can be used for various pleiotropic applications that can benefit humankind.

15.8 Conclusion

The production of chitin from shrimp wastes involving microorganisms is beneficial over other chemical methods. Although there are several reports on microbial shrimp shell degradation, the usage of the environmentally safe microorganisms (GRAS status) for shrimp shell biofermentation is beneficial, as the byproducts like protein hydrolysate derived from them can be used in animal, fish, and poultry feed, without causing risk of any infection. The derived protein hydrolysates from such GRAS organisms can be attempted to cultivate beneficial fungi that produce SCP and other enzymes like chitinases, cellulases, etc. Lactic acid bacteria, being GRAS microorganisms, can be used directly in the demineralization process in shrimp shell processing, producing beneficial products like calcium lactate and lactic acid. Thus, the chitin derived by microbial action of shrimp shell wastes is a safer approach that can resolve the problem of environmental pollution and be beneficial for innumerable applications in various industries.

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References

Abdel-Ghany HM, Salem ME-S (2020) Effects of dietary chitosan supplementation on farmed fish; a review. Rev Aquac 12(1):438–452. https://doi.org/10.1111/raq.12326

Adour L, Arbia W, Amrane A, Mameri N (2008) Combined use of waste materials – recovery of chitin from shrimp shells by lactic acid fermentation supplemented with date juice waste or glucose. J Chem Technol Biotechnol 83:1664–1669. https://doi.org/10.1002/jctb.1980

Akkaya G, Uzun I, Güzel F (2009) Adsorption of some highly toxic dyestuffs from aqueous solution by chitin and its synthesized derivatives. Desalination 249:1115–1123. https://doi.org/10.1016/j.desal.2009.05.014

Allan G, Crospy GD, Lee JH, Miller ML, Reif WM (1980) Proceedings of a symposium on man made polymers in papermaking. In. Helsinki, Finland

Anastopoulos I, Bhatnagar A, Bikiaris DN, Kyzas GZ (2017) Chitin adsorbents for toxic metals: a review. Int J Mol Sci 18(1):114. https://doi.org/10.3390/ijms18010114

Anitha A, Sowmya S, Kumar PTS, Deepthi S, Chennazhi KP, Ehrlich H, Jayakumar R (2014) Chitin and chitosan in selected biomedical applications. Prog Polym Sci 39(9):1644–1667. https://doi.org/10.1016/j.progpolymsci.2014.02.008

Aranaz I, Acosta N, Civera C, Elorza B, Mingo J, Castro C et al (2018) Cosmetics and cosmeceutical applications of chitin, chitosan and their derivatives. Polymers 10:213. https://doi.org/10.3390/polym10020213

Aranday-García R, Román Guerrero A, Ifuku S, Shirai K (2017) Successive inoculation of *Lactobacillus brevis* and *Rhizopus oligosporus* on shrimp wastes for recovery of chitin and added-value products. Process Biochem 58:17–24. https://doi.org/10.1016/j.procbio.2017.04.036

Aranday-García R, Saimoto H, Shirai K, Ifuku S (2019) Chitin biological extraction from shrimp wastes and its fibrillation for elastic nanofiber sheets preparation. Carbohydr Polym 213:112–120. https://doi.org/10.1016/j.carbpol.2019.02.083

Aytekin O, Elibol M (2009) Cocultivation of *Lactococcus lactis* and *Teredinobacter turnirae* for biological chitin extraction from prawn waste. Bioprocess Biosyst Eng 33:393–399. https://doi.org/10.1007/s00449-009-824

Babu CM, Chakrabarti R, Sambasivarao KRS (2008) Enzymatic isolation of carotenoid-protein complex from shrimp head waste and its use as a source of carotenoids. LWT- Food Sci Technol 41:227–235

Bahasan SHO, Satheesh S, Ba-akdah MA (2017) Extraction of chitin from the Shell wastes of two shrimp species *Fenneropenaeus semisulcatus* and *Fenneropenaeus indicus* using microorganisms. J Aquat Food Prod Technol 26:16. https://doi.org/10.1080/10498850.2016.1188191

Bajaj M, Freiberg A, Winter J et al (2015) Pilot-scale chitin extraction from shrimp shell waste by deproteination and decalcification with bacterial enrichment cultures. Appl Microbiol Biotechnol 99:9835–9846. https://doi.org/10.1007/s00253-015-6841-5

Baran A, Biçak E, Baysal H, Önal S (2007) Comparative studies on the adsorption of Cr(VI) ions on to various sorbents. Bioresour Technol 98:661–665. https://doi.org/10.1016/j.biortech.2006.02.020

Barros FCF, Vasconcellos LCG, Carvalho TV, Nascimento RF (2014) Removal of petroleum spill in water by chitin and chitosan. Orbital: The Electronic J Chem 6(1):70–74

Beaney P, Lizardi-Mendoza J, Healy M (2005) Comparison of chitins produced by chemical and bioprocessing methods. J Chem Technol Biotechnol 80:145–150. https://doi.org/10.1002/jctb.1164

Benguella B, Benaissa H (2002) Cadmium removal from aqueous solutions by chitin: kinetic and equilibrium studies. Water Res 36(10):2463–2474. https://doi.org/10.1016/S0043-1354(01)00459-6

Bhaskar N, Suresh PV, Sakhare PZ, Sachindra NM (2007) Shrimp biowaste fermentation with *Pediococcus acidolactici* CFR2182: optimization of fermentation conditions by response surface methodology and effect of optimized conditions on deproteination/demineralization and carotenoid recovery. Enzym Microb Technol 40:1427–1434. https://doi.org/10.1016/j.enzmictec.2006.10.019

Bough WA, Salter WL, Wu ACM, Perkins BE (1978) Influence of manufacturing variables on the characteristics and effectiveness of chitosan products. Chemical composition, viscosity, and molecular-weight distribution of chitosan products. Biotechnol Bioeng 20:1931–1943

Bustos RO, Healy MG (1994) Microbial deproteinization of waste prawn shell. In: proceedings of the second international symposium on environmental biotechnology, Brighton, UK. pp. 15–25

Casadidio C, Peregrina DV, Gigliobianco MR, Deng S, Censi R, Di Martino P (2019) Chitin and Chitosans: characteristics, eco-friendly processes, and applications in cosmetic science. Mar Drugs 17(6):369. https://doi.org/10.3390/md17060369

Charoenvuttitham P, Shi J, Mittal GS (2006) Chitin extraction from black tiger shrimp (*Penaeus monodon*) waste using organic acids. Sep Sci Technol 41:1135–1153. https://doi.org/10.1080/01496390600633725

Cheba BA (2011) Chitin and chitosan: marine biopolymers with unique properties and versatile applications. Biotechnol Biochem 6:149–153

Choorit W, Patthanamanee W, Manurakchinakorn S (2008) Use of response surface method for the determination of demineralization efficiency in fermented shrimp shells. Bioresour Technol 99:6168–6173. https://doi.org/10.1016/j.biortech.2007.12.032

Cira LA, Huerta S, Hall GM, Shirai K (2002) Pilot scale lactic acid fermentation of shrimp wastes for chitin recovery. Process Biochem 37:1359–1366

Damodarasamy A, Baby S, Ramachandran R (2012) Microbial deproteinization of shrimp shell waste for chitin production by wild strains of *Serratia marcescens*. Electronic J Environ Agri Food Chem 11(5):469–476

Değim Z, Celebi N, Sayan H, Babül A, Erdoğan D, Take G (2002) An investigation on skin wound healing in mice with a taurine-chitosan gel formulation. Amino Acids 22(2):187–198. https://doi.org/10.1007/s007260200007

Devi R, Dhamodharan R (2018) Pretreatment in hot glycerol for facile and green separation of chitin from prawn shell waste. ACS Sustain Chem Eng 6:846–853. https://doi.org/10.1021/acssuschemeng.7b03195

Díaz-Rojas E, Argüelles-Monal WM, Higuera-Ciapara I, Hernández J, Lizardi-Mendoza J, Goycoolea FM (2006) Determination of chitin and protein contents during the isolation of chitin from shrimp waste. Macromol Biosci 6:340–347. https://doi.org/10.1002/mabi.200500233

Doan CT, Tran TN, Wen I-H, Nguyen VB, Nguyen AD, Wang S-L (2019a) Conversion of shrimp head waste for production of a Thermotolerant, detergent-stable, Alkaline Protease by *Paenibacillus* sp Catalysts. (9):798. doi:https://doi.org/10.3390/catal9100798

Doan CT, Tran TN, Nguyen VB, Vo TPK, Nguyen AD, Wang SL (2019b) Chitin extraction from shrimp waste by liquid fermentation using an alkaline protease-producing strain, *Brevibacillus parabrevis*. Int J Biol Macromol 131:706–715. https://doi.org/10.1016/j.ijbiomac.2019.03.117

Duan S, Zhang Y, Lu T, Cao D, Chen J (2011) Shrimp waste fermentation using symbiotic lactic acid bacteria. Adv Mater Res 196:2156–2163

Duan S, Li L, Zhuang Z, Wu W, Hong S, Zhou J (2012) Improved production of chitin from shrimp waste by fermentation with epiphytic lactic acid bacteria. Carbohydr Polym 89(4):1283–1288. https://doi.org/10.1016/j.carbpol.2012.04.051

Duong NTH, Nghia ND (2014) Kinetics and optimization of the Deproteinization by pepsin in chitin extraction from white shrimp Shell. J Chitin Chitosan Sci 2:21–28. https://doi.org/10.1166/jcc.2014.1054

Dutta PK, Dutta J, Tripathi V (2004) Chitin and chitosan: chemistry, properties and applications. JSIR, Delhi, India

El Knidri H, El Khalfaouy R, Laajeb A, Addaou A, Lahsini A (2016) Eco-friendly extraction and characterization of chitin and chitosan from the shrimp shell waste via microwave irradiation. Process Saf Environ Prot 104:395–405. https://doi.org/10.1016/j.psep.2016.09.020

Elhussieny A, Faisal M, D'Angelo G, Aboulkhair NT, Everitt NM, Fahim IS (2020) Valorisation of shrimp and rice straw waste into food packaging applications. Ain Shams Eng J. https://doi.org/10.1016/j.asej.2020.01.008

Evers D, Carroll D (1998) Ensiling salt-preserved shrimp waste with grass straw and molasses. Anim Feed Sci Technol 71(3–4):241–249. https://doi.org/10.1016/s0377-8401(97)00145-4

Fadli A, Maulana S, Drastinawati (2018) Shrinking core model of demineralization of chitin isolation from shrimp shell. MATEC Web of Conferences 154:01014. https://doi.org/10.1051/matecconf/201815401014

Fagbenro OA (1996) Preparation, properties and preservation of lactic acid fermented shrimp heads. Food Res Int 29:595–599. https://doi.org/10.1016/s0963-9969(96)00077-4

FAO (2020) The State of World Fisheries and Agriculture, Food and Agricultural Organization of the United Nations. Food and Agriculture Organization of the United Nations

Francisco FC, Simora RMC, Nuñal SN (2015) Deproteination and demineralization of shrimp waste using lactic acid bacteria for the production of crude chitin and chitosan. AACL Bioflux 8:107–115

Ghaffar T, Irshad M, Anwar Z, Aqil T, Zulifqar Z, Tariq A, Kamran M, Ehsan N, Mehmood S (2014) Recent trends in lactic acid biotechnology: A brief review on production to purification. J Radiat Res Appl Sci 7(2):222–229. https://doi.org/10.1016/j.jrras.2014.03.002

Ghorbel-Bellaaj O, Younes I, Maalej H, Hajji S, Nasri M (2012a) Chitin extraction from shrimp shell waste using *Bacillus* bacteria. Int J Biol Macromol 51:1196–1201. https://doi.org/10.1016/j.ijbiomac.2012.08.034

Ghorbel-Bellaaj O, Jridi M, Khaled HB, Jellouli K, Nasri M (2012b) Bioconversion of shrimp shell waste for the production of antioxidant and chitosan used as fruit juice clarifier. Int J Food Sci Technol 47:1835–1841. https://doi.org/10.1111/j.1365-2621.2012.03039.x

Global Chitosan Derivatives Market (2019) By manufacturers, regions, type and application. Global Info Research

Gomaa EZ (2018) Iron nanoparticles α-chitin nanocomposite for enhanced antimicrobial, dyes degradation and heavy metals removal activities. J Polym Environ 26:3638–3654. https://doi.org/10.1007/s10924-018-1247-y

Gopalakannan A, Indra Jasmine G, Shanmugam SA, Sugumar G (2000) Application of proteolytic enzyme, papain for the production of chitin and chitosan from shrimp waste. J Mar Biol Assoc India 42:167–172

Hahn T, Bossog L, Hager T, Wunderlich W, Breier R, Stegmaier T, Zibek S (2019) Chitosan application in textile processing and fabric coating. In: Broek LA, Boeriu CG (eds) chitin and chitosan. doi:https://doi.org/10.1002/9781119450467.ch16

Hamdi M (2017) Chitin extraction from blue crab (*Portunus segnis*) and shrimp (*Penaeus kerathurus*) shells using digestive alkaline proteases from *P. segnis* viscera. Int J Biol Macromol 101:455–463. https://doi.org/10.1016/j.ijbiomac.2017.02.103

Harkin C, Mehlmer N, Woortman DV, Brück TB, Brück WM (2019) Nutritional and additive uses of chitin and chitosan in the food industry, Sustainable agriculture reviews, vol 36. Springer, Cham. https://doi.org/10.1007/978-3-030-16581-9_1

Hayes M, Carney B, Slater J, Brück W (2008) Mining marine shellfish wastes for bioactive molecules: chitin and chitosan and ash; part A: extraction methods. Biotechnol J 3 (7):871–877. https://doi.org/10.1002/biot.200700197

Healy M, Green MH, A. (2003) Bioprocessing of marine crustacean shell waste. Acta Biotechnol 23:151–160

Hongkulsup C, Khutoryanskiy VV, Niranjan K (2016) Enzyme assisted extraction of chitin from shrimp shells (*Litopenaeus vannamei*). J Chem Technol Biotechnol 91:1250–1256. https://doi.org/10.1002/jctb.4714

Hossain MS, Iqbal A (2014) Production and characterization of chitosan from shrimp waste. J Bangladesh Agril Univ 12(1):153–160

Hu Z, Gänzle MG (2019) Challenges and opportunities related to the use of chitosan as a food preservative. J Appl Microbiol 126(5):1318–1331. https://doi.org/10.1111/jam.14131

Huang L, Xiao L, Yang G (2018a) Chitosan application in textile processing. Mini-review. 4 (2):0032-0034. doi:10.19080/CTFTTE.2018.04.555635

Huang WC, Zhao D, Guo N, Xue C, Mao X (2018b) Green and facile production of chitin from crustacean shells using a natural deep eutectic solvent. J Agric Food Chem 66:11897–11901. https://doi.org/10.1021/acs.jafc.8b03847

Hülsey MJ (2018) Shell biorefinery: A comprehensive introduction. Green energy Environ 3:318–327. https://doi.org/10.1016/j.gee.2018.07.007

İlyasoğlu H, Anankanbil S, Nadzieja M (2018) Lipophilization of chitin as novel polymeric stabilizer for improved oil-in-water emulsions. Colloid Polym Sci 296:1841–1848. https://doi.org/10.1007/s00396-018-4410-z

Ioelovich M (2014) Crystallinity and Hydrophility of chitin and chitosan. J Chem 3(3):7–14

Jaafarzadeh N, Mengelizadeh N, Takdastan A, Farsani MH, Niknam N, Aalipour M, Hadei M, Bahrami P (2015) Biosorption of heavy metals from aqueous solutions onto chitin. Int J Environ Health Eng 4:1–7

Jaganathan K, Raffi SM, Soundarapandian P (2016) Extraction and characterization of chitin from marine bycatch crustaceans employing fermentation method. World J Pharm Pharm Sci 5 (1):1290–1301

Junianto WB, Setyahadi S (2013) Selection of methods for microbiological extraction of chitin from shrimp shells. Microbiol Indonesia 7(2):75–83. https://doi.org/10.5454/mi.7.2.5

Kartal SN, Imamura Y (2005) Removal of copper, chromium, and arsenic from CCA-treated wood onto chitin and chitosan. Bioresour Technol 96:389–392. https://doi.org/10.1016/j.biortech.2004.03.004

Kaur S, Dhillon GS (2015) Recent trends in biological extraction of chitin from marine shell wastes: a review. Crit Rev Biotechnol 35(1):44–61. https://doi.org/10.3109/07388551.2013.798256

Kaya M, Baran T, Karaarslan M (2015) A new method for fast chitin extraction from shells of crab, crayfish and shrimp. Nat Prod Res 29:1477–1480

Khanafari A, Marandi R, Sanatei SH (2008) Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods. J Environ Health Sci 5:19–24

Khempaka S, Mochizuki M, Koh K, Karasawa Y (2006) Effect of chitin in shrimp meal on growth performance and digestability in growing broilers. J Poult Sci 43(4):339–343

Khorrami M, Najafpour GD, Younesi H, Amini GH (2011) Growth kinetics and demineralization of shrimp Shell using *Lactobacillus plantarum* PTCC 1058 on various carbon sources. Iran J Energy Environ 2:320–325. https://doi.org/10.5829/idosi.ijee.2011.02.04.2391

Kjartansson GT, Zivanovic S, Kristbergsson K, Weiss J (2006) Sonication-assisted extraction of chitin from North Atlantic shrimps (*Pandalus borealis*). J Agr Food Chem 54:5894–5902. https://doi.org/10.1021/jf060646w

Kumar Gadgey K, Bahekar A (2017) Studies on extraction methods of chitin from crab shell and investigation of its mechanical properties. Int J Mech Eng Technol 8:220–231

Kurita K (2006) Chitin and chitosan: functional biopolymers from marine crustaceans. Mar Biotechnol 8(3):203–226. https://doi.org/10.1007/s10126-005-0097-5

Lertsutthiwong P, How NC, Chandrkrachang S, Stevens WF (2002) Effect of chemical treatment on the characteristics of shrimp chitosan. J Met Mat Miner 12(1):11–18

Lim S-H, Hudson SM (2003) Review of chitosan and its derivatives as antimicrobial agents and their uses as textile chemicals. J Macromol Sci Part C Polym Rev 43:223–269. https://doi.org/10.1081/MC-120020161

Liu P, Liu S, Guo N, Mao X, Lin H, Xue C, Wei D (2014) Cofermentation of *Bacillus licheniformis* and *Gluconobacter oxydans* for chitin extraction from shrimp waste. Biochem Eng J 91:10–15. https://doi.org/10.1016/j.bej.2014.07.004

Longhinotti E, Pozza F, Furlan L, Sanchez MNM, Klug M, Laranjeira MCM, Fávere VT (1998) Adsorption of anionic dyes on the biopolymer chitin. J Braz Chem Soc 9:435–440. https://doi.org/10.1590/S0103-50531998000500005

Madhavan P, Nair KGR (1974) Utilisation of prawn waste-isolation of chitin and its conversion to chitosan. Fish Technol 11:50–53

Mahmoud NS, Ghaly AE, Arab F (2007) Unconventional approach for demineralization of Deproteinized crustacean shells for chitin production. American J Biochem Biotechnol 3 (1):1–9. https://doi.org/10.3844/ajbbsp.2007.1.9

Malerba M, Cerana R (2019) Recent applications of chitin- and chitosan-based polymers in plants. Polymer (Basel) 11:839. https://doi.org/10.3390/polym11050839

Mao X, Guo N, Sun J, Xue C (2017) Comprehensive utilization of shrimp waste based on biotechnological methods: A review. J Clean Prod 143:814–823. https://doi.org/10.1016/j.jclepro.2016.12.042

Maruthiah T, Palavesam A (2017) Characterization of Haloalkalophilic organic solvent tolerant protease for chitin extraction from shrimp Shell waste. Int J Biol Macromol 97:552–560. https://doi.org/10.1016/j.ijbiomac.2017.01.021

Mathew P, Nair KGR (2006) Ensilation of shrimp waste by *Lactobacillus fermentum*. Fish Technol 43:59–64

Meshkat SS, Nezhad MN, Bazmi MR (2019) Investigation of Carmine Dye Removal by Green Chitin Nanowhiskers Adsorbent Emerg Sci J. 3(3):187–194. doi:10.28991/esj-2019-01181

Mizani M, Aminlari M, Khodabandeh M (2005) An effective method for producing a nutritive protein extract powder from shrimp head waste. Food Sci Tech Int 11:49–54. https://doi.org/10.1177/1082013205051271

Moorjani MN, Achutha V, Khasim DI (1975) Parameters affecting the viscosity of chitosan from prawn waste. J Food Sci Technol 12:187–189

Narayan B, Velappan SP, Zituji SP, Manjabhatta SN, Gowda LR (2010) Yield and chemical composition of fractions from fermented shrimp biowaste. Waste Manag Res 28(1):64–70. https://doi.org/10.1177/0734242X09337658

Neves AC, Zanette C, Grade ST, Schaffer JV, Alves HJ, Arantes MK (2017) Optimization of lactic fermentation for extraction of chitin from freshwater shrimp waste. Acta Scientiarum Technol 39(2):125–133

Nishimura S (2001) Chemical biology and biomedicine: general aspects. In: Fraser-Reid BO, Tastuta K, Thiem J (eds) Glycoscience: chemistry and chemical biology. Springer, New York

No HK, Meyers SP (1995) Preparation and characterization of chitin and chitosan—A review. J Aquat Food Prod Technol 4:27–52. https://doi.org/10.1300/J030v04n02_03

Oh Y-S, Shih I-L, Tzeng Y-M, Wang S-L (2000) Protease produced by *Pseudomonas aeruginosa* K-187 and its application in the deproteinization of shrimp and crab shell wastes. Enzym Microb Technol 27(1–2):3–10. https://doi.org/10.1016/s0141-0229(99)00172-6

Pachapur VL, Guemiza K, Rouissi T, Sarma SJ, Brar SK (2016) Novel biological and chemical methods of chitin extraction from crustacean waste using saline water. J Chem Technol Biotechnol 91:2331–2339

Pacheco N, Garnica-Gonzalez M, Gimeno M, Bárzana E, Trombotto S, David L, Shirai K (2011) Structural characterization of chitin and chitosan obtained by biological and chemical methods. Biomacromolecules 12:3285–3290. https://doi.org/10.1021/bm200750t

Parada RY, Egusa M, Aklog YF, Miura C, Ifuku S, Kaminaka H (2018) Optimization of nanofibrillation degree of chitin for induction of plant disease resistance: elicitor activity and systemic resistance induced by chitin nanofiber in cabbage and strawberry. Int J Biol Macromol 118:2185–2192. https://doi.org/10.1016/j.ijbiomac.2018.07.089

Paul T, Halder SK, Das A (2015) Production of chitin and bioactive materials from black tiger shrimp (*Penaeus monodon*) shell waste by the treatment of bacterial protease cocktail. 3. Biotech 5:483–493

Percot A, Viton C, Domard A (2003) Optimization of chitin extraction from shrimp shells. Biomacromolecules 4:12–18. https://doi.org/10.1021/bm025602k

Ploydee E, Chaiyanan S (2014) Production of high viscosity chitosan from biologically purified chitin isolated by microbial fermentation and deproteinization. Int J Polymer Sci 2014:1–8. https://doi.org/10.1155/2014/162173

Prameela K, Mohan CM, Smitha PV, Hemalatha KPJ (2010) Bioremediation of shrimp biowaste by using natural probiotic for chitin and carotenoid production an alternative method to hazardous chemical method. IJABPT 1:903–910

Pusztahelyi (2018) Chitin and chitin-related compounds in plant–fungal interactions. Mycology 9 (3):189–201. https://doi.org/10.1080/21501203.2018.1473299

Qin Y, Lu X, Sun N, Rogers RD (2010) Dissolution or extraction of crustacean shells using ionic liquids to obtain high molecular weight purified chitin and direct production of chitin films and fibers. Green Chem 12(6):968–971. https://doi.org/10.1039/C003583A

Ramirez-Coutino L, Marin-Cervantes MDC, Huerta S, Revah S, Shirai K (2006) Enzymatic hydrolysis of chitin in the production of oligosaccharides using *Lecanicillium fungicola* chitinases. Process Biochem 41:1106–1110. https://doi.org/10.1016/j.procbio.2005.11.021

Rao MS, Stevens WF (2006) Fermentation of shrimp biowaste under different salt concentrations with amylolytic and non-amylolytic *Lactobacillus* strains for chitin production. Food Technol Biotechnol 44:83–87. https://doi.org/10.1007/s002530000449

Rao MS, Muñoz J, Stevens WF (2000) Critical factors in chitin production by fermentation of shrimp biowaste. Appl Microbiol Biotechnol 54:808–813. https://doi.org/10.1007/s002530000449

Ravi Kumar MNV (2000) A review of chitin and chitosan applications. React Funct Polym 46:1–27. https://doi.org/10.1016/S1381-5148(00)00038-9

Regis B, Marius S, Sandrine B, Roux KL, Del Pino RJ, Jean-Pascal B et al (2015) Kinetic study of solid phase demineralization by weak acids in one-step enzymatic bio-refinery of shrimp cuticles, vol 50. Elsevier Ltd, pp 2215–2223. https://doi.org/10.1016/j.procbio.2015.09.017

Robinson-Lora MA, Brennan RA (2009) The use of crab-shell chitin for biological denitrification: batch and column tests. Bioresour Technol 100:534–541. https://doi.org/10.1016/j.biortech.2008.06.052

Sahu BB, Sahu U, Nagesh Kumar Barik AA, Paikaray A, Mohapatra S, Sahu JK (2017) Bio-refinery products from shell fish processing waste: application of chitin, chitosan, Chitooligosaccharides and derivatives in organic agriculture. Int J Fish Aquat Res 2:27–31

Salaberria AM, Labidi J, Fernandes SCM (2015) Different routes to turn chitin into stunning nano-objects. Eur Polym J 68:503–515. https://doi.org/10.1016/j.eurpolymj.2015.03.005

Sánchez R, Stringari GB, Franco JM, Valencia C, Gallegos C (2011) Use of chitin, chitosan and acylated derivatives as thickener agents of vegetable oils for bio-lubricant applications. Carbohydr Polym 85(3):705–714. https://doi.org/10.1016/j.carbpol.2011.03.049

Sedaghat F, Yousefzadi M, Toiserkani H, Najafipour S (2017) Bioconversion of shrimp waste *Penaeus merguiensis* using lactic acid fermentation: an alternative procedure for chemical extraction of chitin and chitosan. Int J Biol Macromol 104:883–888. https://doi.org/10.1016/j.ijbiomac.2017.06.099

Setyahadi S, Hermansyah H, Aruan JB (2014) Chitin extraction fermentation *Penaeus vannamei* Shell wastes with high density cell by recycle culture cells. J Chitin Chitosan Sci 2:209–215. https://doi.org/10.1166/jcc.2014.1061

Shamshina JL, Barber PS, Gurau G, Griggs CS, Rogers RD (2016) Pulping of crustacean waste using ionic liquids: to extract or not to extract. ACS Sustain Chem Engin 4(11):6072–6081. https://doi.org/10.1021/acssuschemeng.6b01434

Shamshina JL, Oldham T, Rogers RD (2019) Applications of chitin in agriculture. In: Sustainable agriculture reviews. vol 36. pp. 125–146

Shimahara K, Takiguchi Y, Ohkouchi K, Kitamura K, Okada O (1984) Chemical composition and some properties of crustacean chitin prepared by use of proteolytic activity of *Pseudomonas maltophilia* LC102. Chitin, chitosan, and related enzymes. Academic press, New York

Sini TK, Santhosh S, Mathew PT (2007) Study on the production of chitin and chitosan from shrimp shell by using *Bacillus subtilis* fermentation. Carbohydr Res 342:2423–2429. https://doi.org/10.1016/j.carres.2007.06.028

Sluyanarayana Rao SV, Yashodha KP, Mahendrakar NSP (1987) Deacetylation of chitin at low temperature by a novel alkali impregnation technique. Indian J Technol 25:194–196

Song Z, Li G, Guan F, Liu W (2018) Application of chitin/chitosan and their derivatives in the papermaking industry. Polymers 10(4):389. https://doi.org/10.3390/polym10040389

Soon CY, Tee YB, Tan CH, Rosnita AT, Khalina A (2018) Extraction and physicochemical characterization of chitin and chitosan from *Zophobas morio* larvae in varying sodium hydroxide concentration. Int J Biol Macromol 108:135–142. https://doi.org/10.1016/j.ijbiomac.2017.11.138

Sorokulova I, Krumnow A, Globa L, Vodyanoy V (2009) Efficient decomposition of shrimp shell waste using *Bacillus cereus* and *Exiguobacterium acetylicum*. J Ind Microbiol Biotechnol 36:1123–1126. https://doi.org/10.1007/s10295-009-0587-y

Srinivasa PC, Tharanathan RN (2007) Chitin/chitosan — safe, ecofriendly packaging materials with multiple potential uses. Food Rev Int 23(1):53–72. https://doi.org/10.1080/87559120600998163

Srinivasan H, Kanayairam V, Ravichandran R (2018) Chitin and chitosan preparation from shrimp shells *Penaeus monodon* and its human ovarian cancer cell line, PA-1. Int J Biol Macromol 107:662–667. https://doi.org/10.1016/j.ijbiomac.2017.09.035

Sumardiono S, Siqhny ZD (2018) Production of fish feed from soy residue and shrimp waste using tapioca as binding agent. In: the 3rd international conference of chemical and materials engineering, Semarang, Indonesia. J Phys Conf Ser

Suryawanshi N, Jujjavarapu SE, Ayothiraman S (2019) Marine shell industrial wastes–an abundant source of chitin and its derivatives: constituents, pretreatment, fermentation, and pleiotropic applications-a revisit. Int J Environ Sci Technol 16:3877–3898. https://doi.org/10.1007/s13762-018-02204-3

Suryawanshi N, Ayothiraman S, Jujjavarapu SE (2020) Ultrasonication mode for the expedition of extraction process of chitin from the maritime shrimp shell waste. Indian J Biochem Bio 57:431–438

Tan YN, Lee PP, Chen WN (2020) Microbial extraction of chitin from seafood waste using sugars derived from fruit waste-stream. AMB Expr 10:17. https://doi.org/10.1186/s13568-020-0954-7

Teng WL, Khor E, Tan TK, Lim LY, Tan SC (2001) Concurrent production of chitin from shrimp shells and fungi. Carbohydr Res 332(3):305–316. https://doi.org/10.1016/s0008-6215(01)00084-2

The Marine Products Export Development Authority [MPEDA] (2013) Annual report

Valdez-Peña AU, Espinoza-Perez JD, Sandoval-Fabian GC (2010) Screening of industrial enzymes for deproteinization of shrimp head for chitin recovery. Food Sci Biotechnol 19:553–557. https://doi.org/10.1007/s10068-010-0077-z

Vandenbergh PA (1993) Lactic acid bacteria, their metabolic products and interference with microbial growth. FEMS Microbiol Rev 12:221–238

Verma ML, Kumar S, Das A, Randhawa JS, Chamundeeswari M (2020) Chitin and chitosan-based support materials for enzyme immobilization and biotechnological applications. Environ Chem Lett 18:315–323

Wang SL, Chio SH (1998) Deproteinization of shrimp and crab shell with the protease of *Pseudomonas aeruginosa* K-187–waste pretreatment, enzyme production, process design, and economic analysis. Enzym Microb Technol 22:629–633

Woods B (1998) Microbiology of fermented foods, vol 1. Blackie, London

Wu ACM, Bough WA (1977) A study of variables in the chitosan manufacturing process in relation to molecular-weight distribution, chemical characteristics and waste-treatment effectiveness. In: Muzzarelli RAA, Pariser ER (eds) Proceedings of the 1st International Conference on Chitin/Chitosan, Boston, USA, 11–13, April, 1978. pp 88–102

Ximenes JCM, Hissa DC, Ribeiro LH, Rocha MVP, Oliveira EG, Melo VMM (2019) Sustainable recovery of protein-rich liquor from shrimp farming waste by lactic acid fermentation for application in tilapia feed. Braz J Microbiol 50(1):195–203. https://doi.org/10.1007/s42770-018-0024-3

Xu Y, Gallert C, Winter J (2008) Chitin purification from shrimp wastes by microbial deproteination and decalcification. Appl Microbiol Biotechnol 79:687–697. https://doi.org/10.1007/s00253-008-1471-9

Xue W, Han Y, Tan J, Wang Y, Wang G, Wang H (2018) Effects of nanochitin on the enhancement of the grain yield and quality of winter wheat. J Agric Food Chem 66:6637–6645. https://doi.org/10.1021/acs.jafc.7b00641

Younes I, Rinaudo M (2015) Chitin and chitosan preparation from marine sources. Structure, properties and applications. Mar Drugs 13:1133–1174. https://doi.org/10.3390/md13031133

Younes I, Ghorbel-Bellaaj O, Nasri R, Chaabouni M, Rinaudo M, Nasri M (2012) Chitin and chitosan preparation from shrimp shells using optimized enzymatic deproteinization. Process Biochem 47(12):2032–2039. https://doi.org/10.1016/j.procbio.2012.07.017

Zakaria Z, Hall GM, Shama G (1998) Lactic acid fermentation of scampi waste in a rotating horizontal bioreactor for chitin recovery. Process Biochem 33(1):1–6. https://doi.org/10.1016/S0032-9592(97)00069-1

Zargar V, Asghari M, Dashti A (2015) A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications. Chem Bioeng Rev 2:204–226. https://doi.org/10.1002/cben.201400025

Zhang H, Yafang Jin Y, Yun Deng Y, Danfeng Wang D, Zhao Y (2012) Production of chitin from shrimp shell powders using *Serratia marcescens* B742 and *Lactobacillus plantarum* ATCC 8014 successive two-step fermentation. Carbohydr Res 362:13–20

Zhang W, Yin B, Xin Y et al (2019) Preparation, mechanical properties, and biocompatibility of graphene oxide-reinforced chitin monofilament absorbable surgical sutures. Mar Drugs 17 (4):210. https://doi.org/10.3390/md17040210

Zhao D, Huang WC, Guo N, Zhang S, Xue C, Mao X (2019) Two-step separation of chitin from shrimp shells using citric acid and deep eutectic solvents with the assistance of microwave. Polym (Basel) 11:409. https://doi.org/10.3390/polym11030409

Zhou D, Zhang L, Guo S (2005) Mechanisms of lead biosorption on cellulose/chitin beads. Water Res 39:3755–3762. https://doi.org/10.1016/j.watres.2005.06.033

Microbes–Surfaces Interactions

16

Udaya Bhat K and Devadas Bhat Panemangalore

Abstract

Microbes are thought to be the first life on the earth. Over the years, microbes have changed considerably to mutate themselves to support human life and a few other strains to change to an extent to pose danger to the human life. Over the years they have also learnt to adopt to various environments. The effectiveness of the microbial strain is highly influenced by the microbe–surface interactions. This chapter deals with various types of microbe–surface interactions and how the environment affects the interactions. The chapter also explores the concepts of engineering the microbe–surface interactions to exploit in various applications, like biosensors, antifouling surfaces, controlling infections on plants and animals, etc.

Keywords

Microbe–surface interactions · Adhesion of microbes · Animal–microbe interactions · Environmental factors · Surface modifications

16.1 Introduction

Bacteria, fungi, virus, archaea, protozoa, algae, etc., belong to the class of microbes, which exist as single-celled or as colonies. These microbes were the first life on Earth and are integral part of human body from cradle to grave and in the evolutionary development and food chain of life. Throughout our lifetime, microbiota changes

U. Bhat K (✉) · D. Bhat Panemangalore
Department of Metallurgical and Materials Engineering, National Institute of Technology Karnataka, Surathkal, India

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occur continuously, some of them benefit us whereas others pose grave challenges. Microbes communicate with plants and animals via different mechanisms, which are either healthy or detrimental. Microbes are vital to many biological processes and for the biochemical adaptation of animals in the environment (McFall-Ngai 2015). Microbe–surface interaction is an important domain of investigation relevant for applications, such as biosensors (Arya et al. 2012), antifouling materials (Chapman et al. 2014), microfluidics (Eland et al. 2016), antimicrobial surfaces (Elbourne et al. 2017), smart materials (Lupitskyy et al. 2005), etc. Symbiosis of the microorganisms with the host could either be obligatory or facultative (neither needs the other to exist), conjunctive (bodily union), or disjunctive. If a parasite (e.g., head lice) is attached to the host's surface, it is called ectosymbiosis, whereas if an organism lives within the cell or body of another (algae in the endoderm of coral), it is called as endosymbiosis. Study of the microbial attachment to the surface is important to understand their mechanisms and it paves way to develop new surfaces to promote or inhibit cell growth. This chapter focuses on different host–microbial interactions, effect of several environmental factors on growth, surface modifications and discusses a few applications.

16.2 Relevance

16.2.1 Biofouling

Industrial biomass combustion in boilers has a tendency of fouling (Romeo and Gareta 2009). Biological processes causing undesired microbial depositions like slime layers in pipelines and biofilms on catheters pose detrimental effects. Membrane bioreactor (MBR) used for wastewater treatment can undergo membrane fouling (Iorhemen et al. 2016) and it is due to the deposition/accumulation of microbial products (Gkotsis and Zouboulis 2019). To inhibit biofouling, disruption of nutrient transport which leads to the starvation of microbial colony using biocides (Rao et al. 2017) can be used. But sometimes biocides can stress the working materials and it is required to adopt an effective antifouling strategy (Flemming 2020). To control membrane biofouling, Sun et al. adopted online chemical cleaning that altered the dominant group on the membrane from Burkholderiaceae to Flavobacteriaceae (Sun et al. 2016). To withstand biofouling, hydrophilic and smooth surfaces should be preferred for microbial electrochemical technology (Koók et al. 2019). Also, carbon-based nanomaterials such as quantum dots and fullerenes show antifouling performance (Wu et al. 2020).

16.2.2 Biofilms

One of the first observations of biofilm was done by Leeuwenhoek, who saw white matter growing between his teeth using his first microscope. Bacterial surface colonization, prior to developing biofilm undergoes preferential attachment that improves fitness under stress such as antibiotics and predation (Grinberg et al. 2019). Microorganisms develop biofilm via four steps, i.e. attachment to the surface, formation of microcolonies, biofilm maturation and dispersion that further leads to repetition of these steps (Dos Santos et al. 2018). Biofilm formation is one of the defenses of microorganisms that consist of extracellular polymeric substances (EPS). Biofilms cater further attachment to the surface, render communication between each other, and also optimize the environment (Cuadros 2017). There are several detection and measuring techniques involved, such as microscopy, spectroscopy, reflectometry, phospholipid based analysis, dye-staining, etc. (Subramanian et al. 2020).

16.2.3 Infection of Plants and Animals

Several diagnostic symptoms of bacterial infections in plants include bacterial spots in the leaves, gummosis in twigs, scabs in potatoes, etc. Since these infections that spread to diseases cannot be easily controlled, it is preferred to prevent the spread rather than healing via crop rotation, using hybrids, application of chemicals, etc. Pathogenic microbes also depend on the environment such as rainfall and atmospheric humidity (Xin et al. 2016) to spread infections (Aung et al. 2018). These microbes are also capable of altering the plant nutrient pathways at the molecular level (Plett and Martin 2018). Several polymicrobial interactions develop antibiotic tolerance to chronic infections related to bone (osteomyelitis), gum (periodontitis), urinary tract, etc. (Ibberson and Whiteley 2020). Microbial interactions can also affect our mental health (Hayes et al. 2020). Virulence is a term that defines the disease inducing capability of a microbial agent. Genome type, biochemical characteristics, structure, interaction with the host, and environmental factors can influence the infection rate and its spread to animals. Bovine tuberculosis, anthrax, pneumonia are some examples of bacterial cattle diseases (Abdelhay Kaoud 2019). Zoonotic bacterial diseases are caused by Staphylococcus. Campylobacter could be transmitted from animals to humans via food consumption and vice-versa (Shin and Park 2018). Hence, disease preventive measures in veterinary science could be improved by understanding the animal–microbe interactions.

16.2.4 Plant Decay

Several microorganisms along with insects and worms aid plant decay, i.e. breaking down its composition (decomposition). It is an important phenomenon that releases CO_2 necessary for photosynthesis and other chemicals, which are important for life. Optimum oxygen and nitrogen levels are important for the microbes to break the bonds holding the molecules of cellulose and enhance the rate of decomposition. Increased global warming could increase the reaction rates but lower the microbial efficiency. Microbial communities can also adapt to changing climates via feedback mechanism (Glassman et al. 2018).

16.2.5 Machinery

Microbes are important in dairy and beverage production throughout the world (Dos Santos et al. 2020). Fermentation is an important process for dairy and ethanol production and Candida yeasts have significant biotechnological use in the food industry (Kieliszek et al. 2017). Several physical and chemical cleaning and disinfection procedures must be adopted to clean tanks and tubes to prevent infectious diseases. Otherwise, microbial spoilage in the case of beer brewing, presence of Campylobacter while mishandling meat production can shorten the lifespan of machines and lead to poor food hygiene. Metal working industries suffer the problem of microbial contamination in metalworking fluids. Incorporation of a biocide was not effective and the only solution was proper cleaning and disinfection before usage (Desrousseaux et al. 2013).

16.2.6 Mineral Weathering

Mechanical and chemical processes lead to the dissolution of minerals via microbes that has shaped our lithosphere (Mapelli et al. 2012) and deep biosphere below seafloor (Edwards et al. 2005). Dissolution of metals from minerals via prokaryotic acidophiles is defined as biomining, which is used in the recovery of copper and gold from low grade ores (Johoson 2008). Acidithiobacillus, Leptospirillum, Ferroplasma, and Sulfolobales are some of the microorganisms that govern this process (Johoson 2008). Mineral weathering can increase fertility of the soil via transportation of mineral nutrients, even in a high Arctic desert (Borin et al. 2010). It is also possible to understand the cell–mineral interface via TEM analysis to study the bioweathering of biotite with Hassallia byssoidea (cyanobacterium) (Ward et al. 2013), using focused-ion-beam preparation of electron-transparent specimens.

16.2.7 Mineralization

Precipitation of an inorganic material in an organic matrix is defined as mineralization, which can be controlled by different processes in a microbe at the cellular level. Microbes can alter the physical and chemical state of minerals and their role in geological processes is of great significance. Redox transformations of the elements, specially belonging to the transition series can be a part of the microbial metabolism (Geoffrey 2010). Liu et al. (2020) studied the biomineralization aspect of the desert soil microbes by converting atmospheric CO_2 into soil inorganic carbon. Magnetotactic bacteria that consist of magnetic iron nanominerals align along geomagnetic field lines (Yan et al. 2012). These bacteria are capable of producing magnetite (Fe_3O_4) (Frankel et al. 1979) particles that find several biotechnological applications such as MRI, drug delivery, etc. (Arakaki et al. 2008). Shinohara et al. (2011) used microbial mineralization via novel hydroponic culture method to convert organic nitrogen to nitrate via ammonification and nitrification.

16.2.8 Microbial Interactions

There are several theories and mechanisms of bacterial adhesion that are influenced by the surface chemistry and topology of the material (Moriarty et al. 2017). The interactions such as mutualism (+,+), syntrophism (Schink and Stams 2012) are positive interactions that are co-operative and the organisms get mutually benefitted, whereas negative interactions such as amensalism (0,-), parasitism, competition (−,-), and predation (+,-) lead to negative affection. Gut bacteria is an example for mutualism where both species are benefitted. Syntrophy is the mechanism pertained to the association of two different microorganisms which perform a function (Schink and Stams 2012) synergetically, otherwise that cannot be done alone. Amensalism is when there is negative interaction of A to B, when B has no detectable impact on A (Kitching and Harmsen 2008). It is different from commensalism (+, 0) where the symbiont gets benefitted without the host getting affected (Hartel 2005). Guven-Maiorov et al. (2020) developed Host–Microbe Interaction PREDictor (HMI-PRED) to predict structural host–microbe interactions via interface mimicry.

16.2.9 Biomedical Devices

Several infections such as dental caries, periodontitis, bacterial prostatitis, etc., involve biofilm bacterial species such as *E. coli*, *Streptococcus,* etc., that commonly occur on medical devices (Costerton et al. 1999). Implant devices, such as pacemakers, contact lenses, catheters can develop biofilm on their surfaces (Chen et al. 2013). A clean and sterile implant when placed inside the body interacts with the bodily fluids and several components diffuse towards the implant. These macromolecules form the conditioning film which is the first step in the biofilm formation (Habash and Reid 1999). This step is absent during in-vitro tests and hence the implant loses its efficacy during in-vivo tests (Cormio et al. 1996). During the initial microbial approach, forces such as van der Waal's interactions, interplay of attractive and repulsiveness take place (Habash and Reid 1999). This is followed by growth and colonization and finally the formation of a biofilm which creates a microenvironment which is immune to many antimicrobial agents (Habash and Reid 1999). Although a lot of research has been made to study the microbial interaction with the implants inside the human body, so far it is not possible to completely prevent microbial adhesion (Habash and Reid 1999).

16.3 Adhesion of Microbes to the Surface

The outer walls of a bacteria consists of biomacromolecules and they are covered with polysaccharides or lipopolysaccharides if the bacteria is gram-positive or gram-negative, respectively (Harvey 2007). Cell adhesion to the surface depends on parameters such as hydrophobicity and electrokinetic potential (Van Loosdrecht et al. 1987). Adhesion studies of cells to polystyrene was studied by van Loosdrecht et al. (1987) who attributed the initial instantaneous interaction as weak and reversible. In this phase, the cells exhibit Brownian motion and they could be removed by washing using a fluid due to shear action (Marshall et al. 1971). The irreversible attachment which is usually stable is due to strong bonding forces like covalent bonding (Jones and Isaacson 1982; Elbourne et al. 2019) between the material surface and bacterial organelles, such as curli fibers, pili (fimbriae) (Pratt and Kolter 1998), and flagella (Tuson and Weibel 2013). Yoshihara et al. (2015) estimated the adhesive forces of *E. coli* microbial flagella on a glass substrate and found that a greater force was required for cell detachment from the surface. After initial adhesion, interfacial rearrangements (Busscher et al. 2010) and bond strengthening via hydrogen bond (Boks et al. 2008) can strengthen the microbial attachment.

16.3.1 Clay–Humus–Microbes Interactions

Soil consists of substratum at the bottom, followed by subsoil and surface at the top. Humus is the topsoil horizon (up to 30 centimeters) that consists of disintegration of organic matter followed by their combinations and it is amorphous in nature. Humification is a process by which the stable string of organic polymers (humus) is formed from the organic matter that did not contribute to mineralization process by a combination of microbes. Clay–humus interaction takes place between the inorganic mineral particles of clay and with the proteins, amino acids of humus via adsorption mechanism. With respect to the adsorption behavior, the linkage will be unstable if humic acid is attached to the micelle of the clay and when the cations of clay (Al^{3+}, Mg^{2+}, Ca^{2+}) take part in the interaction, the linkage will be stable (Khan and Schnitzer 1972; Head and Zhou 2000). The plant growth and farming is directly related to this interaction and a simple operation such as soil tillage will affect the clay-humus stability. Dubey and Dwivedi (1985) saw that the kaolinite ($Al_2Si_2O_5(OH)_4$), Zn, and Mn reduced the toxicity of Cd on the growth of *Macrophomina phaseolina* and this was attributed to ion antagonism. Playter et al. studied the microbe–clay interactions that preserved the trace metal signatures of marine planktonic cyanobacterium in black shales (Playter et al. 2017). Tazaki and Asada carried out TEM studies of clay minerals attached to exopolysaccharides of bacteria resistant to Hg that is present in gold mines (Tazaki and Asada 2007). Extracellular polymeric substance (EPS), that is cohesive in nature, adsorbed Fe, Hg, and Au within the smectite, kaolinite, and halloysite of clay, which can get released into air (Tazaki and Asada 2007). Clay minerals can inhibit respiration of fungal species (Stotzky and Rem 1967). Clay minerals have an effect on nitrogen cycle, carbon cycle, humification processes (Filip 1973). Interaction takes place via sorption and this reversible process depends on the type of sorbent, microbe, environment, etc. (Filip 1973). Bentonite can impact the microbial activity and can influence the starch mineralization (Filip 1973). The activity of clay minerals with soil affects the activity of soil microbes. Nitrogen, potassium, sodium, magnesium are some of the important metal nutrients absorbed by the microorganisms (Landeweert et al. 2001). Bacteria are instrumental in precipitating clay from solutions and also help in weathering of silicate minerals (Douglas and Beveridge 1998). Altering mineral composition of the soil with mica, basalt, and rock phosphate addition both separately and together was tested by Carson et al. that attributed to a change in the microbial community structure (Carson et al. 2007). Other interactions with the microorganisms include hydration state modification on clay interlayer expansion and organic compound intercalation (Alimova et al. 2009).

16.3.2 Plant–Microbe Interactions

Plant–microbe interactions occur on many levels (foliage, roots), and different ways (destructive, neutral, etc.). They develop interrelationships among different microorganisms. A representation of insect–plant–microbial interaction is shown in Fig. 16.1. The most important symbiosis occurring in major crops, the Arbuscular mycorrhizal fungi supplies nitrates and phosphates from the soil to plants. The plants in return supply nutrients in the form of carbon to these fungi that are associated with the roots. Their association dates back to several million years which also might have played a role in plant colonialization of land surface (Redecker et al. 2000). The plasma membrane nanodomains in plants that function as exchange medium for ion and signaling molecule transport, play a major role in plant–bacterial interactions (Bhat et al. 2005; Haney and Long 2010; Lefebvre et al. 2010). This membrane raft keeps control over incoming harmful pathogens at the interface and with recent

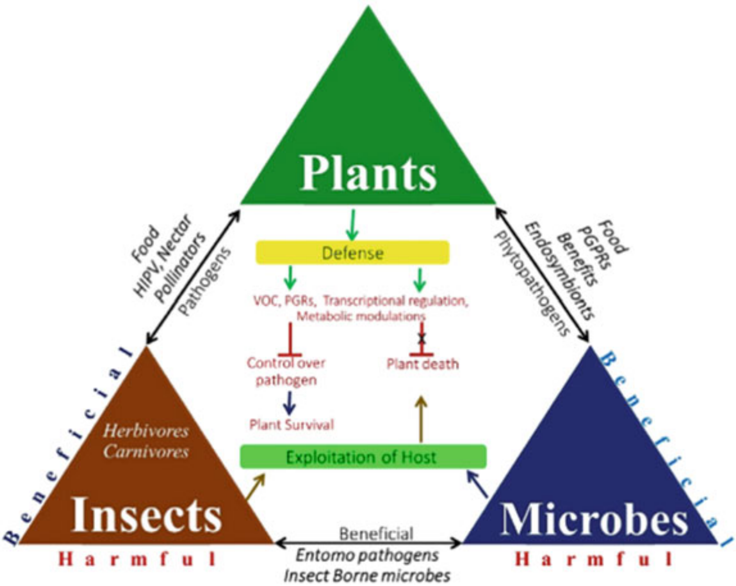

Fig. 16.1 Pictorial representation of the beneficial and harmful plant–insect–microbial interactions (Reproduced with permission from ELSEVIER) (Noman et al. 2020)

advances in high resolution microscopic techniques, such interactions could be directly observed (Ziomkiewicz et al. 2015).

A process that is used to transform the target pollutants into inert substances via stimulating microbial activity and modifying environment conditions is known as bioremediation. Rhizoremediation takes place in the rhizosphere (soil around plant roots) which remediates pollutants such as polycyclic aromatic hydrocarbons (PAHs). Several rhizobacteria belonging to Serratia, Bacillus, and Pseudomonas species provide host with defense and protect the plants from pathogens like fungi (Tikhonovich and Provorov 2007). Transport of nutrients vary along the length of root and so does its surface characteristics and therefore rhizosphere consists of a variety of microenvironments (Ramey et al. 2004).

Increased CO_2 concentrations in the atmosphere, rise in temperature, metal polluted soil, drought, land salinity, and other climate changing events can alter the plant–microbe relationship (Rajkumar et al. 2013). The microbial structure could be altered due to the presence of soil pollutants, which can have contrasting effects on different plants as studied by Feng et al. (2020) for maize and rice. Lindane, an organochloric pollutant present in agricultural pesticides is removed significantly by maize roots from soil as compared to rice, which affects plant growth that in turn affects the microbial environment in the rhizosphere. Mayton et al. (2019) studied the effects of water chemistry and nutrient availability on *E. coli* and *S. typhi* adhesion to spinach leaf surfaces. Climate change can have an adverse effect on the plant–microbe interactions (Aamir et al. 2019; Singh et al. 2019). Ranganathan presented beneficial and harmful plant–microbe interactions from the plant's defense mechanism perspective, also known as "innate immunity" (Janeway 1989).

Nitrogen-Fixing Symbioses

French scientist Antoine Lavoisier named nitrogen as "azote," which means "without life" in French. Humans cannot survive without plants and N_2 is an important constituent of plant mineral nutrition. Chlorophyll, amino acids, ATP, and nucleic acids consist of nitrogen, but plants can use the reduced forms (e.g., NH_3) and not the predominantly available N_2 gas. Among several means of combined nitrogen procurement, symbiotic nitrogen fixation is the association between nitrogen-fixing bacteria (also called diazotrophs), such as Azospirillum that provides fixed nitrogen and the host plant that provides fixed carbon in exchange. The Gunnera–Nostoc plant-N_2 fixing cyanobacteria endosymbiosis is also facultative where the bacteria enter the cells of the Gunnera angiosperm via mucilage-secreting glands (Khamar et al. 2010). Nostoc provides reduced N_2 via its heterocysts that create an environment to synthesize nitrogenase and different proteins (Wolk et al. 1994) to generate fixed N_2.

Legume-Rhizobia Symbioses

A plant belonging to the bean family or Fabaceae, legumes consists of root nodules that develop symbiosis with rhizobia and play an important role in crop rotation. *Rhizobium leguminosarum* is one of the first and most thoroughly studied species that exhibit mutually beneficial association. The symbiosis is initiated by the mediation of signaling molecules such as lipo-chitooligosaccharides by the plant, also called nod factor that are secreted by rhizobia (Dénarié et al. 1996; Van Zeijl et al. 2015). The intermediary barrier between the symbionts, a temporary plant organelle, namely symbiosome dictates the transport processes (Clarke et al. 2014). Nod factors which promote the modulation of legume roots activate bacterial infection at the epidermis and nodule organogenesis via different processes, such as calcium oscillations, gene expression, cytokinin signaling, inhibition of polar auxin transport at the inner or mid cortex (Oldroyd et al. 2011), both these developments are coordinated. Curling of root hairs and induction of the cortical cell divisions comprise pre-infection steps. Bacterial infection mainly occurs via root hairs, but in some legumes it can occur via cracks in the root epidermis. At this point, there is an initiation of composite structures called infection threads and its progression to the inner cortex of the host root. It is then followed by autoregulation of nodulation, nodule tissue differentiation, release of bacteria into the plant cells, and bacteroid differentiation. Nodule organogenesis is initiated by the flavonoids produced by the host and recognized by the rhizobia via NodD receptor-transcription factor (Gifford et al. 2018).

Defensive Symbioses

This mechanism constitutes an indirect interaction between the host, microbe (symbiont), and the enemy (Clay 2014). This involves the host (plant) supplying its energy to microbes for its metabolic demands in order to protect itself from biotic stresses against pests and pathogens (Tikhonovich and Provorov 2007). The principle behind this mechanism is similar to few insect species which host symbiotic bacteria that help them to defend against its enemies (Nakabachi et al. 2013). Seaweeds also hosts epiphytic bacteria that protect them from secondary colonization (Egan et al. 2013). Cordier et al. studied the effect of Glomus mosseae fungus on the bioprotection of tomato roots against parasites (Cordier et al. 1998).

16.3.3 Animal–Microbe Interactions

Eisthen and Theis (2016) reviewed the influence of this interaction on the evolution of the nervous system of animals. Significant research on whether the microbes can influence our cognition and social behaviors is underway. Journal of Animal ecology presents several research works on the host–microbe interactions (Hoye and Fenton 2018). Microorganisms on the outer layer of epidermis can be permanent which grow and multiple or transient which remain for a short period of time (James et al. 2008). Diapause is a period of suspended development in animals and insects. Mushegian and Tougeron (2019) reviewed the microbial factors that affect this process. During diapause, microbes can induce antibiotic production, regulate nutrients, and tolerate heat, cold, and stress. These diapausing hosts can serve as pathogen reservoirs but in some cases, it can cause innate immunity.

Humans need microbes to stay healthy and there are several locations of microbiome such as oral, nasal cavities, genito-urinary, gastrointestinal tracts, etc.

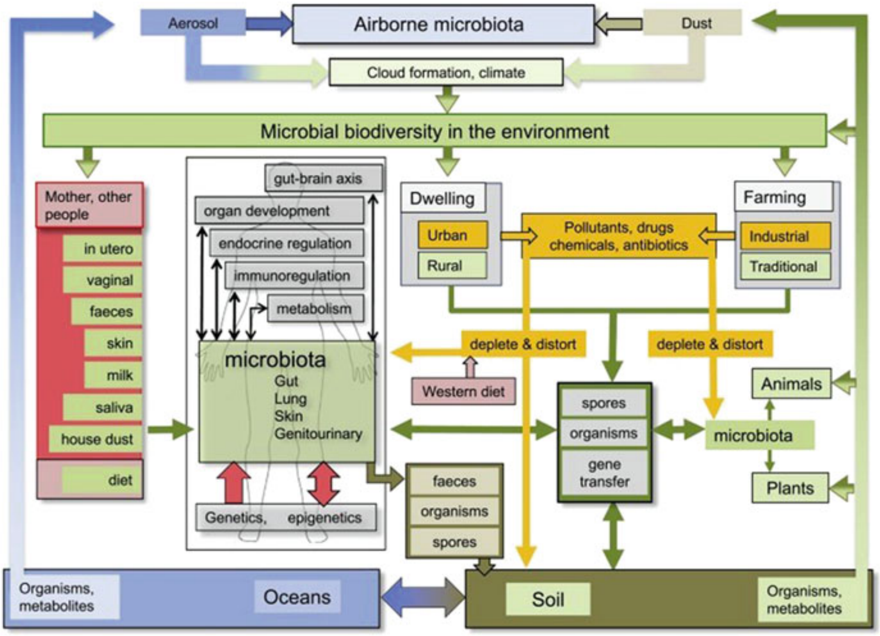

Fig. 16.2 Microbes–human–environment *(Reproduced with permission from ELSEVIER)* (Flandroy et al. 2018)

Bacillus subtilis spores, which are metabolically dormant protect the skin against environmental oxidative stress produced by inorganic trivalent arsenic that otherwise can lead to keratosis and carcinoma (Petruk et al. 2018). Houghteling and Walker presented several factors such as natural prebiotics, breast milk (bifidobacterium), etc., that help in normal colonization of bacteria that will aid in adult microbiome (2015). The birth mode is also a factor that can alter the bacterial colonization (Dominguez-Bello et al. 2010). Our body is sterile when we were born and during the process of birth, we first come in contact with bacteria. Kaplan et al. (2011) reviewed factors in development of immunologic programming during several stages of development such as pre-pregnancy, in-utero, etc. With several factors such as maternal nutrition, nutrient transfer, parental genes, delivery method, in-utero priming contribute to the infinitely complex material-fetal system (Kaplan et al. 2011). Gut microbiota plays a very important role in our gastrointestinal tract that is home to several health-promoting microbes, which otherwise could lead to intestinal dysbiosis. The human wellbeing is directly influenced by the microbial world and a pictorial representation can be seen in Fig. 16.2.

16.3.4 Microbe–Microbe Interactions

Such interactions take place in the human body on the skin (Gallo 2017), in the gastrointestinal tract, in the lung, etc. (Hörmannsperger et al. 2012; Skaar and Zackular 2020). Intra/interspecies signaling that includes indole, cyclic dipeptides, and other signaling molecules and interkingdom signaling are different modes of interaction among microbes. Bacteria communicate via quorum sensing, a method that allows them to coordinate several processes such as virulence, bioluminescence, competence, etc. (Rutherford and Bassler 2012). Several signaling molecules such as 2-alkyl-4(1H)-quinolones produced by *Pseudomonas aeruginosa* (Rampioni et al. 2016), cyclic dipeptides by *P. fluorescens* (Bellezza et al. 2014), etc., are examples of plant associated microbes. Simultaneous nutrient transportation and colony protection take place via this mechanism in *Vibrio cholerae*, the human pathogen leading to infamous cholera disease (Cámara et al. 2002). Hulkova et al. (2020) cocultured *P. aeruginosa* with *Candida albicans* and saw the efficacy of polyethylene surface modified with silver nanoparticles getting undermined (Fig. 16.3).

16.4 Interaction in Attached and Unattached Forms

Ahmed and Holmström (2015) studied the effect of fungal and bacterial attachment on mineral weathering. Microbial cell separation from the biotite surface was ensured using PET track etched devices. The mineral biotite on weathering led to dissolution of elements, such as Fe, Al, and Si. It was more prone to surface attached microorganisms. Aerobic degradation experiments conducted by Holm et al. (1992) to study the xenobiotic organic contaminants in an aquifer sediment such as benzene, toluene, etc., determined that the unattached bacteria in the microbial biomass was also important in determining degradation potential. Lehman et al. (2001) observed compositional differences between the attached and unattached bacterial communities in *122 m corehole* from a buried chalcopyrite ore. He also deduced that sampling method influences the aquifer microbiology (Lehman 2007). Size-selective predation of aquifer nanoflagellates on bacterial community was estimated by Kinner et al. (1998) using time series incubation experiments and found that 74% of the unattached bacterial community consumption in a day. For airborne bacteria, four important environmental parameters like temperature, relative humidity, occupant density, and air exchange rate measurements are important for data analysis (Fujiyoshi et al. 2017). Airborne bacterial at-a-distance interactions are mediated by bacterial volatile compounds (BVCs) along with secondary metabolites (Audrain et al. 2015a, b). Jung et al. (2018) studied the cooperation of attached bacteria and unattached fungal interaction on disease progression on rice plants and this interaction also promotes aerial dispersal of the bacteria.

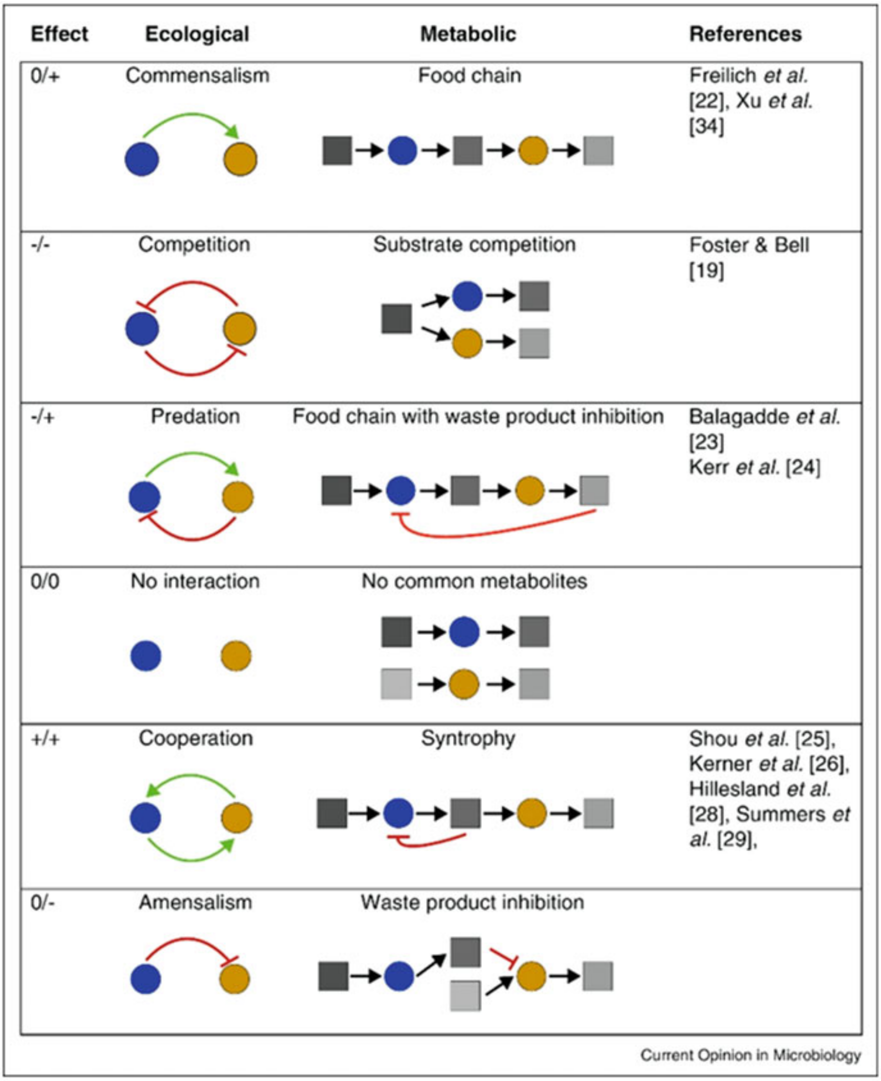

Fig. 16.3 Six basic motifs of microbial interactions. Circles blue and yellow represent microbial stains, whereas boxes denote metabolites (Großkopf and Soyer 2014)

16.5 Effect of Environmental Factors

Microorganisms exhibit "cold-shock response" against abrupt change in any environmental factor, such as pH, temperature, etc., and the stress responses will act towards its survival (Beales 2004). Yang et al. (2017) proposed a metagenomics Lognormal-Dirichlet-Multinomial (mLDM) to investigate the association between microbe–microbe and environmental factors–microbe interactions. The microenvironment in the vicinity of the bacterial surface adhesion with respect to ionic strength, pH, osmolarity is different as compared to the bulk of the material (Berne et al. 2018). Salt concentration also could possibly affect the microbial growth that can lead to change in pH and hence lead to spoilage of food products (Röling et al. 1994).

16.5.1 pH

The thermodynamics and kinetics related to microbial growth depend on the effect of hydrogen ion concentration or pH. Any change in the environmental pH will set the feedback loops that will affect its metabolism. Typical bacteria like *E. coli* and *staphylococci* prefer neutral pH values (neutrophils), whereas acid loving microbes with pH < 5 are called acidophiles. A few examples like *archaean genus Ferroplasma* that finds applications in trace metal extraction via bioleaching, milk fermentation into yogurt via lactobacillus, sulfur oxidizing *Sulfolobus*, etc. A neutrophile *Helicobacter pylori* that causes peptic ulcers survives in the acidic environment of the stomach via releasing ammonium ions due to internal urease activity (Rektorschek et al. 1998). Alkaliphile like *Vibrio cholerae* can survive with pH > 8 (Preiss et al. 2015). Jones et al. (2015) reviewed the effect of pH on biofilms and specify that every microbe has its own pH range (5.5–8.0) for its growth and indicated an optimum pH at which it displays highest growth. Study of microbial activity in acidic soil is important as nitrification takes place in the alkaline pH range and it is inhibited below pH $= 7.5$ (Lynch 1995). Also, microbes can alter the pH of the environment, an example is *Paenibacillus sp*. that lowers pH of the environment when its population density is high. This negative interaction leads to its extinction—its ecological suicide (Ratzke et al. 2018), whereas addition of bactericidal substances such as disinfectants, antiseptics could save them.

16.5.2 Temperature

Low temperature could possibly slow down the cellular process and high temperature can cause the cells extinct. An example is that of fever that activates our immune system, it is harder for the virus or bacteria to survive. On the other hand, microorganisms are capable of raising the body temperature due to the microbial metabolism. For their optimal growth, one can state the upper and lower limits of temperature and a particular temperature in this range with prime growth characteristics. An Arrhenius plot of the logarithmic value of microbial generations per hour versus temperature can give an idea of permissive temperatures for growth. Depending on the microbes that live in the temperature range between 20 and 45 °C are referred to as mesophiles and they live in the human microbiome. The category of microbes beyond this temperature upwards are termed thermophiles and hyperthermophiles. The reaction rates of enzymes that govern nutrient metabolism can be correlated to the temperature of a microbe to form a biofilm (Stepanović et al. 2003). Climate changes can account for changes in the behavior of microorganisms such as production and removal of greenhouse gases, affect the microbes living in the permafrost layers that could possibly melt. Sposob et al. studied the changes in temperature affecting the Proteobacteria microbial community that affected the sulfur accumulation (Sposob et al. 2018). *Thermus aquaticus* is a thermophile (heat-loving) that grows in hot springs at 70 °C and its DNA polymerase led to the invention of Polymerase Chain Reaction (PCR) (Innis et al. 1988). *Pyrolobus fumarii* is a hyperthermophile that grows optimally at 106 °C (Anderson et al. 2011) which could survive the autoclave used to kill bacteria during sterilization.

16.5.3 Adhesiveness of Biofilms

Chavant et al. (2002) assessed the biofilm formation and adhesiveness by *L. monocytogenes LO28* on PTFE surfaces. At 37 °C, the hydrophobic PTFE led to the detachment of biofilms, they were stable at 20 °C due to the flagellation but at 8 °C, the colonization power of the strain decreased and hence PTFE could be a possible candidate to be used in cold rooms for food storage. Development of high resolution microscopes has led to study the dynamic behavior in bacterial biofilms with respect to migration, attachment, and detachment via digital time-lapse imaging (Stoodley et al. 2001). Ponomareva et al. discussed several factors like nutrient availability, ferrous concentration, osmolarity, temperature that affect the microbial biofilms, but it is capable of developing its own local environment after maturity (Ponomareva et al. 2018). These biofilms can be resistant to antibiotics by innate resistance factors via alteration of metabolic activity such as oxygen and nutrient availability (Anderson and O'Toole 2008). Bacteria could possess different adhesins that are environment specific (Macklaim et al. 2011). Adhesion can also be preferential adherence to same genotype cells apart to surfaces (Queller et al. 2003).

16.5.4 Extreme Environments

Extremophiles are a class of microbes that survive in extreme environments (polyextremophiles can withstand more than one extreme environment). Research on life in earth's extreme environments is ongoing for potential habitats in other planets and moons (Marion et al. 2003). Bacteria such as *Deinococcus radiodurans* are radioresistant, they can withstand radiations of up to 5000 Gy (up to 5 Gy can kill a human being) via using Mn (II) complexes as antioxidants, rapid DNA repair, and several copies of its genome (Battista et al. 1999). Several applications such as information storage surviving a nuclear catastrophe, study related to aging (Slade and Radman 2011) is observed. Low temperatures up to −20 °C could be withstood by some of the microbes, regulate metabolite transport, ATP synthesis, etc., and demonstrate microbial metabolism (Clarke et al. 2013). *Thermococcus barophilus,* belonging to a class of piezophilic bacteria (Yayanos 2008) isolated from the depths of about 3550 m on the Mid-Atlantic Ridge can withstand high hydrostatic pressures (Marteinsson 1999). A strain belonging to Halomonadaceae family, *GFAJ-1* (Erb et al. 2012) is a metallotolerant bacteria that is capable of resisting high levels of arsenic. Halophiles are microbes that survive in saline environments and some of them such as bacterioruberin (Rodrigo-Baños et al. 2015) produce pigments such as carotenoids that impart color to water bodies.

16.6 Surface Modifications to Manage Microbe–Surface Interactions

Ma et al. (2011) studied the hydrophobic surfaces of lotus and taro leaves and attributed its superhydrophobicity to the dense nanostructures that are found around epidermal papilla. Such nanostructured topological surfaces can be engineered to eradicate bacterial fouling under completely wetted conditions.

For implant related infection, several strategies (Wang and Tang 2019) such as prevention of bacterial adhesion (Park et al. 2013), contact killing (Jose et al. 2005), antibiofilm (Tan et al. 2015), release killing (Braem et al. 2015) have been developed. TiO_2 coated with antifouling agent and mussel-inspired catechol-grafted dextran (Park et al. 2013), antibiotic vancomycin covalently bonded with Ti that kills susceptible bacteria (Jose et al. 2005), enzyme immobilization to enhance antibiofilm efficacy (Tan et al. 2015), bio-active toremifene molecule leading to release killing are some of the examples of novel surface modifications against infection. Graphene oxide coatings with sharpened edges of the nanowalls showed enhanced antibacterial activity (Akhavan and Ghaderi 2010). Drug eluting hydrogels are an example of a biocide-based strategy to kill the microbial cells on contact (Bazaka et al. 2015). Varaprasad et al. designed Ag nanoparticle-curcumin composite films for antimicrobial applications and due to the synergistic effect, it displayed inhibition of *E. coli* growth (Varaprasad et al. 2011).

Carobolante et al. (2018) carried out anodic oxidation of Ti10Mo8Nb alloy to develop nanostructured TiO_2 on the surface for biomedical applications. This nanoporous layer exhibited reduced proliferation of *Staphylococcus epidermidis* due to increased surface area of the anatase structure. Lorenzetti et al. (2015) synthesized anatase coatings via hydrothermal treatment that provided nanoroughness features which led to reduced contact area at the interface and hence reduced *E. coli* adhesion.

Delaviz et al. (2015) discussed the development of antibacterial mechanisms and coatings in designing infection resistant biomaterials.

16.7 Microbe–Materials Interaction

Bohinc et al. (2014) studied the bacterial adhesion using atomic force microscopy and spectrophotometric measurements onto different glass surfaces. They attributed increased adhesion to surface roughness and other factors like hydrophobicity, surface charges had little impact on bacterial adhesion. Farahat et al. studied the adhesion of *E. coli* onto oxide minerals such as hematite, corundum, and quartz (Farahat et al. 2009) using contact angle measurements. At lower pH values, *E. coli*-quartz combination has the highest affinity, followed by corundum and hematite surfaces. The contact angle measurements showed that the mineral surfaces were hydrophilic and its interaction with *E. coli* rendered them to be more hydrophilic. Zhou et al. studied the adsorption characteristics of *Acidianus manzaensis YN25* on chalcopyrite and found increased adhesion at lower pH values, that increased with initial cell concentration but the rate decreased with time (Zhou et al. 2019). Solar energy driven carbon dioxide bioelectrosynthesis, which is an artificial photosynthesis system relies on the material–microbe interface (Sahoo et al. 2020). For solar to chemical energy conversion, the interface can be intercellular (Zhang et al. 2018) or extracellular (Holmes et al. 2008; Rosenbaum et al. 2011). Extracellular CdS crystallites produced by bacterium Klebsiella aerogenes in Cd^{2+} environment absorb ultra-violet rays and develop a photoprotective layer (Holmes et al. 2008). Intracellular Au nanoclusters aid in enhancing the electron transfer kinetics of photosynthetic biohybrid systems for solar fuel production, as studied by Zhang et al. (2018).

Shen et al. (2019) studied the microbial adhesion of *Pseudomonas aeruginosa* onto the cosmetic contact lenses, which if not treated can cause ocular infectious diseases such as microbial keratitis that can result in blindness. The microbial adhesion was found to be greater with surface roughness. Gordesli and Abu-Lail (2012) studied the adhesion energies of *L. monocytogenes* to the surface of Si_3N_4 using AFM. Garrett et al. (2008) reviewed instrumental methods used to understand the adhesive properties of bacteria. Weiss (1961) carried out cell-counting technique to study bacterial adhesion by implementing distractive techniques to separate the adherends from the surface. Fang et al. (2000) used AFM to quantify bacterial adhesion forces. They calculated adhesion forces between Si_3N_4 tip of the AFM and the bacterial surface, cell–cell interface and quantified these interactions using topographical images. Rahnamaee et al. (2020) developed TiO_2 nanostructures for biomedical implant applications. The bioinspired hierarchical micro/nano wettable surfaces with adequate roughness were developed to inhibit bacterial adhesion, which otherwise could lead to clinical infection and septic loosening. Dewald et al. (2018) studied the microbial adhesion on nanostructured surfaces with COOH-functionalized gold nanoparticles. The contact point density as seen using FIB-SEM was minimum and this led to a reduced microbial adhesion. Duch et al. (2019) modified the graphitic sheets via oxygen plasma treatment and evaluated its electrodonor properties on bacterial adhesion. Biochar is a charcoal-like constituent produced from agricultural crop residues via pyrolysis. Zhu et al. (2017) reviewed the mechanisms of microbe–biochar interactions in soil that enhances its fertility, quality, carbon storage, and pollution remediation. Biological Nitrogen Removal from high ammonia nitrogen wastewater is important to enhance oxygen concentration in industrial wastewater and urban sewage. Peng et al. (2018) introduced N-acyl homoserine lactones for increased microbial adhesion and formation of biofilm on biocarrier surfaces. Limsuwan et al. (2014) studied the antimicrobial effect of Rhodomyrtus tomentosa leaf extract for human buccal epithelial cells.

16.7.1 Promote Healing

Wounds can be classified into acute and chronic depending upon the time required for healing. Bacteria that grow in the presence of oxygen such as *staphylococci, diphtheroids*, etc., are found in the superficial surface of skin, whereas anaerobic bacteria such as *Clostridium* are found in deeper wounds. Infection in a wound can be caused by bacterial colonization and dead tissues such as necrosis and eschar and they can aid as nutrition source for bacterial species (Mertz and Ovington 1993). There are four stages of healing that are overlapping, namely hemostasis, inflammatory, proliferative, and maturation. Healing can be promoted by understanding the host–microbial interactions and manipulate the microbiome environment. Role of specific microorganism promoting healing among large populations remains unclear (James et al. 2008). Variola et al. (2009) discussed different nanoscale surface modification methods in implantable metals to enhance biocompatibility. Several processes such as oxidative nanopatterning, chemical vapor deposition, plasma spray could be used to alter the surfaces to exhibit antimicrobial properties. Scales and Huffnagle (2013) discussed the microbiome in repairing wound and tissue fibrosis and highlighted different host factors such as diet and nutrient availability, temperature/pH gradients, surfactants, physical abrasion, oxygen concentration, host defense, etc., that can modulate the bacterial microbiome. Karrasch and Jobin (2009) discussed the wound healing at epithelial cells of gastrointestinal tract and stated the microbial metabolism as one of the luminal factors that contribute to the intestinal restitution. Some antiseptics like Hibiclens can kill the microflora around the wound and povidone-iodine was found ineffective to do so (Mertz and Ovington 1993).

16.7.2 Food Storage and Processing

Several factors such as temperature, oxygen concentration, hydrodynamic effects, food matrix composition, etc., affect the formation of bacterial biofilm (García-Gonzalo and Pagán 2015). Di Ciccio et al. (2015) studied the interaction of *S. aureus* bacteria on food processing surfaces and found preferential biofilm formation on polystyrene surfaces compared to stainless steel and it was attributed to the hydrophobicity of polystyrene that minimizes the forces of repulsion. Sinde and Carballo (2000) studied the attachment of Listeria monocytogenes and Salmonella spp. (higher hydrophobicity) to the surfaces of rubber, stainless steel, and PTFE. The bacteria preferred more hydrophobic (PTFE) material and it was concluded to prefer stainless steel in food industry. Fellows (2017) discussed fermentation technology in which lactic acid, alcohol, acetic acid, and CO_2 are the main components. These microorganisms preserve foods by producing organic acids and lower the pH of the food (Adams and Nicolaides 1997). During fermentation, it also leads to bio-enrichment with protein, essential amino acids, vitamins and reduces toxins. Leuconostoc mesenteroides and Streptococcus faecalis help in natural fermentation of Indian idlis, which produce lactic acid and CO_2. The naturally present bacilli in ingredients and utensils render the batter anaerobic and puffs it up with time. Lactobacillus bulgaricus present in the raw milk ferments lactose to lactic acid and it becomes semi-solid. Low pH will inhibit the action of harmful disease producing microorganisms.

16.7.3 Self-Defensive Coatings

Nandakumar et al. (2019) used an alternate approach analogous to hand sanitizers by designing functional microparticle enabled removal of bacteria from surfaces. Tiller et al. (2001) attached long chains of N-alkylated poly (4-vinylpyridine) covalently bonded to the glass surface that killed airborne bacteria on contact. Kugler et al. (2005) proposed an electrostatic mechanism for the biocidal surfaces containing quaternized poly(vinylpyridine) groups on glass surfaces which induce bacterial death. Gao et al. attributed the biocidal mechanism as phospholipid sponge effect by developing a typical poly quarternary "-onium" coating which is an efficient antimicrobial agent (Gao et al. 2017).

16.8 Conclusions

Microbial ecosystem is a complex subject to understand due to its interrelation with other microorganisms, plants, animals, and humans. The ecological balance depends on their performance in different processes such as nitrogen and carbon cycle, human digestive processes, etc. Till date, no clear understanding of different mechanisms governing microbial adhesion is properly understood. Several analytical, microscopic, and biochemical techniques that are under developmental stage can help us to understand several biological phenomena aiding microbial interaction with substrate surfaces. Metagenomics, i.e., the study of genetic material (DNA sequence) of microbes from an environmental sample can help us to understand the microbial diversity. This has a great assurance to help us answer several questions in understanding the complex world of host–microbe interactions.

References

Aamir M, Rai KK, Dubey MK et al (2019) Impact of climate change on soil carbon exchange, ecosystem dynamics, and plant-microbe interactions. Elsevier, New York

Abdelhay Kaoud H (2019) Introductory chapter: bacterial cattle diseases - economic impact and their control. In: Bacterial cattle diseases. IntechOpen

Adams MR, Nicolaides L (1997) Review of the sensitivity of different foodborne pathogens to fermentation. Food Control 8:227–239. https://doi.org/10.1016/s0956-7135(97)00016-9

Ahmed E, Holmström SJM (2015) Microbe-mineral interactions: the impact of surface attachment on mineral weathering and element selectivity by microorganisms. Chem Geol 403:13–23. https://doi.org/10.1016/j.chemgeo.2015.03.009

Akhavan O, Ghaderi E (2010) Toxicity of graphene and graphene oxide nanowalls against bacteria. ACS Nano 4:5731–5736. https://doi.org/10.1021/nn101390x

Alimova A, Katz A, Steiner N et al (2009) Bacteria-clay interaction: structural changes in smectite induced during biofilm formation. Clay Clay Miner 57:205–212. https://doi.org/10.1346/CCMN.2009.0570207

Anderson GG, O'Toole GA (2008) Innate and induced resistance mechanisms of bacterial biofilms. Curr Top Microbiol Immunol 322:85–105

Anderson I, Göker M, Nolan M et al (2011) Complete genome sequence of the hyperthermophilic chemolithoautotroph Pyrolobus fumarii type strain (1A T). Stand Genomic Sci 4:381–392. https://doi.org/10.4056/sigs.2014648

Arakaki A, Nakazawa H, Nemoto M et al (2008) Formation of magnetite by bacteria and its application. J R Soc Interface 5:977–999

Arya SK, Saha S, Ramirez-Vick JE et al (2012) Recent advances in ZnO nanostructures and thin films for biosensor applications: review. Anal Chim Acta 737:1–21

Audrain B, Farag MA, Ryu CM, Ghigo JM (2015a) Role of bacterial volatile compounds in bacterial biology. FEMS Microbiol Rev 39:222–233

Audrain B, Létoffé S, Ghigo JM (2015b) Airborne bacterial interactions: functions out of thin air? Front Microbiol 6:1476

Aung K, Jiang Y, He SY (2018) The role of water in plant–microbe interactions. Plant J 93:771–780. https://doi.org/10.1111/tpj.13795

Battista JR, Earl AM, Park MJ (1999) Why is Deinococcus radiodurans so resistant to ionizing radiation? Trends Microbiol 7:362–365

Bazaka K, Jacob MV, Chrzanowski W, Ostrikov K (2015) Anti-bacterial surfaces: natural agents, mechanisms of action, and plasma surface modification. RSC Adv 5:48739–48759. https://doi.org/10.1039/c4ra17244b

Beales N (2004) Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. Compr Rev Food Sci Food Saf 3:1–20. https://doi.org/10.1111/j.1541-4337.2004.tb00057.x

Bellezza I, Peirce MJ, Minelli A (2014) Cyclic dipeptides: from bugs to brain. Trends Mol Med 20:551–558

Berne C, Ellison CK, Ducret A, Brun YV (2018) Bacterial adhesion at the single-cell level. Nat Rev Microbiol 16:616–627

Bhat RA, Miklis M, Schmelzer E et al (2005) Recruitment and interaction dynamics of plant penetration resistance components in a plasma membrane microdomain. Proc Natl Acad Sci U S A 102:3135–3140. https://doi.org/10.1073/pnas.0500012102

Bohinc K, Dražić G, Fink R et al (2014) Available surface dictates microbial adhesion capacity. Int J Adhes Adhes 50:265–272. https://doi.org/10.1016/j.ijadhadh.2014.01.027

Boks NP, Busscher HJ, Van Der Mei HC, Norde W (2008) Bond-strengthening in staphylococcal adhesion to hydrophilic and hydrophobic surfaces using atomic-force microscopy. Langmuir 24:12990–12994. https://doi.org/10.1021/la801824c

Borin S, Ventura S, Tambone F et al (2010) Rock weathering creates oases of life in a high Arctic desert. Environ Microbiol 12:293–303. https://doi.org/10.1111/j.1462-2920.2009.02059.x

Braem A, De Cremer K, Delattin N et al (2015) Novel anti-infective implant substrates: controlled release of antibiofilm compounds from mesoporous silica-containing macroporous titanium. Colloids Surfaces B Biointerfaces 126:481–488. https://doi.org/10.1016/j.colsurfb.2014.12.054

Busscher HJ, Norde W, Sharma PK, van der Mei HC (2010) Interfacial re-arrangement in initial microbial adhesion to surfaces. Curr Opin Colloid Interface Sci 15:510–517. https://doi.org/10.1016/j.cocis.2010.05.014

Cámara M, Hardman A, Williams P, Milton D (2002) Quorum sensing in vibrio cholerae. Nat Genet 32:217–218

Carobolante JPA, Pereira CA, Dias-Netipanyj MF et al (2018) Cell and bacteria-Baterial interactions on the Ti10Mo8Nb alloy after surface modification. Mater Res 21:3–7. https://doi.org/10.1590/1980-5373-mr-2017-0508

Carson JK, Rooney D, Gleeson DB, Clipson N (2007) Altering the mineral composition of soil causes a shift in microbial community structure. FEMS Microbiol Ecol 61:414–423. https://doi.org/10.1111/j.1574-6941.2007.00361.x
Chapman J, Hellio C, Sullivan T et al (2014) Bioinspired synthetic macroalgae: examples from nature for antifouling applications. Int Biodeterior Biodegrad 86:6–13. https://doi.org/10.1016/j.ibiod.2013.03.036
Chavant P, Martinie B, Meylheuc T et al (2002) Listeria monocytogenes LO28: surface physico-chemical properties and ability to form biofilms at different temperatures and growth phases. Appl Environ Microbiol 68:728–737. https://doi.org/10.1128/AEM.68.2.728-737.2002
Chen M, Yu Q, Sun H (2013) Novel strategies for the prevention and treatment of biofilm related infections. Int J Mol Sci 14:18488–18501
Clarke A, Morris GJ, Fonseca F et al (2013) A low temperature limit for life on earth. PLoS One 8:66207. https://doi.org/10.1371/journal.pone.0066207
Clarke VC, Loughlin PC, Day DA, Smith PMC (2014) Transport processes of the legume symbiosome membrane. Front Plant Sci 5:699. https://doi.org/10.3389/fpls.2014.00699
Clay K (2014) Defensive symbiosis: a microbial perspective. Funct Ecol 28:293–298. https://doi.org/10.1111/1365-2435.12258
Cordier C, Pozo MJ, Barea JM et al (1998) Cell defense responses associated with localized and systemic resistance to Phytophthora parasitica induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant-Microbe Interact 11:1017–1028. https://doi.org/10.1094/MPMI.1998.11.10.1017
Cormio L, Vuopio-Varkila J, Siitonen A et al (1996) Bacterial adhesion and biofilm formation on various double-J stents in vivo and in vitro. Scand J Urol Nephrol 30:19–24. https://doi.org/10.3109/00365599609182343
Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science (80-). 284:1318–1322
Cuadros J (2017) Clay minerals interaction with microorganisms: a review. Clay Miner 52:235–261. https://doi.org/10.1180/claymin.2017.052.2.05
Delaviz Y, Santerre JP, Cvitkovitch DG (2015) Infection resistant biomaterials. Woodhead Publishing Limited
Dénarié J, Debellé F, Promé J-C (1996) Rhizobium Lipo-Chitooligosaccharide nodulation factors: signaling molecules mediating recognition and morphogenesis. Annu Rev Biochem 65:503–535. https://doi.org/10.1146/annurev.bi.65.070196.002443
Desrousseaux C, Sautou V, Descamps S, Traoré O (2013) Modification of the surfaces of medical devices to prevent microbial adhesion and biofilm formation. J Hosp Infect 85:87–93. https://doi.org/10.1016/j.jhin.2013.06.015
Dewald C, Lüdecke C, Firkowska-Boden I et al (2018) Gold nanoparticle contact point density controls microbial adhesion on gold surfaces. Colloids Surfaces B Biointerfaces 163:201–208. https://doi.org/10.1016/j.colsurfb.2017.12.037
Di Ciccio P, Vergara A, Festino AR et al (2015) Biofilm formation by Staphylococcus aureus on food contact surfaces: relationship with temperature and cell surface hydrophobicity. Food Control 50:930–936. https://doi.org/10.1016/j.foodcont.2014.10.048
Dominguez-Bello MG, Costello EK, Contreras M et al (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 107:11971–11975. https://doi.org/10.1073/pnas.1002601107
Dos Santos ALS, Galdino ACM, de Mello TP et al (2018) What are the advantages of living in a community? A microbial biofilm perspective! Mem Inst Oswaldo Cruz 113:180212
Dos Santos Morais R, Gaiani C, Borges F, Burgain J (2020) Interactions microbe-matrix in dairy products. In: Reference module in food science. Elsevier
Douglas S, Beveridge TJ (1998) Mineral formation by bacteria in natural microbial communities. FEMS Microbiol Ecol 26:79–88. https://doi.org/10.1111/j.1574-6941.1998.tb00494.x

Dubey R, Dwivedi R (1985) Toxicity of cadmium on the growth of Macrophomina azXphaseolina causing charcoal rot of soybean as influenced by kaolinite, pH, zinc and manganese. Proc Indian Natl Sci Acad U S A B51:259–264

Duch J, Golda-Cepa M, Kotarba A (2019) Evaluating the effect of oxygen groups attached to the surface of graphenic sheets on bacteria adhesion: the role of the electronic factor. Appl Surf Sci 463:1134–1140. https://doi.org/10.1016/j.apsusc.2018.08.237

Edwards KJ, Bach W, McCollom TM (2005) Geomicrobiology in oceanography: microbe-mineral interactions at and below the seafloor. Trends Microbiol 13:449–456

Egan S, Harder T, Burke C et al (2013) The seaweed holobiont: understanding seaweed-bacteria interactions. FEMS Microbiol Rev 37:462–476

Eisthen HL, Theis KR (2016) Animal-microbe interactions and the evolution of nervous systems. Philos Trans R Soc B Biol Sci 371. https://doi.org/10.1098/rstb.2015.0052

Eland LE, Wipat A, Lee S, et al (2016) Microfluidics for bacterial imaging. In: Methods in microbiology. Academic Press Inc., pp 69–111

Elbourne A, Crawford RJ, Ivanova EP (2017) Nano-structured antimicrobial surfaces: from nature to synthetic analogues. J Colloid Interface Sci 508:603–616

Elbourne A, Chapman J, Gelmi A et al (2019) Bacterial-nanostructure interactions: the role of cell elasticity and adhesion forces. J Colloid Interface Sci 546:192–210. https://doi.org/10.1016/j.jcis.2019.03.050

Erb TJ, Kiefer P, Hattendorf B, et al (2012) GFAJ-1 is an arsenate-resistant, phosphate-dependent organism. Science (80-) 337:467–470. doi:https://doi.org/10.1126/science.1218455

Fang HHP, Chan KY, Xu LC (2000) Quantification of bacterial adhesion forces using atomic force microscopy (AFM). J Microbiol Methods 40:89–97. https://doi.org/10.1016/S0167-7012(99)00137-2

Farahat M, Hirajima T, Sasaki K, Doi K (2009) Adhesion of Escherichia coli onto quartz, hematite and corundum: extended DLVO theory and flotation behavior. Colloids Surfaces B Biointerfaces 74:140–149. https://doi.org/10.1016/j.colsurfb.2009.07.009

Fellows PJ (2017) Food biotechnology. In: Food processing technology. Elsevier, pp. 387–430

Feng J, Shentu J, Zhu Y et al (2020) Crop-dependent root-microbe-soil interactions induce contrasting natural attenuation of organochlorine lindane in soils. Environ Pollut 257:113580. https://doi.org/10.1016/j.envpol.2019.113580

Filip Z (1973) Clay minerals as a factor influencing the biochemical activity of soil microorganisms. Folia Microbiol (Praha) 18:56–74. https://doi.org/10.1007/BF02884250

Flandroy L, Poutahidis T, Berg G et al (2018) The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems. Sci Total Environ 627:1018–1038

Flemming HC (2020) Biofouling and me: my Stockholm syndrome with biofilms. Water Res 173:115576

Frankel RB, Blakemore RP, Wolfe RS (1979) Magnetite in freshwater magnetotactic bacteria. Science (80-) 203:1355–1356. https://doi.org/10.1126/science.203.4387.1355

Fujiyoshi S, Tanaka D, Maruyama F (2017) Transmission of airborne bacteria across built environments and its measurement standards: a review. Front Microbiol 8:2336

Gallo RL (2017) Human skin is the largest epithelial surface for interaction with microbes. J Invest Dermatol 137:1213–1214. https://doi.org/10.1016/j.jid.2016.11.045

Gao J, White EM, Liu Q, Locklin J (2017) Evidence for the phospholipid sponge effect as the biocidal mechanism in surface-bound polyquaternary ammonium coatings with variable cross-linking density. ACS Appl Mater Interfaces 9:7745–7751. https://doi.org/10.1021/acsami.6b14940

García-Gonzalo D, Pagán R (2015) Influence of environmental factors on bacterial biofilm formation in the food industry: a review. Postdoc J 3:. doi:https://doi.org/10.14304/surya.jpr.v3n6.2

Garrett TR, Bhakoo M, Zhang Z (2008) Bacterial adhesion and biofilms on surfaces. Prog Nat Sci 18:1049–1056. https://doi.org/10.1016/j.pnsc.2008.04.001

Geoffrey MG (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643. https://doi.org/10.1099/mic.0.037143-0
Gifford I, Battenberg K, Vaniya A, et al (2018) Distinctive patterns of flavonoid biosynthesis in roots and nodules of datisca glomerata and Medicago spp. Revealed by metabolomic and gene expression profiles. Front Plant Sci 9. doi:https://doi.org/10.3389/fpls.2018.01463
Gkotsis PK, Zouboulis AI (2019) Biomass characteristics and their effect on membrane bioreactor fouling. Molecules 24:2867. https://doi.org/10.3390/molecules24162867
Glassman SI, Weihe C, Li J et al (2018) Decomposition responses to climate depend on microbial community composition. Proc Natl Acad Sci U S A 115:11994–11999. https://doi.org/10.1073/pnas.1811269115
Gordesli FP, Abu-Lail NI (2012) Impact of ionic strength of growth on the physiochemical properties, structure, and adhesion of Listeria monocytogenes polyelectrolyte brushes to a silicon nitride surface in water. J Colloid Interface Sci 388:257–267. https://doi.org/10.1016/j.jcis.2012.08.048
Grinberg M, Orevi T, Kashtan N (2019) Bacterial surface colonization, preferential attachment and fitness under periodic stress. PLoS Comput Biol 15:1–17. https://doi.org/10.1371/journal.pcbi.1006815
Großkopf T, Soyer OS (2014) Synthetic microbial communities. Curr Opin Microbiol 18:72–77
Guven-Maiorov E, Hakouz A, Valjevac S et al (2020) HMI-PRED: a web server for structural prediction of host-microbe interactions based on interface mimicry. J Mol Biol 432:3395–3403. https://doi.org/10.1016/j.jmb.2020.01.025
Habash M, Reid G (1999) Microbial biofilms: their development and significance for medical device-related infections. J Clin Pharmacol 39:887–898. https://doi.org/10.1177/00912709922008506
Haney CH, Long SR (2010) Plant flotillins are required for infection by nitrogen-fixing bacteria. Proc Natl Acad Sci U S A 107:478–483. https://doi.org/10.1073/pnas.0910081107
Hartel P (2005) Encyclopedia of soils in the environment. Encycl Soild Environ:448–455
Harvey RA (2007) Microbiology, 2nd edn. Lippincott Williams and Wilkins
Hayes CL, Peters BJ, Foster JA (2020) Microbes and mental health: can the microbiome help explain clinical heterogeneity in psychiatry? Front Neuroendocrinol 58:100849. https://doi.org/10.1016/j.yfrne.2020.100849
Head MJ, Zhou WJ (2000) Evaluation of NaOH leaching techniques to extract humic acids from palaeosols. Nucl Instruments Methods Phys Res Sect B Beam Interact with Mater Atoms 172:434–439. https://doi.org/10.1016/S0168-583X(00)00221-4
Holm PE, Nielsen PH, Albrechtsen HJ, Christensen TH (1992) Importance of unattached bacteria and bacteria attached to sediment in determining potentials for degradation of xenobiotic organic contaminants in an aerobic aquifer. Appl Environ Microbiol 58
Holmes JD, Smith PR, Evans-Gowing R et al (2008) Bacterial photoprotection through extracellular cadmium sulfide crystallites. Photochem Photobiol 62:1022–1026. https://doi.org/10.1111/j.1751-1097.1995.tb02403.x
Hörmannsperger G, Clavel T, Haller D (2012) Gut matters: microbe-host interactions in allergic diseases. J Allergy Clin Immunol 129:1452–1459. https://doi.org/10.1016/j.jaci.2011.12.993
Houghteling PD, Walker WA (2015) Why is initial bacterial colonization of the intestine important to infants' and children's health? J Pediatr Gastroenterol Nutr 60:294–307
Hoye BJ, Fenton A (2018) Animal host–microbe interactions. J Anim Ecol 87:315–319. https://doi.org/10.1111/1365-2656.12788
Hůlková M, Soukupová J, Carlson RP, Maršálek B (2020) Interspecies interactions can enhance Pseudomonas aeruginosa tolerance to surfaces functionalized with silver nanoparticles. Colloids Surfaces B Biointerfaces 192:111027. https://doi.org/10.1016/j.colsurfb.2020.111027
Ibberson CB, Whiteley M (2020) The social life of microbes in chronic infection. Curr Opin Microbiol 53:44–50

Innis MA, Myambo KB, Gelfand DH, Brow MAD (1988) DNA sequencing with Thermus aquaticus DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA. Proc Natl Acad Sci U S A 85:9436–9440. https://doi.org/10.1073/pnas.85.24.9436

Iorhemen OT, Hamza RA, Tay JH (2016) Membrane bioreactor (Mbr) technology for wastewater treatment and reclamation: membrane fouling. Membranes (Basel) 6:33. https://doi.org/10.3390/membranes6020033

James G, Swogger E, deLancey-Pulcini E (2008) Microbial ecology of human skin and wounds. In: The role of biofilms in device-related infections. Springer, Berlin Heidelberg, pp 1–14

Janeway CA (1989) Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harbor Symposia on Quantitative Biology. Cold Spring Harbor Laboratory Press, In, pp 1–13

Johoson DB (2008) Biodiversity and interactions of acidophiles: key to understanding and optimizing microbial processing of ores and concentrates. Trans nonferrous met Soc China (English Ed 18:1367–1373). doi:https://doi.org/10.1016/S1003-6326(09)60010-8

Jones GW, Isaacson RE (1982) Proteinaceous bacterial adhesins and their receptors. Crit Rev Microbiol 10:229–260. https://doi.org/10.3109/10408418209113564

Jones EM, Cochrane CA, Percival SL (2015) The effect of pH on the extracellular matrix and biofilms. Adv Wound Care 4:431–439. https://doi.org/10.1089/wound.2014.0538

Jose B, Antoci V, Zeiger AR et al (2005) Vancomycin covalently bonded to titanium beads kills Staphylococcus aureus. Chem Biol 12:1041–1048. https://doi.org/10.1016/j.chembiol.2005.06.013

Jung B, Park J, Kim N et al (2018) Cooperative interactions between seed-borne bacterial and air-borne fungal pathogens on rice. Nat Commun 9:1–11. https://doi.org/10.1038/s41467-017-02430-2

Kaplan JL, Shi HN, Walker WA (2011) The role of microbes in developmental immunologic programming. Pediatr Res 69:465–472

Karrasch T, Jobin C (2009) Wound healing responses at the gastrointestinal epithelium: a close look at novel regulatory factors and investigative approaches. Z Gastroenterol 47:1221–1229

Khamar HJ, Breathwaite EK, Prasse CE et al (2010) Multiple roles of soluble sugars in the establishment of Gunnera-Nostoc endosymbiosis. Plant Physiol 154:1381–1389. https://doi.org/10.1104/pp.110.162529

Khan SU, Schnitzer M (1972) The retention of hydrophobic organic compounds by humic acid. Geochim Cosmochim Acta 36:745–754. https://doi.org/10.1016/0016-7037(72)90085-3

Kieliszek M, Kot AM, Bzducha-Wróbel A et al (2017) Biotechnological use of Candida yeasts in the food industry: a review. Fungal Biol Rev 31:185–198

Kinner NE, Harvey RW, Blakeslee K et al (1998) Size-selective predation on groundwater bacteria by nanoflagellates in an organic-contaminated aquifer. Appl Environ Microbiol 64:618–625. https://doi.org/10.1128/aem.64.2.618-625.1998

Kitching R., Harmsen R (2008) Encyclopedia of ecology. In: Reference module in earth systems and environmental sciences. pp 160–162

Koók L, Bakonyi P, Harnisch F et al (2019) Biofouling of membranes in microbial electrochemical technologies: causes, characterization methods and mitigation strategies. Bioresour Technol 279:327–338

Kügler R, Bouloussa O, Rondelez F (2005) Evidence of a charge-density threshold for optimum efficiency of biocidal cationic surfaces. Microbiology 151:1341–1348. https://doi.org/10.1099/mic.0.27526-0

Landeweert R, Hoffland E, Finlay RD et al (2001) Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. Trends Ecol Evol 16:248–254

Lefebvre B, Timmers T, Mbengue M et al (2010) A remorin protein interacts with symbiotic receptors and regulates bacterial infection. Proc Natl Acad Sci U S A 107:2343–2348. https://doi.org/10.1073/pnas.0913320107

Lehman RM (2007) Understanding of aquifer microbiology is tightly linked to sampling approaches. Geomicrobiol J 24:331–341. https://doi.org/10.1080/01490450701456941

Lehman RM, Roberto FF, Earley D et al (2001) Attached and unattached bacterial communities in a 120-meter Corehole in an acidic, crystalline rock aquifer. Appl Environ Microbiol 67:2095–2106. https://doi.org/10.1128/AEM.67.5.2095-2106.2001

Limsuwan S, Homlaead S, Watcharakul S et al (2014) Inhibition of microbial adhesion to plastic surface and human buccal epithelial cells by Rhodomyrtus tomentosa leaf extract. Arch Oral Biol 59:1256–1265. https://doi.org/10.1016/j.archoralbio.2014.07.017

Liu Z, Sun Y, Zhang Y et al (2020) Desert soil sequesters atmospheric CO_2 by microbial mineral formation. Geoderma 361:114104. https://doi.org/10.1016/j.geoderma.2019.114104

Lorenzetti M, Dogša I, Stošicki T et al (2015) The influence of surface modification on bacterial adhesion to titanium-based substrates. ACS Appl Mater Interfaces 7:1644–1651. https://doi.org/10.1021/am507148n

Lupitskyy R, Roiter Y, Tsitsilianis C, Minko S (2005) From smart polymer molecules to responsive nanostructured surfaces. Langmuir 21:8591–8593. https://doi.org/10.1021/la050404a

Lynch JM (1995) Microbial activity in acid soils. In: Plant-soil interactions at low pH: principles and management. Springer Netherlands, pp 167–172

Ma J, Sun Y, Gleichauf K et al (2011) Nanostructure on taro leaves resists fouling by colloids and bacteria under submerged conditions. Langmuir 27:10035–10040. https://doi.org/10.1021/la2010024

Macklaim JM, Gloor GB, Anukam KC et al (2011) At the crossroads of vaginal health and disease, the genome sequence of lactobacillus iners AB-1. Proc Natl Acad Sci U S A 108:4688–4695. https://doi.org/10.1073/pnas.1000086107

Mapelli F, Marasco R, Balloi A et al (2012) Mineral-microbe interactions: biotechnological potential of bioweathering. J Biotechnol 157:473–481. https://doi.org/10.1016/j.jbiotec.2011.11.013

Marion GM, Fritsen CH, Eicken H, Payne MC (2003) The search for life on Europa: limiting environmental factors, potential habitats, and earth analogues. Astrobiology 3:785–811. https://doi.org/10.1089/153110703322736105

Marshall KC, Stout R, Mitchell R (1971) Mechanism of the initial events in the sorption of marine bacteria to surfaces. J Gen Microbiol 68:337–348. https://doi.org/10.1099/00221287-68-3-337

Marteinsson VT (1999) Thermococcus barophilus sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int J Syst Bacteriol 49:351–359. https://doi.org/10.1099/00207713-49-2-351

Mayton HM, Marcus IM, Walker SL (2019) Escherichia coli O157:H7 and salmonella typhimurium adhesion to spinach leaf surfaces: sensitivity to water chemistry and nutrient availability. Food Microbiol 78:134–142. https://doi.org/10.1016/j.fm.2018.10.002

McFall-Ngai MJ (2015) Giving microbestheir due - animal life in amicrobially dominant world. J Exp Biol 218:1968–1973. https://doi.org/10.1242/jeb.115121

Mertz PM, Ovington LG (1993) Wound healing microbiology. Dermatol Clin 11:739–747

Moriarty TF, Poulsson AHC, Rochford ETJ, Richards RG (2017) Bacterial adhesion and biomaterial surfaces. Compr Biomater II 4:101–129. https://doi.org/10.1016/B978-0-08-100691-7.00106-3

Mushegian AA, Tougeron K (2019) Animal-microbe interactions in the context of diapause. Biol Bull 237:180–191. https://doi.org/10.1086/706078

Nakabachi A, Ueoka R, Oshima K et al (2013) Defensive bacteriome symbiont with a drastically reduced genome. Curr Biol 23:1478–1484. https://doi.org/10.1016/j.cub.2013.06.027

Nandakumar V, Huang C, Pulgar A et al (2019) Particle assisted removal of microbes from surfaces. J Colloid Interface Sci 533:190–197. https://doi.org/10.1016/j.jcis.2018.08.043

Noman A, Aqeel M, Qasim M et al (2020) Plant-insect-microbe interaction: a love triangle between enemies in ecosystem. Sci Total Environ 699:134181. https://doi.org/10.1016/j.scitotenv.2019.134181

Oldroyd GED, Murray JD, Poole PS, Downie JA (2011) The rules of engagement in the legume-Rhizobial Symbiosis. Annu Rev Genet 45:119–144. https://doi.org/10.1146/annurev-genet-110410-132549

Park JY, Kim JS, Nam YS (2013) Mussel-inspired modification of dextran for protein-resistant coatings of titanium oxide. Carbohydr Polym 97:753–757. https://doi.org/10.1016/j.carbpol.2013.05.064

Peng P, Huang H, Ren H et al (2018) Exogenous N-acyl homoserine lactones facilitate microbial adhesion of high ammonia nitrogen wastewater on biocarrier surfaces. Sci Total Environ 624:1013–1022. https://doi.org/10.1016/j.scitotenv.2017.12.248

Petruk G, Donadio G, Lanzilli M et al (2018) Alternative use of Bacillus subtilis spores: protection against environmental oxidative stress in human normal keratinocytes. Sci Rep 8. https://doi.org/10.1038/s41598-018-20153-2

Playter T, Konhauser K, Owttrim G et al (2017) Microbe-clay interactions as a mechanism for the preservation of organic matter and trace metal biosignatures in black shales. Chem Geol 459:75–90. https://doi.org/10.1016/j.chemgeo.2017.04.007

Plett JM, Martin FM (2018) Know your enemy, embrace your friend: using omics to understand how plants respond differently to pathogenic and mutualistic microorganisms. Plant J 93:729–746. https://doi.org/10.1111/tpj.13802

Ponomareva AL, Buzoleva LS, Bogatyrenko EA (2018) Abiotic environmental factors affecting the formation of microbial biofilms. Biol Bull 45:490–496. https://doi.org/10.1134/S106235901805014X

Pratt LA, Kolter R (1998) Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol Microbiol 30:285–293. https://doi.org/10.1046/j.1365-2958.1998.01061.x

Preiss L, Hicks DB, Suzuki S et al (2015) Alkaliphilic bacteria with impact on industrial applications, concepts of early life forms, and bioenergetics of ATP synthesis. Front Bioeng Biotechnol 3. https://doi.org/10.3389/fbioe.2015.00075

Queller DC, Ponte E, Bozzaro S, Strassmann JE (2003) Single-gene greenbeard effects in the social amoeba Dictyostelium discoideum. Science (80-) 299:105–106. doi:https://doi.org/10.1126/science.1077742

Rahnamaee SY, Bagheri R, Vossoughi M et al (2020) Bioinspired multifunctional TiO_2 hierarchical micro/nanostructures with tunable improved bone cell growth and inhibited bacteria adhesion. Ceram Int 46:9669–9679. https://doi.org/10.1016/j.ceramint.2019.12.234

Rajkumar M, Prasad MNV, Swaminathan S, Freitas H (2013) Climate change driven plant-metal-microbe interactions. Environ Int 53:74–86. https://doi.org/10.1016/j.envint.2012.12.009

Ramey BE, Koutsoudis M, Bodman SBV, Fuqua C (2004) Biofilm formation in plant-microbe associations. Curr Opin Microbiol 7:602–609. https://doi.org/10.1016/j.mib.2004.10.014

Rampioni G, Falcone M, Heeb S et al (2016) Unravelling the genome-wide contributions of specific 2-Alkyl-4-quinolones and PqsE to quorum sensing in Pseudomonas aeruginosa. PLoS Pathog 12:e1006029. https://doi.org/10.1371/journal.ppat.1006029

Rao TS, Kumar R, Balamurugan P, Vithal GK (2017) Microbial fouling in a water treatment plant and its control using biocides. Biocontrol Sci 22:105–119

Ratzke C, Denk J, Gore J (2018) Ecological suicide in microbes. Nat Ecol Evol 2:867–872. https://doi.org/10.1038/s41559-018-0535-1

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science (80-) 289:1920–1921. https://doi.org/10.1126/science.289.5486.1920

Rektorschek M, Weeks D, Sachs G, Melchers K (1998) Influence of pH on metabolism and urease activity of helicobacter pylori. Gastroenterology 115:628–641. https://doi.org/10.1016/S0016-5085(98)70142-8

Rodrigo-Baños M, Garbayo I, Vílchez C et al (2015) Carotenoids from Haloarchaea and their potential in biotechnology. Mar Drugs 13:5508–5532

Röling WFM, Timotius KH, Stouthamer AH, van Verseveld HW (1994) Physical factors influencing microbial interactions and biochemical changes during the Baceman stage of Indonesian Kecap (soy sauce) production. J Ferment Bioeng 77:293–300. https://doi.org/10.1016/0922-338X(94)90237-2

Romeo LM, Gareta R (2009) Fouling control in biomass boilers. Biomass Bioenergy 33:854–861. https://doi.org/10.1016/j.biombioe.2009.01.008
Rosenbaum M, Aulenta F, Villano M, Angenent LT (2011) Cathodes as electron donors for microbial metabolism: which extracellular electron transfer mechanisms are involved? Bioresour Technol 102:324–333
Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med 2
Sahoo PC, Pant D, Kumar M et al (2020) Material–microbe interfaces for solar-driven CO_2 Bioelectrosynthesis. Trends Biotechnol 1. https://doi.org/10.1016/j.tibtech.2020.03.008
Scales BS, Huffnagle GB (2013) The microbiome in wound repair and tissue fibrosis. J Pathol 229:323–331
Schink B, Stams AJM (2012) Syntrophism among prokaryotes. In: The prokaryotes: prokaryotic communities and ecophysiology. Springer, Berlin, pp 471–493
Shen EP, Chu HS, Hsieh YT, et al (2019) Analysis of P. aeruginosa disinfectant sensitivity and microbial adhesions to worn cosmetic contact lenses. Contact Lens Anterior Eye 0–1. doi: https://doi.org/10.1016/j.clae.2019.10.137
Shin B, Park W (2018) Zoonotic diseases and phytochemical medicines for microbial infections in veterinary science: current state and future perspective. Front Vet Sci 5:166. https://doi.org/10.3389/fvets.2018.00166
Shinohara M, Aoyama C, Fujiwara K et al (2011) Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. Soil Sci Plant Nutr 57:190–203. https://doi.org/10.1080/00380768.2011.554223
Sinde E, Carballo J (2000) Attachment of salmonella spp. and Listeria monocytogenes to stainless steel, rubber and polytetrafluorehtylene: the influence of free energy and the effect of commercial sanitizers. Food Microbiol 17:439–447. https://doi.org/10.1006/fmic.2000.0339
Singh VK, Shukla AK, Singh AK (2019) Impact of climate change on plant-microbe interactions under agroecosystems. In: Climate change and agricultural ecosystems: current challenges and adaptation. Elsevier, pp 153–179
Skaar EP, Zackular JP (2020) Editorial overview: Microbe–microbe interactions: the enemy of my enemy is my friend. Curr Opin Microbiol 53:iii–v. doi:https://doi.org/10.1016/j.mib.2020.05.001
Slade D, Radman M (2011) Oxidative stress resistance in Deinococcus radiodurans. Microbiol Mol Biol Rev 75:133–191. https://doi.org/10.1128/mmbr.00015-10
Sposob M, Cydzik-Kwiatkowska A, Bakke R, Dinamarca C (2018) Temperature-induced changes in a microbial community under autotrophic denitrification with sulfide. Process Biochem 69:161–168. https://doi.org/10.1016/j.procbio.2018.03.006
Stepanović S, Ćirković I, Mijač V, Švabić-Vlahović M (2003) Influence of the incubation temperature, atmosphere and dynamic conditions on biofilm formation by salmonella spp. Food Microbiol 20:339–343. https://doi.org/10.1016/S0740-0020(02)00123-5
Stoodley P, Hall-Stoodley L, Lappin-Scott HM (2001) Detachment, surface migration, and other dynamic behavior in bacterial biofilms revealed by digital time-lapse imaging. Methods Enzymol 337:306–319. https://doi.org/10.1016/S0076-6879(01)37023-4
Stotzky G, Rem L (1967) Influence of clay minerals on microorganisms. IV. Montmorillonite and kaolinite on fungi. Can J Microbiol 13:1535–1550
Subramanian S, Huiszoon RC, Chu S et al (2020) Microsystems for biofilm characterization and sensing – a review. Biofilms 2:100015. https://doi.org/10.1016/j.bioflm.2019.100015
Sun Y, Fang Y, Liang P, Huang X (2016) Effects of online chemical cleaning on removing biofouling and resilient microbes in a pilot membrane bioreactor. Int Biodeterior Biodegrad 112:119–127. https://doi.org/10.1016/j.ibiod.2016.05.010
Tan Y, Ma S, Liu C et al (2015) Enhancing the stability and antibiofilm activity of DspB by immobilization on carboxymethyl chitosan nanoparticles. Microbiol Res 178:35–41. https://doi.org/10.1016/j.micres.2015.06.001

Tazaki K, Asada R (2007) Transmission electron microscopic observation of mercury-bearing bacterial clay minerals in a small-scale gold mine in Tanzania. In: Geomicrobiology Journal. Taylor & Francis Group, pp 477–489

Tikhonovich IA, Provorov NA (2007) Beneficial plant-microbe interactions. Compr Mol Phytopathol 12:365–420. https://doi.org/10.1016/B978-044452132-3/50018-3

Tiller JC, Liao CJ, Lewis K, Klibanov AM (2001) Designing surfaces that kill bacteria on contact. Proc Natl Acad Sci U S A 98:5981–5985. https://doi.org/10.1073/pnas.111143098

Tuson HH, Weibel DB (2013) Bacteria-surface interactions. Soft Matter 9:4368–4380. https://doi.org/10.1039/c3sm27705d

Van Loosdrecht MCM, Lyklema J, Norde W et al (1987) Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. Appl Environ Microbiol 53:1898–1901

Van Zeijl A, Op Den Camp RHM, Deinum EE et al (2015) Rhizobium Lipo-chitooligosaccharide signaling triggers accumulation of Cytokinins in Medicago truncatula roots. Mol Plant 8:1213–1226. https://doi.org/10.1016/j.molp.2015.03.010

Varaprasad K, Vimala K, Ravindra S et al (2011) Fabrication of silver nanocomposite films impregnated with curcumin for superior antibacterial applications. J Mater Sci Mater Med 22:1863–1872. https://doi.org/10.1007/s10856-011-4369-5

Variola F, Vetrone F, Richert L et al (2009) Improving biocompatibility of implantable metals by nanoscale modification of surfaces: an overview of strategies, fabrication methods, and challenges. Small 5:996–1006

Wang M, Tang T (2019) Surface treatment strategies to combat implant-related infection from the beginning. J Orthop Transl 17:42–54

Ward MB, Kapitulčinová D, Brown AP et al (2013) Investigating the role of microbes in mineral weathering: nanometre-scale characterisation of the cell-mineral interface using FIB and TEM. Micron 47:10–17. https://doi.org/10.1016/j.micron.2012.12.006

Weiss L (1961) The measurement of cell adhesion. Exp Cell Res 8:141–153. https://doi.org/10.1016/0014-4827(61)90345-7

Wolk CP, Ernst A, Elhai J (1994) Heterocyst metabolism and development. In: The molecular biology of Cyanobacteria. Springer Netherlands, pp 769–823

Wu Y, Xia Y, Jing X et al (2020) Recent advances in mitigating membrane biofouling using carbon-based materials. J Hazard Mater 382:120976. https://doi.org/10.1016/j.jhazmat.2019.120976

Xin XF, Nomura K, Aung K et al (2016) Bacteria establish an aqueous living space in plants crucial for virulence. Nature 539:524–529. https://doi.org/10.1038/nature20166

Yan L, Zhang S, Chen P et al (2012) Magnetotactic bacteria, magnetosomes and their application. Microbiol Res 167:507–519

Yang Y, Chen N, Chen T (2017) Inference of environmental factor-microbe and microbe-microbe associations from metagenomic data using a hierarchical Bayesian statistical model. Cell Syst 4:129–137.e5. doi:https://doi.org/10.1016/j.cels.2016.12.012

Yayanos AA (2008) Piezophiles. In: Encyclopedia of life sciences. Wiley, Chichester

Yoshihara A, Nobuhira N, Narahara H et al (2015) Estimation of the adhesive force distribution for the flagellar adhesion of Escherichia coli on a glass surface. Colloids Surfaces B Biointerfaces 131:67–72. https://doi.org/10.1016/j.colsurfb.2015.04.038

Zhang H, Liu H, Tian Z et al (2018) Bacteria photosensitized by intracellular gold nanoclusters for solar fuel production. Nat Nanotechnol 13:900–905

Zhou W, Zhang L, Cheng H et al (2019) Adsorption characteristics of Acidianus manzaensis YN25 on chalcopyrite: surface thermodynamics and the extended DLVO theory. Miner Eng 135:105–110. https://doi.org/10.1016/j.mineng.2019.02.039

Zhu X, Chen B, Zhu L, Xing B (2017) Effects and mechanisms of biochar-microbe interactions in soil improvement and pollution remediation: a review. Environ Pollut 227:98–115. https://doi.org/10.1016/j.envpol.2017.04.032

Ziomkiewicz I, Sporring J, Pomorski TG, Schulz A (2015) Novel approach to measure the size of plasma-membrane nanodomains in single molecule localization microscopy. Cytom Part A 87:868–877. https://doi.org/10.1002/cyto.a.22708

Microbial Activities and their Importance in Crop Production

17

Anuradha and Jagvir Singh

Abstract

Today, the entire society is in the grip of the most serious crisis of modern farming system. Due to faulty agricultural activities, the health and fertility of the land are decreasing, the quality of crop products is reduced, global warming, weather inequalities are coming out. Also, improper and excessive use of agricultural chemicals in agriculture land is leading to a continuous increase in air, water, and soil pollution, resulting in adverse effects on human health. This problem is becoming more serious due to lack of knowledge among farmers and inadequate agricultural spread. The possibility of increasing the area of agricultural land in the future is negligible. The growing population, which is expected to increase from 7.6 billion to 9.510 billion by 2050, has posed a serious threat to scientists, governments, and the human race around the world. Using microorganisms, today we have created an innovative technology that is simple, inexpensive, and sustainable for our ecosystem. This in many ways prevents the quality of the crop, its yield increase, as well as the fertility of the soil from becoming barren such as biofertilizer, bio-stimulants, and biopesticides. Therefore, in the near future, further increase in food production can only be achieved through natural resources such as soil and water and better management of agricultural inputs which are possible only through microorganisms.

Anuradha
Department of Zoology, Raghuveer Singh Government Degree College, Lalitpur, UP, India

J. Singh (✉)
Department of Chemistry, ARSD College (University of Delhi), New Delhi, India

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17.1 Introduction

Soil is a very important natural resource. The root of agriculture is soil and water. The sum of these two is a guarantee of good crop production (Berg 2009; Butt and Copping 2000). The future prospects of the present society should also be considered while deciding the scale of development and prosperity. If we cannot do this, then it will not take long to suffer the ill effects of unreasonable actions and decisions. If proper attention is not paid to soil management, the next century will not survive starvation, malnutrition, and hunger-borne diseases (Nelson 2004; Oerke 2006).

In view of the present environment, it is absolutely necessary to save the declining fertility of the soil and sustainable production can be possible only when microorganisms are present in the agricultural land in sufficient quantity (Vyas et al. 2008). The productivity of crops has been stagnant or decreasing for the last several years due to deteriorating health and decreasing fertility of agricultural land. In modern farming, dwarf, semi-dwarf, and hybrid varieties of food crops, intensive farming system, reduction in the use of organic fertilizers, unbalanced use of chemical fertilizers, and excessive use of agricultural chemicals are adversely affecting soil fertility (McQuilken et al. 1998). The use of excessive and unbalanced agricultural chemicals in the soil has also changed the physical, chemical, and biological properties of the soil, which has an impact on the crops grown on the soil. Undoubtedly agriculture production has increased due to the above factors, but soil productivity is decreasing due to adverse effects of agricultural chemicals on soil fertility where soil fertility means that the physical, chemical, and biological conditions of the soil remain favorable for crop production (Panpatte et al. 2015).

Recently we have a serious problem of crop production, which has arisen due to various inorganic and organic factors, limited land availability (Parr and Hornick 1992; Patel 2014). There are many microorganisms in nature, such as viruses, bacteria, and fungi, which cause diseases in enemy pests and destroy them, these viruses, bacteria, and fungi are identified by scientists and multiplied and used in the laboratory. They are being provided, which farmers can take advantage of. The rhizosphere, rhizoplane, endosphere, and phyllosphere are the types of microorganisms found where individual cells of plants take over. Hence, they are also known as their secondary genome, which informs plants and its microbes to act like meta organisms (Das and Adhya 2014; Das et al. 2018). A microbiome is a group of microbes that have been shown to be useful in improving crop yield and health in limited conditions. Therefore, these zones facilitate the acquisition of nutritional diversity of plant microbes, diseases, keto and abiotic and biotic components such as unpredictable weather patterns in global climate change, tolerating drought, salinity, and high temperature and helping it grow (Delgado-Baquerizo et al. 2016). However, this type of technology is still not used on a full scale as only 1–5% of the germs on the earth are left, 95–9% of the germs are indispensable. For sustainable production, it is necessary to keep the land healthy so that we can take care of the food supply of the current population as well as the need of future children (Vyas et al. 2014; Waites et al. 2001), which are supported only by microorganisms.

17.2 Status of Agriculture in India

India is a vast country where climatic conditions such as temperature, humidity, and rainfall vary from one area to another. According to this, there is a rich variety of crops grown in different parts of the country (Bisoyi 2006; Dugad and Sudhakar 2006; Adrian et al. 2009). Despite this diversity, two broad cropping patterns can be identified. These are:

17.2.1 Kharif Crops

India's agriculture is based on the monsoon. The crop is good when a good monsoon arrives, but the year the drought falls, the crop of farmers is destroyed that year. Kharif crops are grown in the month of June–July. The plant is planted between May and July. The crop is harvested between September and October. These are also called "rainy season crops." Humidity is high at the time when crops are sown but when the weather is dry at the time of harvesting. These types of crops require higher temperatures and more water. Jowar, millet, soybean, cowpea, cotton, groundnut, paddy, maize, sugarcane, tobacco, jute are the major Kharif crops.

17.2.2 Rabi Crops

Crops grown in the winter season are called Rabi crops. Their time period is usually from October to March. Examples of Rabi crops are gram, pea, mustard, linseed, and wheat. Apart from these, pulses and vegetables are grown in many places during summer.

About half of the country's workforce is employed in agriculture. However, its contribution to GDP is 17.5%. During the last few decades, the contribution of manufacturing and service sectors to the growth of the economy has increased rapidly, while the contribution of the agriculture sector has declined. While agriculture accounted for 50% of GDP in the 1950s, it fell to 15.4% in 2015–2016. India's food production is increasing every year and the country is one of the main producers of crops like wheat, rice, pulses, sugarcane, and cotton (Mondal and Tewari 2007; Chand 2017). It is first in milk production and second in fruits and vegetables production. India accounts for 25% of the total cotton production along with being the second largest cotton exporter for the last several years. However, in the case of many crops, India's agricultural yield is low compared to large agricultural producing countries like China, Brazil, and America.

For example, the yield of rice in Brazil was 1.3 tonnes per hectare in 1981, which increased to 4.9 tonnes per hectare in 2011. In comparison, India's yield increased from 2.0 tonnes per hectare to 3.6 tonnes per hectare. Rice productivity in China also increased from 4.3 tons per hectare to 6.7 tons per hectare during this period (Chand et al. 2011; Mondal and Tewari 2007). The growth rate of agricultural productivity in India has been very slow compared to other countries. Agricultural productivity depends on many factors (Shibusawa 1998; Auernhammer 2001).

17.3 Required Nutrients

According to agricultural principles, 20 natural nutrients are required for proper growth and production of any plant, these elements are divided into following four classes as in Fig. 17.1.

17.3.1 Basic Element

After chemical analysis of any plant, it is known that it contains 98.6% carbon, hydrogen, and oxygen. While the percentage of other essential, minor, and micro elements is only 1.4%. The basic source of carbon is air. The green leaves of the

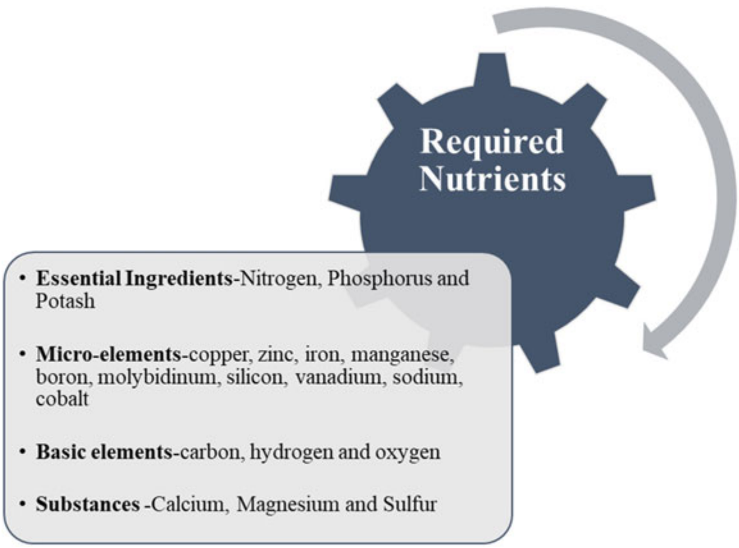

Fig. 17.1 The classification of essential element for plant growth and responsible for crop production

plant synthesize carbon-oxide located in the air through the process of photosynthesis, absorb carbon, and release oxygen back into the air. Plants contain 45–55% carbon content Noble (Noble and Ruaysoongnern 2010; Edgar 2010). The carbon of these dried leaves, stalks, and the residues of plant-dependent organisms in the food chain is eventually added to or mixed with the soil (Vyas et al. 2010). In this way carbon accumulates in the soil. While the main source of oxygen and hydrogen is water, which is mainly obtained from the soil through the roots of plants. The plant also uses small amounts of oxygen and hydrogen in the air. The plant does not use carbon located in the soil, but this carbon is essential for the growth and reproduction of microorganisms. Normally 10 kg of carbon is the food of 1 kg of microorganisms. 100 kg of microorganisms can be functional in the soil.

17.3.2 Essential Nutrients

Nitrogen, phosphorus, and potash are essential nutrients. These three nutrients are mainly used in chemical farming, which are popularly known as NPK. The main role of nitrogen is considered for plant growth and good production (Wood 2001; Yang et al. 2009). The main source of nitrogen in organic farming is the organic matter present in the soil. In which the roots of the last crop, the remnants of the stem, the dried leaves, and the remains of microorganisms and the remains and compost manure made from cattle dung and urine are the main ones. With 10 kg of carbon, 1 kg of nitrogen is automatically available. The ratio of carbon and nitrogen in soil is 10:1, and 78.4% nitrogen is present in the atmosphere which is the key source of nitrogen. The natural technique of storing this atmospheric nitrogen in the soil is used in organic farming (Provorov and Tikhonovich 2003). It is known that the roots of pulses crops (urad, mung, cowpea, rapeseed, rajma, frachbein, soybean, linen, and dhencha) have a type of glands, which are full of nitrogen. In fact, Rhizobium bacteria located in the soil enter the roots of the plant through the root foramen in the roots of pulses. These bacteria enter the roots of the plant. These bacteria reach inside the roots and reproduce rapidly and produce glands. The specialty of this Rhizobium bacterium is that it is made available in nitrogen by using nitrogen located in the roots of the plants and providing them to the plant. In return, it gets sugars and carbohydrates from the plant for its growth, which we know as symbiosis. The pulp plant uses nitrogen as per its requirement, and the residual nitrogen is stored in the roots and stem of the plant which eventually gets mixed into the soil. In this way the amount of nitrogen available in the soil increases, which is useful for the next crop (Franche et al. 2009; Glick 1995).

In organic farming, we have to ensure that we include pulses in the annual crop cycle. By producing pulses crops, we can increase the amount of nitrogen in the soil by 40–60% kg per acre. Different species also have different nitrogen storage capacities. In a seed leaf crops like paddy, wheat, maize, madwa, some bacteria pull nitrogen from the air and provide it to the plant such as Azotobacter, Azospirillum, Pseudomonas. These bacteria do not act as symbiosis with the plant but are located near the roots. However, these bacteria collect very little nitrogen from the air, which can range from 5 to 20 kg per acre. Brazilian scientists have discovered a bacterium that draws nitrogen from the air in sugarcane and grapes and makes the plant available. It is known as Acetobacter diazotrophicus. This bacterium collects 45–60 kg of nitrogen from the air in an acre area and makes it available to the plant. Dissolution of this type of organic matter and nitrifying bacteria can provide nitrogen up to 80–150 kg per acre, which is more than enough for the growth and production of any crop (Jenkins and Medsken 1964).

17.3.3 Substances

Sulfur is essential for the production of amino acids and vitamins, similarly magnesium is essential for the manufacture of chlorophyll, magnesium has a major role in greening the leaves. Deficiency of calcium stops the growth of the plant (Zafar et al. 2007; Panpatte et al. 2016). These three minor elements require small amounts. These are basically insoluble in soil and organic matter and are available continuously to the crop due to the activities of microorganisms and earthworms.

Sulfur

It plays an important role in the formation of protein and fat. It is present in organic remains and soil in a basic way. Seventy percent of the sulfur in the soil is not soluble in water and plants cannot use it in this form. Therefore, when sulfuric acid is present in the cells of microorganisms like mycobacteria, penicillin in the soil, it becomes ready to taken up by the plants. Garlic, cabbage, turnip, mustard like oilseeds plants contain a lot of sulfur. Therefore, the inclusion of these crops in the crop cycle increases the availability of sulfur in the soil. 8.11 kg of sulfur is required for one acre (Guo et al. 2018). To increase the availability of sulfur, oilseeds should be grown in the field at least once or twice every 2–3 years. Oilseeds (sesame seeds, mustard seeds, flaxseeds) have high amounts of sulfur. Oilseed husk should be used in the soil as mulching or manure.

Calcium

It is important for cell division. It plays an important role in transporting nutrients to different parts of the plant. Calcium remains as a chosen stone in the soil. It is also abundant in organic remains. It is also found in large quantities in vermicast or vermicompost obtained from earthworms. Generally, calcium is easily available in organic farming. An acre of crop requires 40 kg of calcium.

Magnesium

It has an important role in photosynthesis. It is necessary for the greenness of the leaves. It accumulates in large quantities in the roots of plants. Magnesium is available in plenty in soil. Plants are easily available from 16 kg of magnesium reserves for an acre crop.

17.3.4 Microbial Elements

Copper, iron, zinc, manganese, boron, sodium, nickel, chlorine, cobalt, molybdenum are the major micronutrients due to the action of earthworms and microorganisms in organic farming. Out of the 11 subtle elements, 5 subtle elements, iron, zinc, copper, molybdenum, and boron have an important role in the growth of the plant.

Iron

It acts as an important catalyst in the process of photosynthesis. It is necessary to have iron to make chlorophyll. 800 grams of iron is required for one acre of crop. Abundant iron is available in the remains of the plants and soil (Huang et al. 2009).

Zinc

Yellowing of the lower leaves of the plant and red spots are the symptoms of zinc deficiency. The plant does not get enough food from the sun's rays, due to which the production is affected. It keeps the leaves of plants green which helps in obtaining chlorophyll from the sun's rays. 100 grams of zinc are required for one acre of crop. It is abundant in bio-residues and basically in soil.

Copper

This element is abundant in soil and bio-residues. But in the soil where the air circulation is reduced, the plant cannot accept copper. Therefore, the immunity of the plant to fungal disease is reduced. In such a situation the crop repeatedly suffers from fungal disease. For an acre crop, only 400 grams of copper is required.

Boron

It is important for cell division. Due to its deficiency, the crop in the field is reduced and the plant growth stops. Boron is necessary for the development of crop roots. It is abundant in soil and bio-residues.

Molybdenum

Plants absorb molybdenum in the form of molybdate. Molybdenum is mainly located in the phloem and vascular parenchyma and is the mobile element in plants. Molybdenum is required for the chemical conversion of nitrogen into plants (Kozhemyakov et al. 2004). In organic farming, the management of micro and minor elements is done continuously, so we do not need to put anything from outside.

17.4 Microorganisms Versus Sustainable Plant Growth

The relationship between plants and microorganisms is very old and close. Microorganisms along with the growth of plants nourish them and protect them from diseases occurring in plants (He et al. 2014). With the help of the diagram 17.2 that shows their usefulness in different areas of the microorganisms.

17.4.1 Plant Growth Promotion

Plant organs are grown in a sterilized state on a nutrient medium using various techniques in plant tissue culture. In this, clones of plants of particularly good flowers, fruit production, or other desirable traits are produced (Jin et al. 2015).

17.4.2 Control of Plant Diseases

Generally, the use of other organisms for the control of plant disease factors is called biological control. Biological control is the process in which more than one microorganism is used to reduce or prevent disease. Those microorganisms that are used to control factors are called biological pathogens (Fig. 17.2).

17.4.3 Accelerates Use of Available Resources

There is a symbiotic relationship between mycorrhiza fungi and plant roots. This relationship is found in about 95% of plant species. Mycorrhiza plays an important role in soil biology and soil chemistry. Mycorrhiza help plants to absorb various types of nutrients such as phosphorus, nitrogen, and other microbial nutrients from the soil. Hence, they play an important role in increasing crop yield. Increasing the rate of water ingestion by mycorrhiza plants makes them resistant to drought conditions. For this reason, they are also known as natural organic fertilizers (Vora

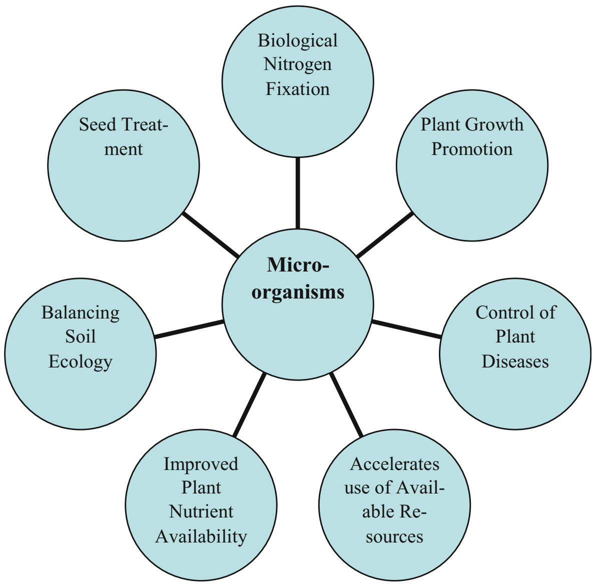

Fig. 17.2 Microorganisms help in continuous and improved growth of plants. The presented figure shows their multi-utility

et al. 2008), as well as providing protection to the plant from disease causing microbes.

17.4.4 Seed Treatment

Two types of seed-borne diseases are found in crops. These are internal and external borne diseases. Bean anthracnose, black leg of tied cabbage, bacterial leaf blight of rice, bacterial blight of cotton, exposed trunk of wheat and barley and obligate root of paddy inter-seed and alternaria (early blight of potato and tomato, blight of mustard family, and blight in onion), “Eugerium, Helminthosporium, and Cercospora” are external seed-borne disease agents. They cause a lot of damage to cereal and vegetable crops. If we talk about crop protection, by reducing seed losses in crops by seed treatment, net profit can be made by 15–20%.

17.4.5 Balancing Soil Ecology

The goal of ecological agriculture is to produce food as well as to protect soil health, to promote water and climate, biodiversity, and not to contaminate the environment by actions such as chemical inputs or genetic engineering. Ecological agriculture usually involves a diversity of animals, crops, and methods. Its management techniques include cover crop mulching, organic manure, crop cycle, green manure, and use of animal waste or dung.

17.4.6 Improved Plant Nutrient Availability

Microorganisms carry out a wide variety of functions in soil, all microorganisms start growing rapidly which break down organic matter and convert it into organic acids and amino acids. Then, they start trickling with various other substances including organic acids and ammo acid salts (Shtark et al. 2010; Singh 2006). This is known as chelating. Chelating occurs when a coating is formed around a substance such as salt and metal. This coating allows plants to absorb more nutrients while consuming less salt. As bacteria continue to grow and chelating continues, water begins to penetrate deeper which causes salt to move downstream. This chain reaction allows water in the soil to reach depths while removing salt from the soil above and transferring it deeper into the ground to allow more nutrients to reach the plants.

17.4.7 Biological Nitrogen Fixation

Nitrogen has an important place in various metabolic activities of plants. Therefore, plants get most of the nitrogen from the substances dissolved in their roots. In addition to this, fixation of free nitrogen present in the atmosphere is done by the plant, due to which the plants get maximum amount of nitrogen. The change in ammonia is called nitrogen fixation (Rojas et al. 2001; Sanguin et al. 2009). This stabilization is done by microbes. In this way, fixation is called biological stabilization when it is combined with plant roots. Biological nitrogen fixation consists of two groups of microbes.

Symbiotic

Rhizobium bacteria are already present in the soil but later infect the root pores of plants of the Leguminosae family and gradually enter them (Rengel 2002). These bacteria enter the cells of the cortex of the bacterial root and continue to proliferate in number. In addition, the cells of the cortex continuously divide. As a result of which irregular nodules are formed. Bacteria present in these glands perform the fixation of dinitrogen.

Non-Symbiotic

Under this, there are independent microorganisms such as Azetobacter, Chlorobium, Enterobacter, Rhodospirillum. Many bacteria are found in soil from free form, which can be classified as follows.

Aerobic Bacteria

Azotobacter Chroococcum, a bacterium called Azotobacter Agilis, is found in nature, which is often motile or non-motile.

Anaerobic Bacteria

Clostridium called bacteria are rod-shaped, which survive even in the absence of oxygen and stabilize nitrogen.

Photosynthetic Bacteria

Bacteria, known as Rhodospirillus, are found in marine and freshwater. This bacterium performs photosynthesis in the absence of oxygen.

Chemosynthetic Bacteria

Thiobacillus and Desulforibrio Desulfuricans carry out anaerobic respiration called bacteria that take sulfate in the place of oxygen and organic matter. Often, the above bacteria stay in acidic soils and fixation of free N_2 in the atmosphere.

Ammonification

Plants and animals have a lot of nitrogen in the fecal urine, which is decomposed into the soil by bacteria called Bacillus Vulgaris and B. Mycoides bacteria, which results in the formation of ammonia, which is mixed with the soil and receives nitrogen.

Nitrification

Ammonia (NH_3) present in soil is converted into nitrates with the help of bacteria called Nitrosomonas and Nitrobacter (Mohanty et al. 2006).

Blue Green Algae by Indigo Green Algae

According to Winogradeasky, members of the Blue Green Algae also fix atmospheric free nitrogen (N_2). When the soil is deficient in oxygen, bacteria present in the water are helpful in the stabilization of nitrogen (Smith and Read 2008; Vance 1998). Thus, many soils are alkaline or neutral.

17.5 Factors Preventing Crop Growth

17.5.1 Improper and Unbalanced Use of Chemical Fertilizers

Improper and unbalanced use of chemical fertilizers in farming is adversely affecting soil fertility. Chemical imbalances are so much more imbalanced that the ill effects are now visible. Nitrogen, phosphorus, and potash, the three main nutrients for plants, are being used in an indefinite proportion in many agricultural areas of the country. The ratio of nitrogen, phosphorus, and potash in our country in the last years has been 9:3:1, which is very unbalanced (Kempers and Kok 1989). The more use of chemical fertilizers mainly providing nitrogen in crop is causing some secondary and micronutrient deficiencies in the soil, resulting in adverse effects on the physical, chemical and biological properties of the soil. At the same time, the quality and yield of crops are also declining.

17.5.2 Faulty Irrigation System

Decreasing soil fertility remains a concern in our country. The faulty irrigation system is directly or indirectly responsible for this. Today, farmers are using irrigation water in many parts of the country without any understanding. As a result, the cost of production in agriculture not only increases but also has an adverse effect on soil fertility. Irrational and uncontrolled use of irrigation water is causing problems like water stagnation, soil salinity, loss of nutrients, decreasing soil fertility, and soil erosion. The physical condition of that part of the field where the irrigation water remains filled for a long time is deteriorated. The soil structure is severely deformed. Eventually, soil productivity and fertility decline significantly.

17.5.3 Improper and Excessive Exploitation of Intensive Cropping System

At present, soil fertility is decreasing due to improper and excessive exploitation of the soil under intensive cropping system which is adversely affecting the yield of crops. After each crop, there is a shortage of nutrients in the land, which is very important to compensate; otherwise, the soil fertility and productivity decrease (Liu et al. 2012).

17.5.4 Increased Use of Agricultural Chemicals in Farming

In the last several decades, excessive and unbalanced use of toxic agricultural chemicals such as herbicides, pesticides, and plant regulators have been adversely affecting soil fertility. Weeds, pests, and diseases are controlled by using the above-mentioned chemicals, but these toxic agricultural chemicals are adversely affecting the physical, chemical, and biological properties of the soil, which reduces soil fertility (Rural Development Administration [RDA] 1999). Today, the fertile land is turning into barren land due to farmers not having the right knowledge of the use of these chemicals. In addition, soil fertility is also decreasing due to use of adulterated and spurious agricultural chemicals. Excessive use of these chemicals being used in agriculture is also adversely affecting the natural resources: ground water, surface water, soil, fauna, and environment.

17.5.5 Low Quality Irrigation Water

Irrigation water is a very expensive resource in agriculture, due to which the ratio of cost and yield is becoming unbalanced. Due to the continuous use of such water for a long time in crop production, at first it starts gradually decreasing the yield and later the land becomes infertile.

17.5.6 Low Use of Organic Fertilizers

Nowadays the number of livestock in agriculture is decreasing. Previously, farming was dependent on oxen. Due to the mechanization of farming, the whole village does not see a pair of oxen. Due to which the cow dung manure and animal excreta are being used very little in the fields, as a result there is a shortage of bacterial substance in the soil. In addition, the inclusion of pulses and crop residues is being used less frequently in the crop cycle. Farmers are using leaves of multipurpose plants as fuel instead of manure. In modern farming, the combination of organic fertilizers and chemical fertilizers is deteriorating. Instead of compost manure and green manures, the use of single element fertilizers is increasing, which has a direct effect on soil fertility. In this way, due to the lack of bacterial substance in the soil, the number of many beneficial bacteria is decreasing. This type of microorganism take an active part in soil decomposition and decomposition, which ultimately prove fatal to soil fertility.

17.5.7 Declining Level of Agricultural Land

Soil productivity in organic farming depends on the amount of organic matter in it. A good organic soil requires up to 5% organic matter. When ideal temperature and humidity are available inside the soil and in the presence of abundant organic matter, many types of microbial and chemical activities are carried out continuously. The number of microorganisms (like bacteria, fungi, algae, fungi, protozoa) in the soil increases rapidly. Due to the activity and biochemical actions of these microorganisms, essential nutrients from various sources in nature are available to the plant in soluble state. As much as possible efforts should be made to increase reproduction and activity of microorganisms on the soil surface so that the top surface of the soil can be protected from direct sunlight. Efforts are made to keep the soil surface covered by using dried twigs, leaves, and pre-crop residues of tree and plants. If this is not possible, then along with the main crop, several types of supporting crops are grown and the soil surface is covered.

17.6 Microorganism and their Use

These microorganisms can prove to be very useful for proper growth of crops, their good yields, environmentally friendly, airborne life and sustaining them. It describes the use of some such microorganisms as follows.

17.6.1 Bacteria

Friendly bacteria are also found independently in nature, but to simplify their use, they are artificially prepared in the laboratory and transported to the market, to protect them from insects that could harm the crop.

Bacillus thuringiensis

It is a bacteria-based biological insecticide. Its protein-forming crystals have insecticidal properties, a deadly poison of the stomach of the insect. It is effective on over 90 species of Lepidoptera and Colioptera classes. Due to this effect, the mukhang of the Sundaris gets paralyzed, due to which the Sundaris stop eating and become lethargic and die in 4–5 days. Four other species of Bacillus populi, Bacillus sphaerix, Bacillus moiety, Bacillus lentimorbus have also been found for pest management (Bravo et al. 2011; Du et al. 2012).

It is an alternative bacterium which gives good results when used at the rate of 1 kg per hectare against enemy insect pests like gram beet, tobacco beetle, semi-looper, red hairy beetle, soldier insect, and diamond back moth. The time for spraying should be chosen in such a way that when the Sundi is coming out of the eggs. Mixing stickers and spreaders using organic pesticides in solution gives good results. This organic pesticide should not be stored at a temperature higher than 35 °C. Mix this organic pesticide in some water first and then mix the required amount of powder and make the solution and spray it in the evening (Guo et al. 2009; Jia et al. 2014). They are available in the market under the names Bio Lop, Bio Hospital, Bio Pail, Delphin, Bio Bit, Halt.

17.6.2 Virus

Nuclear Polyhedrosis Virus

It is a microbial based on a naturally present virus. Those microorganisms that are made up of only nucleic acids and proteins are called viruses. It is effective for a particular species of insect. This is used for gram bean and tobacco bean. By eating the leaf affected by these viruses used for pest management, Sundi dies within 4–7 days. At first the infected Sundi becomes dull, giving up food (Chiu et al. 2012) Sundi first changes to white color and later to black and hangs upside down on the leaf. It is available in the market in the name of Helicide, Bio-Virus-H, Heliocele, Bio-Virus-S, Spoide Side, Prodex. They are available in the market in the name of Bio Rin, Larvo Seal, Daman, and Anmol Boss. Metarhizium anisopliae is a very useful biological mildew, used against about 300 insect species such as termites, grasshoppers, plant hoppers, woolly aphids, bugs, and beetles. Spores of this mildew germinate on the body of the insect in sufficient moisture, which grow by entering the body through the skin. This mildew eats the insect's body and when the insect dies, there are white molds on the first body of the insect which later turns dark green (Boucias and Nordin 1977).

Some microorganisms co-live with bacteria, which are collectively useful in pest control. Sutrakrami DD136 can be successfully used to control various harmful pests of paddy, sugarcane, and fruit trees. Trichoderma products about six species of Trichoderma are available. But only two species such as Trichoderma virdi and Trichoderma harzianum are found in abundance in the soil. It is available in the market in the name of Bioderma, Diprot, Anmolderma, Trico-P.

17.6.3 Fungus

Numeria Rileyi

This is also a type of fungus that causes diseases in pests and destroys them. It affects insects of all types of Lepidoptera group, but it particularly affects chickpea, helicoverpa armigera of arhar, soldier insect, spodoptera litura of cabbage and tobacco, and semi-looper insect (Yergeau et al. 2014; Gilbert and Gill 2010). The spores of fungi stick to the body of insects after spraying. When they come in contact with the fungi on the crop, they bio-act and enter the body of the insects, where it develops the fungal body on the liquid element and spreads the fungal trap and makes them dead (Faria and Wraight 2007; Shahid et al. 2012).

17.7 Precautions in the Use of Microorganisms

The sun's anti-violet (ultraviolet) rays have the opposite effect on microbes, so it is advisable to use them in the evening. Adequate moisture and humidity are required for the proper development of microorganisms, especially pesticide mildew. The number of insects required in micro-biological control should be above a threshold.

Their self-life is short, so before using them, attention must be paid to the production date (Tedersoo et al. 2014). A large part of the tea produced in India is exported and occupies an important position in the economy. However, the demand for chemicals-free tea is leading to a decline in its exports. Indian scientists have now identified microorganisms found in the cells of tea plants that can be helpful in tea production without the use of chemical fertilizers. It is necessary to test the soil to make agriculture field the business of benefits. By doing this, the farmers get to know the fertile power of their fields and in what proportion the chemical fertilizer has to be used so that they help in increasing the crop yield.

17.8 Conclusion

Organic farming, as the name itself suggests, is farming done with the help of organisms. Food is produced by keeping the fertile strength of soil intact with maximum support of the organisms. It is necessary to have general knowledge about these microorganisms present in the soil. Increasing population all over the world is a serious problem, with increasing population, the use of various types of chemical fertilizers, poisonous pesticides, in order to obtain maximum production in the food production race by humans for the supply of food. The cycle of interchange between biological and abiotic materials (ecology system) affects, which degrades the fertility of the land, as well as pollutes the environment and degrades human health. In ancient times, agriculture was cultivated in accordance with human health and in accordance with the natural environment. There is a continuous exchange of organic and inorganic substances so that water, land, air and environment are not polluted. Now instead of using chemical fertilizers, toxic pesticides, we can get maximum production by using organic fertilizers and medicines, which will keep the land, water, and environment clean and humans and every living organism will be healthy. The use of microorganisms increases the fertility of agricultural land and the irrigation gap. Decreasing dependence on chemical fertilizer reduces cast cost and increases the productivity of crops.

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Conflict of Interest The authors declare that they have no conflict of interest.

References

Adrian AM, Shannon H, Norwood SH et al (2009) Producers' perceptions and attitudes toward precision agriculture technologies. Comput Electron Agric 48:256–271

Auernhammer H (2001) Precision farming the environmental challenge. Comput Electron Agric 30: 31–43. https://doi.org/10.1016/S0168-1699(00)00153-8

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18. https://doi.org/10.1007/s00253-009-2092-7

Bisoyi LK (2006) Inter linking of river basins of India-risks vs benefits? In: proc of 19th national convention of agricultural engineers on role of information technology in high-tech agriculture and horticulture, Bangalore. India 218
Boucias DG, Nordin GL (1977) Interinstar susceptibility of the fall webworm, Hyphantria cunea, to its nucleopolyhedrosis and granulosis viruses. J Invertebr Pathol 30(1):68–75. https://doi.org/10.1016/0022-2011(77)90148-3
Bravo A, Likitvivatanavong S, Gill SS, Soberon M (2011) Bacillus thuringiensis: a story of a successful bioinsecticide. Insect Biochem Mol Biol 41:423–431. https://doi.org/10.1016/j.ibmb.2011.02.006
Butt TM, Copping LG (2000) Fungal biological control agents. Pesticide Outlook-October 2000. Accessed 25 Apr 2016
Chand R (2017) Doubling Farmers' income: rationale, strategy, prospects and action plan. NITI policy paper 01/2017. National Institution for transforming India, government of India, New Delhi
Chand R, Raju SS, Pandey LM (2011) Growth crisis in agriculture: severity and options at national level and state level. In: Balakrishnan P, Orient BS (eds) Economic reforms and growth in India. New Delhi
Chiu E, Coulibaly F, Metcalf P (2012) Insect virus polyhedra, infectious protein crystals that contain virus particles. Curr Opin Struct Biol 22(2):234–240. https://doi.org/10.1016/j.sbi.2012.02.003
Das S, Adhya TK (2014) Effect of combine application of organic manure and inorganic fertilizer on methane and nitrous oxide emissions from a tropical flooded soil planted to rice. Geoderma 213:185–192. https://doi.org/10.1016/j.geoderma.2013.08.011
Das S, Jeong ST, Das S, Kim PJ (2018) Composted cattle manure increases microbial activity and soil fertility more than composted swine manure in a submerged rice paddy. Front Microbiol 8:1702. https://doi.org/10.3389/fmicb.2017.01702
Delgado-Baquerizo M, Maestre FT, Reich PB, Jeffries TC, Gaitan JJ, Encinar D et al (2016) Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat Commun 7:10541. https://doi.org/10.1038/ncomms10541
Du L, Qiu L, Peng Q, Lereclus D, Zhang J, Song F, Huang D (2012) Identification of the promoter in the intergenic region between orf1 and cry8Ea1 controlled by sigma H factor. Appl Environ Microbiol 78:4164–4168. https://doi.org/10.1128/AEM.00622-12
Dugad SV, Sudhakar MS. (2006) Application of information technology in irrigated agriculture. In: Proc of 19th national convention of agricultural engineers on role of information technology in high-tech agriculture and horticulture, Bangalore, India. 197–202
Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461. https://doi.org/10.1093/bioinformatics/btq461
Faria MR, Wraight SP (2007) Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol Control 43:237–256. https://doi.org/10.1016/j.biocontrol.2007.08.001
Franche C, Lindstrom K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. https://doi.org/10.1007/s11104-008-9833-8
Gilbert LI, Gill SS, (2010) Insect control: biological and synthetic agents. Academic Press/Elsevier, London, p. 1–5
Glick B (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117. https://doi.org/10.1139/m95-015
Guo S, Ye S, Liu Y, Wei L, Xue J, Wu H, Song F, Zhang J, Wu X, Huang D, Rao Z (2009) Crystal structure of Bacillus thuringiensis Cry8Ea1: an insecticidal toxin toxic to underground pests, the larvae of Holotrichia parallela. J Struct Biol 168:259–266. https://doi.org/10.1016/j.jsb.2009.07.004
Guo X, Zhou X, Hale L, Yuan M, Feng J, Ning D et al (2018) Taxonomic and functional responses of soil microbial communities to annual removal of above-ground plant biomass. Front Microbiol 9:954. https://doi.org/10.3389/fmicb.2018.00954

He Z, Xiong J, Kent AD, Deng Y, Xue K, Wang G et al (2014) Distinct responses of soil microbial communities to elevated CO2and O3 in a soybean agro-ecosystem. ISME J 8:714–726. https://doi.org/10.1038/ismej.2013.177

Huang B, Yu K, Gambrell RP (2009) Effects of ferric iron reduction and regeneration on nitrous oxide and methane emissions in a rice soil. Chemosphere 74:481–486. https://doi.org/10.1016/j.chemosphere.2008.10.015

Jenkins D, Medsken L (1964) A brucine method for the determination of nitrate in ocean, estuarine, and fresh waters. Anal Chem 36:610. https://doi.org/10.1021/ac60209a016

Jia Y, Zhao C, Wang Q, Shu C, Feng X, Song F, Zhang J (2014) A genetically modified broad-spectrum strain of Bacillus thuringiensis toxic against Holotrichia parallela, Anomala corpulenta and Holotrichia oblita. World J Microbiol Biotechnol 30:595–603. https://doi.org/10.1007/s11274-013-1470-6

Jin J, Tang C, Sale P (2015) The impact of elevated carbon dioxide on the phosphorus nutrition of plants: a review. Ann Bot 116:987–999. https://doi.org/10.1093/aob/mcv088

Kempers AJ, Kok CJ (1989) Re-examination of the determination of ammonium as the indophenol blue complex using salicylate. Anal Chim Acta 221:147–155. https://doi.org/10.1016/S0003-2670(00)81948-0

Kozhemyakov AP, Provorov NA, Zavalin AA, Shott PR (2004) Analysis of interactions between different barley and wheat cultivars with rhizospheric growth promoting bacteria on the variable nitrogen background. Agrokhimia 3:33–40

Liu S, Zhang L, Liu Q, Zou J (2012) Fe(III) fertilization mitigating net global warming potential and greenhouse gas intensity in paddy rice-wheat rotation systems in China. Environ Pollut 164:73–80. https://doi.org/10.1016/j.envpol.2012.01.029

McQuilken MP, Halmer P, Rhodes DJ (1998) Application of microorganisms to seeds. In: Burges HD (ed) Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments. Kluwer Academic Publishers, Dordrecht, pp 255–285. https://doi.org/10.1007/978-94-011-4926-6_8

Mohanty SR, Bodelier PLE, Floris V, Conrad R (2006) Differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils. Appl Environ Microbiol 72:1346–1354. https://doi.org/10.1128/AEM.72.2.1346-1354.2006

Mondal P, Tewari VK (2007) Present status of precision farming: a review. Int J Agric Res 2(1):1–10. https://doi.org/10.3923/ijar.2007.1.10

Nelson EB (2004) Biological control of oomycetes and fungal pathogens. In: Goodman RM (ed) encyclopedia of plant and crop science. Marcel Dekker, USA.137-140. doi:https://doi.org/10.1081/E-EPCS-120019935

Noble AD, Ruaysoongnern S (2010) The nature of sustainable agriculture. In: Dixon R, Tilston E (eds) Soil microbiology and sustainable crop production. Springer, Berlin, pp 1–25. https://doi.org/10.1007/978-90-481-9479-7_1

Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43. https://doi.org/10.1017/S0021859605005708

Panpatte DG, Shelat HN, Jhala YK (2015) Compatibility of biocontrol bacteria with Phyto-extracts. J Pure Appl Microbiol 9(4):3083–3087

Panpatte DG, Shelat HN, Jhala YK, Dhole AM (2016) Inhibition of multiple fungal phyto-pathogens by biocontrol bacteria. Nat J Life Sci 13(1):29–31

Parr JF, Hornick SB (1992) Agricultural use of organic amendments: a historical perspective. Am J Altern Agric 7:181–189. https://doi.org/10.1017/S0889189300004781

Patel KT (2014) Mass Production Technology of Azotobacter in Laboratory Fermentor on Agro-industrial Wastes with Assessment of Alginate and Poly-β-hydroxybutyrate (PHB) Production Potential- A Ph.D. thesis submitted to Anand Agricultural University, Anand, Gujarat

Provorov NA, Tikhonovich IA (2003) Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. Genet Resour Crop Evol 50:89–99. https://doi.org/10.1023/A:1022957429160

Rengel Z (2002) Breeding for better symbiosis. Plant Soil 245:147–162. https://doi.org/10.1023/A:1020646011229
Rojas A, Holguin G, Glick BR, Bashan Y (2001) Synergism between Phyllobacterium sp. (N2-fixer) and Bacillus licheniformis (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microbiol Ecol 35:181–187. https://doi.org/10.1111/j.1574-6941.2001.tb00802.x
Rural Development Administration [RDA] (1999) Fertilization standard of crop plants, National Institute of agricultural science and technology, Suwon. Korea pp 148
Sanguin H, Sarniguet A, Gazengel K, Moenne-Loccoz Y, Grundmann GL (2009) Rhizosphere bacterial communities associated with disease suppressiveness stages of take-all decline in wheat monoculture. New Phytol 184:694–707. https://doi.org/10.1111/j.1469-8137.2009.03010.x
Shahid AA, Rao AQ, Bakhsh A, Husnain T (2012) Entomopathogenic fungi as biological controllers: new insights into their virulence and pathogenicity. Archiv Biol Sci, Belgrade 64(1):21–42. https://doi.org/10.2298/ABS1201021S
Shibusawa S. (1998) Precision farming and terra-mechanics. In: The fifth ISTVS Asia-Pacific regional conference in Korea, October 20–22
Shtark OY, Borisov AY, Zhukov VA, Provorov NA, Tikhonovich IA (2010) Intimate associations of beneficial soil microbes with host plants. In: Dixon R, Tiltson E (eds) soil microbiology and sustainable crop production. Springer Science and Business Media, Berlin. 119-196. doi:https://doi.org/10.1007/978-90-481-9479-7_5
Singh OV (2006) Proteomics and metabolomics: the molecular make-up of toxic aromatic pollutant bioremediation. Proteomics 6(20):5481–5492. https://doi.org/10.1002/pmic.200600200
Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press, London, pp. 503–512
Tedersoo L, Bahram M, Polme S, Koljalg U, Yorou NS, Wijesundera R et al (2014) Global diversity and geography of soil fungi. Science 346:107. https://doi.org/10.1126/science.1256688
Vance CP (1998) Legume symbiotic nitrogen fixation: agronomic aspects. In: Spaink HP, Kondorosi A, Hooykaas PJJ (eds) The Rhizobiaceae. Molecular biology of model plant-associated bacteria. Kluwer Academic, Dordrecht, pp 509–530. https://doi.org/10.1007/978-94-011-5060-6_26
Vora MS, Shelat HN, Vyas RV (2008) Handbook of biofertilizers and microbial pesticides. Satish Serial Publishing House, Delhi
Vyas RV, Shelat HN, Vora MS (2008) Biofertilizers techniques for sustainable production of major crops for second green revolution in Gujarat - an overview. Green Farming 1:68–72
Vyas RV, Shelat HN, Jhala YK (2010) Microbial pesticides - an alternative tool to combat insect pests in GOI organic farming. Org Farming News Lett 6(4):14–17
Vyas RV, Singh B, Shelat HN and Shekh AM (2014) Present scenario & future prospects: AAU BPDU, approaches to promote Agri-business by technology transfer and public-private
Waites MJ, Morgan NL, Rockey JS, Higton G (2001) Industrial microbiology: an introduction. Blackwell Science Ltd 288
Wood N (2001) Nodulation by numbers: the role of ethylene in symbiotic nitrogen fixation. Trend Plant Sci 6:501–502. https://doi.org/10.1016/S1360-1385(01)02128-8
Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4. https://doi.org/10.1016/j.tplants.2008.10.004
Yergeau E, Sanschagrin S, Maynard C, St-Arnaud M, Greer CW (2014) Microbial expression profiles in the rhizosphere of willows depend on soil contamination. ISMEJ 8:344–358. https://doi.org/10.1038/ismej.2013.163
Zafar S, Aqil F, Ahmad I (2007) Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour Technol 98:2257–2261. https://doi.org/10.1016/j.biortech.2006.09.051

Potential Application of Agriculturally Promising Microorganisms for Sustainable Crop Production and Protection

18

Vasavi Rama Karri

Abstract

Modern agriculture entails utilization of agrochemicals to boost the food output worldwide. Though these inorganic-based fertilizers are essential as a nutrient addendum to plants and consisted of phosphorus (P), potassium (K), and nitrogen (N) as their primary components, their continuous reliance causes environmental and human health risks, viz., interruption of ecological recycling and elimination of advantageous microbial consortium required to increase production of crops. In the past few years, microorganisms that reside in the soil were largely employed for increase of quality and quantity of crop production along with management of plant and soil health. In addition, greater yields are recorded in crop plants, when they are inoculated with plant growth-promoting microorganisms (PGPMs) during their cultivation. So, utilization of these PGPMs is an effective and promising approach to raise the grade of food production with no harm to the environment or human health. Further, research studies also supported application of these beneficial microorganisms as marvelous choice to chemical fertilizers and pesticides because they can supply nutrients via atmospheric nitrogen fixation and phosphorus hydrolyzation and prompt the growth of plants by synthesizing the substances needed for plant growth and protection. Moreover, to improve agricultural produce, modern biotechnology is employing recent methods of gene alteration to produce genetically engineered novel transgenic microbial strains. Thus, exploitation of microbial inoculants can be a profitable strategy to intensify the crop production by accumulating more nutrients from soil with limited usage of agrochemicals. The present study investigates current research and developments related to the application of

V. R. Karri (✉)
Department of Biotechnology, GITAM Institute of Technology, GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India

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microorganisms as versatile tools to boost the outgrowth and produce of various crop plants in an ecofriendly manner through sustainable agriculture.

Keywords

Plant growth-promoting microorganisms (PGPMs) · Biofertilizers · Biopesticides · Chemical fertilizers · Genetically engineered organisms · Agricultural sustainability

18.1 Introduction

The worldwide population pertaining to humans is anticipated to rise approximately from 7.8 billion to 9 billion by the year 2050 (Rodriguez and Sanders 2015). So, to provide sufficient food material to the growing population, crop productivity should increase twofold (Bruinsma 2017) until 2050 year. The Agrarian Revolution prompted the application of pesticides, fertilizing substances, and genic alteration for assuring required nutrients for the growing population. In this consequence, extensive utilization of inorganic fertilizers and agrochemicals for higher crop yields remains a regular exercise in crop production. But, majority of these products are petroleum originated, and their too much use slowly deteriorates the grade of the soil. Therefore, further attempts must be underlined in a safe and environmentally friendly manner. The ascending demand for food production has led to the extension of traditional farming practices which are neither ecofriendly nor economic (Trivedi et al. 2017). These developments recommend a sequence of novel contention to global agricultural yield leading to steadily improve the production of food and agriculture and search for resolutions to encounter various abiotic and biotic stresses. Under these circumstances, employment of biological additives such as fulvic acid, humic acid, seaweed extracts, chitosan, protein hydrolysates, and desirable microorganisms can be an appropriate approach which not only supports development and nutritional level of plants but also incites stress resistance in plants (Yakhin et al. 2017).

Since the last decade, knowledge of association between plant and microbes has advanced tremendously. Despite that, it is broadly affirmed that usage of equitable fertilizer along with organic sources and agriculturally important microorganisms is pivotal in the attainment of greater crop output (Imran and Inamullah 2016; Imran et al. 2016). The expostulations related to agricultural production under coarse and adverse environmental changes during the twenty-first century would merely be overwhelmed by biofertilizer application (Imran 2017). Use of enviable microorganisms is supported by the existence of more number of plant roots for meliorated absorption of nutrients. Ahmad et al. (2018) reported that crops treated with microbial formulations may advance crop vigor and expansion and amend their ability to utilize nutrients successfully.

Among various biologically derived products, microbes of agricultural significance acquired global attention and acquiescence on sustainable farming boons.

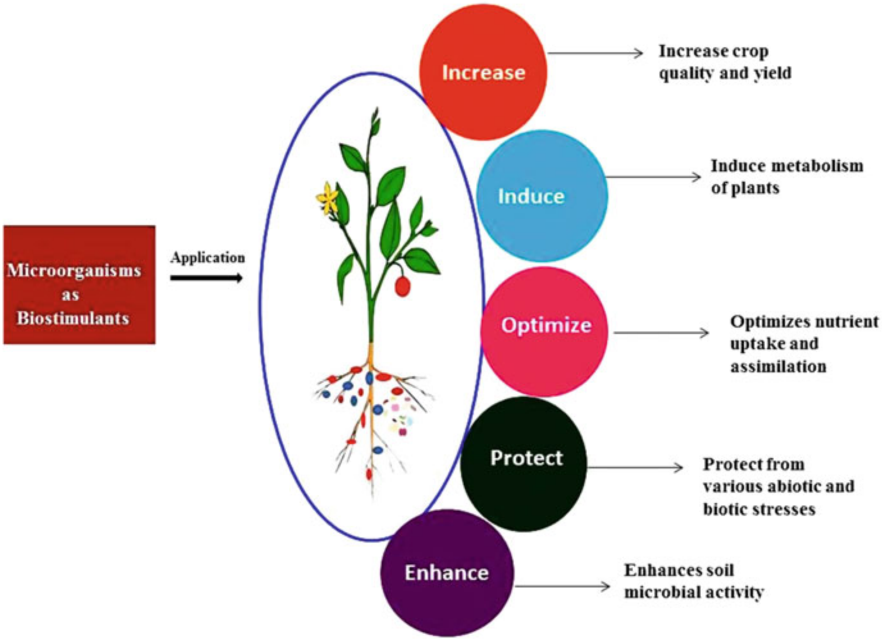

Fig. 18.1 Impact of microbial inoculants on various aspects of plant growth and promotion

Such microbial organisms were progressively integrated into crop cultivation strategies with the objective of maximizing productivity without causing adverse effects. Microorganisms such as bacteria and fungi secrete enzymes with hydrolytic activity that decompose the soil organic matter and control net carbon accumulation in soil carbon sequestration process (Shelake et al. 2019). Administration of microbial additives activate and enhance naturalistic activities like texture and structure of soil, capacity of holding water, maintenance of sanitary conditions, and microbial biomass and finally elevate plant's nutrient and water intake ability and improve photosynthetic rate and level of forbearance to varied environmental constraints (Bhogal et al. 2018; Shelake et al. 2019) (Fig. 18.1). Furthermore, microorganisms indispensible in agriculture are identified as competent microbial contenders in the zone of rhizoplane and rhizosphere. These microbes are also exploited to suppress plant pathogens and are also wielded in the process of rhizoremediation. The aforementioned microbial organisms are exercised by way of application to soil, seed treatment, and foliar spray.

18.2 Function of Microbes that Promote Plant Growth-Promoting Microorganisms (PGPMs) for Promoting Sustained Cultivation

There exist a lot of bacteria, cyanobacteria, actinobacteria, mycorrhizae, and fungi which augment plants' development and maturity via various processes. This encompasses inorganic compounds' solubilization, fixation of atmospheric nitrogen, organic compounds' mineralization, phytohormone generation, production of siderophores, activity of ACC deaminase, production of hydrolytic enzymes, antimicrobial compounds and hydrogen cyanide (HCN), etc. (Fig. 18.2). Employment of PGPM in the cultivation of various crops depicts as a cost-effective and environmentally amiable alternate to comprehensive chemical fertilization in farming. One of the goals of agricultural biotechnology is to acquire potent microorganism inoculants, which may boost proliferation and development of cultivated plants that simultaneously repress onset of disease, with a crucial objective of minimizing credence on inorganic pesticides and fertilizers (Adesemoye et al. 2009). Large-scale economic utility of these PGPM needs a right preliminary examination and mass multiplying methods in order to foster grade, quantity, and formulation of product with improved bioactivity and durability (Gopalakrishnan et al. 2016). In addition, several aspects like collection of suitable plant growth-supporting microorganisms in

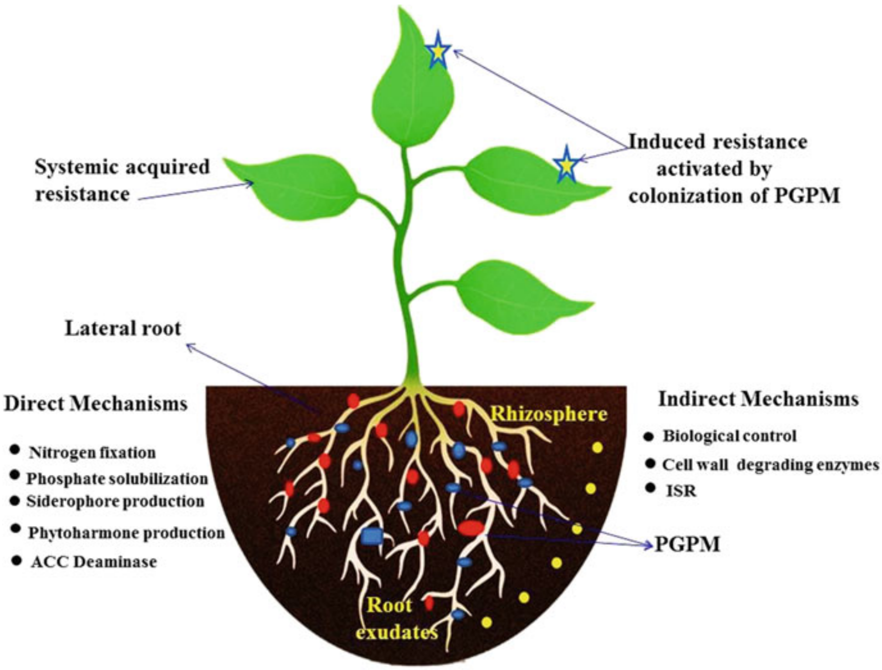

Fig. 18.2 Mechanisms employed by PGPM (plant growth-promoting microorganisms) to improve plant growth

accordance with selected host plants, nature of soil, autochthonous microbial groups, ecological circumstances, concentration of inoculants, and consistency with integrated management of cultivated crops should be taken into account while formulating microbial inoculants at commercial level (Berg 2009). Research on PGP microorganisms expresses that multifunctional nature is the most distinguishing character of these agriculturally advantageous microbial organisms (Vassilev et al. 2006; Avis et al. 2008) that enables their efficacy in agriculture. Thus, application of microbes or bioaugmentation has a premium effect on soil-microbe interactions that constitute control of disease development (biocontrol), improvement of nutrient accessibility (biofertilization), and induction of plant hormone synthesis (Martinez-Viveros et al. 2010; Bhattacharyya and Jha 2012).

In accordance with mechanism of action, plant growth-promoting microorganisms (PGPMs) are subdivided into biofertilizers, biopesticides, phytostimulators, and bioprotectors. PGPMs pertain to numerous genera as follows: *Azospirillum*, *Arthrobacter*, *Azotobacter*, *Enterobacter*, *Bacillus* species, *Pseudomonas* species, *Serratia*, *Rhizobium*, etc. Among fungi, *Aspergillus*, *Trichoderma*, *Beauveria*, *Metarhizium*, *Penicillium*, and AMF (arbuscular mycorrhizal fungi) were crucial (Choudhary et al. 2016).

18.3 Biofertilizers

Biofertilizer is a substance that includes microbes like bacteria and fungi that were used along with a carrier in the agricultural sector. Biofertilizers are simple to apply, affordable, and environmentally amiable. These are applied either by the method of soil inoculation or through seed treatment where these biofertilizers accumulate and support in the cycling of nutrients by atmospheric nitrogen fixation and solubilization of potassium and phosphate or through mineralization. Moreover, microorganisms living in biofertilizers liberate substances enhancing plant growth, develop antibiotics, decay soil organic matter, and finally promote the production of crops. In this way, various kinds of biofertilizers such as microbes fixing nitrogen, hydrolyzing phosphate, and bacteria solubilizing potassium and zinc are stated to improve the fertility of soil and ultimately accelerate the crop yield (Kumar 2018). Distinct categories of microorganisms that function as biofertilizers are represented in Table 18.1.

18.3.1 Microorganisms Fixing Atmospheric Nitrogen

Generally atmospheric nitrogen is fixed by microorganisms in two separate paths: (1) free-living and (2) symbiotic mode of nitrogen fixation. Discrete kinds of microorganisms were identified to carry out the process of nitrogen fixation. *Rhizobium* bacteria are properly recorded that they can effectively fix nitrogen in the atmosphere into soluble form through symbiotic mode of association in root nodules of host plant. Majority of these bacteria are in obligate relation with the host and are

Table 18.1 List of microorganisms that serve as biofertilizers to promote plant growth and development (Source: Reddy and Saravanan (2013))

S. No	Group	Example
N_2 fixers		
1	Free-living	*Azotobacter*, *Beijerinckia*, *Klebsiella*, *Burkholderia*, *Enterobacter*, *Clostridium*, *Anabaena*, *Nostoc*, *Herbaspirillum*
2	Associative symbiotic	*Azospirillum* sp.
3	Symbiotic	*Methylobacterium*, *Rhizobium*, *Anabaena azollae*, *Frankia*
Phosphate solubilizers		
1	Fungi	*Trichoderma harzianum* T-22, *Aspergillus awamori*, *Penicillium* sp.
2	Bacteria	*Bacillus*, *Pseudomonas*, *Rhizobium*, *Agrobacterium*, *Micrococcus*, *Burkholderia*, *Acetobacter*, *Flavobacterium*
Phosphate mobilizers		
1	Ectomycorrhiza	*Pisolithus* sp., *Boletus* sp., *Laccaria* sp., *Amantia* sp.,
2	Arbuscular mycorrhiza	*Gigaspora* sp., Glomus sp., *Sclerocystis* sp., *Scutellospora* sp., *Acaulospora* sp., *Rhizophagus* sp.
Micronutrient nutrient suppliers		
1	Potassium solubilizers	*Bacillus mucilaginosus*, *Azotobacter chroococcum*, *Aspergillus awamori*
2	Silicate and zinc solubilizers	*Bacillus*, *Acinetobacter*, *Burkholderia*
3	Iron sequesters	*Pseudomonas*, *Azotobacter*, *Rhodococcus*, *Mycobacteria*, *Rhizobia*, *Bacillus*, *Burkholderia*, *Arthrobacter*, *Actinobacteria*

capable of fixing 500–300 kg N/ha per year in the case of legumes. For example, soybean is observed to consociate with *Bradyrhizobium japonicum*, and chickpea is associated with *Mesorhizobium cicero*; likewise, a lot of other interrelationships subsists in legume plants (Vaishnav et al. 2017a, b). Furthermore, few free-living organisms like *Azotobacter* can fix nitrogen up to 15–20 kg N/ha per year. *A. chroococcum* is a familiar species of *Azotobacter* occurring in cultivated agricultural lands (Wang et al. 2018). These were also noted to enhance the germination potential, exuberance in young plants, and biocontrol function toward numerous pathogens of plant leading to better crop returns. Species of *Azotobacter* were amply reported in the rhizospheric part of soil in various crops such as maize, rice, bajra, and sugarcane and in some plantation and vegetable crops (Jnawali et al. 2015). Additionally, *Azospirillum* is another beneficial microorganism participating in nitrogen fixation in the case of non-leguminous plants and is capable of fixing around 20–40 kg/h per ha nitrogen and is also proficient to generate different substances encouraging growth of plants (Steenhoudt and Vanderleyden 2000). In the case of cereals, bacteria belonging to *Herbaspirillum* genus were identified to exist as endophytes in intracellular space and are involved in nitrogen fixation process. This particular genus was reported in sorghum, rice, and maize roots for the first time, and about 20–30% of the nitrogen is fixed biologically in these crops (rice and sorghum) (Carvalho et al. 2014). Afterwards, a pathogen of sugarcane called *Pseudomonas rubrisubalbicans* was reported to fix nitrogen biologically, which is later recharacterized as *Herbaspirillum rubrisubalbicans*. In this way, about 40–60% of nitrogen fixation occurs through biological process in different varieties of sugarcane.

Further, cyanobacteria or blue-green algae are prokaryotic phototrophic organisms that have competency in the conversion of molecular nitrogen into usable form through asymbiotic and symbiotic interactions. Well-known examples of these organisms are *Nostoc*, *Anabaena*, *Plectonema*, etc. Blue-green algae, *Anabaena*, associate in a symbiotic mode with a fern *Azolla* in submersed paddy fields and participate in the fixation of around 2–30 kg/ha nitrogen under brighter sunlight and can also transform insoluble form of phosphate into soluble form. Generally, for field applications, cyanobacterial inoculums prepared with a combination of two or more strains are used for better performance (Berman-Frank et al. 2003). In Vietnam, during rice cultivation, *Azolla* is used as a biofertilizer for enhanced production, but in India the same is not practiced because of its unsuitableness in rainfed regions (need appropriate and more supply of water), high−/low-temperature lenience, and highest level of vulnerability for diseases and insects.

Among fungi, *Trichoderma* species have significant function in organic matter decomposition which improves nourishing level attributed to soil and simultaneously accelerates accessibility of nutrients to the plants. It was reported the secondary metabolites produced from *Trichoderma* have numerous agroindustrial uses (Ram et al. 2016; Singh et al. 2012). It became noticed such that plants' nitrogen utilization efficiency (NUE) was improved when seeds were treated and primed with *Trichoderma* species and also minimized the requirement of inorganic nitrogen containing fertilizing substances down to 30–50% (Zhang et al. 2018; Singh 2014). Furthermore, fungi of mycorrhizae establish root hyphal communication which extends the area of absorption and facilitates in the acculturation of meagerly accessible nutrient materials. Additionally, through infection, mycorrhiza can render plants accessibility for nitrogenous sources, which are usually not available to roots without mycorrhizal interaction (Rillig et al. 2016).

18.3.2 Microorganisms Actively Involved in Solubilization and Mineralization of Phosphate

Major portion of soil phosphorus is inaccessible for plants. Soils with acidulous nature contain phosphorus in immovable condition and present as twain inorganic and organic states. Out of this, organic type of phosphorus accounts up to 70–80% of the whole fixed phosphate of the soil. Specific type of bacteria called PSB (phosphate-solubilizing bacteria) liberates organic acids into soil that decrease pH and unleash phosphate from its bounded state. Insoluble form of phosphorus is solubilized by phosphate-solubilizing bacteria (PSB) and makes it reachable to plants. It was noticed that plant's inoculation with PSB enhanced the yield of crops (Datta et al. 2015). Bacteria solubilizing or mobilizing phosphate mostly relates to *Flavobacterium*, *Erwinia*, *Micrococcus*, *Aerobacter*, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Achromobacter*, *Burkholderia*, and others. This set of microorganisms has proficiency to hydrolyze many compounds of inorganic phosphate like rock phosphate, hydroxyapatite, dicalcium phosphate, tricalcium phosphate, etc. It was also noticed that indissoluble phosphorus could be transformed to dissoluble with no secretion of organic acids. For instance, *Trichoderma harzianum* T-22 exhibited phosphate (P)-mobilizing activity without production of organic acid (Khan et al. 2014). This approach of P-solubilization with fungal strains is also beneficial in the management of plant pathogen management. Certain mycorrhizae are also stated to hydrolyze and mineralize the phosphate from fixed state to easily available form to the plants resulting in better upgrowth and production (Sharma et al. 2013).

18.3.3 Microbes Concerned with Hydrolyzation of Zinc

Zinc is one of the important micronutrients that has crucial activity in numerous plant metabolic reactions. Zinc is participated in various processes like biological membrane integration, steps involved in the production of auxins, reactions of chlorophyll, and enzymatic activities involving superoxide dismutase and carbonic anhydrase enzymes. Because of these many functions, plants rely on Zn nutrition during their development. It also has a major role in improving the quality of grains and is also involved in the synthesis of lipids, proteins, and nucleic acids. Deficiency of zinc is directly linked to category of soils like neutral, sandy, saline, calcareous, sodic, etc (Vaishnav et al. 2016a, b). Zn occurs in various insoluble states like zincite, smithsonite, franklinite, hopeite, zinkosite, etc. in soil. These different forms of insoluble zinc were checked for solubilization using PGPR. It was reported that bacteria that reside in the rhizosphere can solubilize bounded form of zinc and aid in its improved uptake by host plants. In this manner, fluorescent *pseudomonads* were noticed to increase intake of zinc in genotypes of wheat (Abaid-Ullah et al. 2015). Similarly, other types of PGPRs like *Acinetobacter* SG3 (AB), *Acinetobacter* SG2 (AX), and *Burkholderia* SG1 (BC) were observed to release gluconic acid into rhizosphere part of soil and support zinc intake by the crop plants of rice (Vaid et al. 2014). Furthermore, plants' coalition with mycorrhiza was observed to prompt the absorption of minerals such as zinc in the case of wheat, pigeon pea, tomato, and soybean (Srivastava et al. 2015).

18.3.4 Microorganisms Solubilizing Potassium (K)

Potassium (K) has a significant role in plant defense mechanism and also in different processes like synthesis of proteins and enzymes and photosynthesis. In soil this mineral occurs in both accessible (soluble in water) and inaccessible (illite, micas, and orthoclase) forms. Special types of bacteria known as KSB (potassium-solubilizing bacteria) are efficient in mobilizing potassium in rocks and also involved in silicon ion chelation. So, these bacteria (KSB) have prominence in improving the potassium absorption potency of plants, by which they can facilitate decreased usage of expensive inorganic synthetic fertilizers (Ahmad et al. 2016). Apart from this, *A. awamori* (*Aspergillus awamori*) fungus had shown to be utilized for the solubilization and composting of rock phosphate and mica that supplies usable form of potassium which could be harnessed to enhance farm production (Biswas and Narayanasamy 2006). In the similar manner, microorganisms, viz., *Bacillus mucilaginosus*, *Rhizobium* sp., and *Azotobacter chroococcum*, have been stated to hydrolyze refused form of mica during wheat and maize cultivation through hydroponics (Singh et al. 2010).

18.3.5 Microorganisms Sequestering Iron

In plants, iron acts as a cofactor in different enzymatic reactions. Despite its abundance, iron is not easily obtainable to microbes and plants. Generally, solubility of iron is diminished in soils with alkaline nature (Vaishnav et al. 2016a, b). Microorganisms present in the soil have competency to retrieve iron from inaccessible sources via various processes such as conversion of iron from ferric to ferrous state, utilization of stored form like ferritin, and breakup of complex form of iron by enzymatic reactions. Nevertheless, among these processes, in microbes, production of siderophores was better investigated. Siderophores were low m.wt water-dissolvable composites which exhibit high Fe (iron) binding capacity. Production of these compounds is a significant characteristic feature of PGPR to support progress of plants and guard them from phytopathogens. Further, roots of plants can directly absorb siderophores as iron source (Khan et al. 2018). Moreover, bacteria actively producing siderophores were observed in paddy fields in consociation with rice plants (Loaces et al. 2011). Additionally, broad array of siderophores were generated in distinct species of fungi (Winkelmann 2007). Their type relies upon the nature of backbone structures similar to tri- and di-aminoalkane, lipopeptide, peptide, and citric acid. Microorganisms of *Azotobacter*, *Arthrobacter*, *Mycobacteria*, *Burkholderia*, *Rhodococcus*, *Actinomycetes*, *Rhizobia*, *Pseudomonas*, *Bacillus*, etc genera are well explored with respect to siderophore formation.

18.3.6 Mycorrhizal Collaboration

In majority of the plant families (80%), arbuscular mycorrhizal fungal interactions were noticed where they are concerned with cycling of nutrients. Furthermore, arbuscular mycorrhizal fungi (AMF) were also demonstrated in improving plants' resilience towards discrete categories of abiotic and biotic stresses (Lone et al. 2017). *Glomerales* is the best examined order of AMF along with family *Glomaceae* grouped together with *Gigasporaceae* and *Acaulosporaceae* under monophyletic clade. Remaining clades, specifically, *Paraglomus* and *Archaeospora*, are segregated out of *Glomaceae* (Schwarzott et al. 2001). Normally, two distinct categories concerned with mycorrhizae interactions were recorded in plants that have diverse physiological and structural relevance with hosts. Mycorrhizal collaboration changes both physical and chemical features of rhizospheric ground and is associated with cycling of mineral nutrients through the accumulation of glomalin (Ahanger et al. 2014). Glomalin represents a material with proteinaceous nature which stimulates agglomeration and solidity of soil. In the symbiotic interaction, hyphae of fungi enhance plants' root area that traverse greater soil volume and increase the proficiency of intake of nutrients. Association with these arbuscular fungi is primarily accountable for carbon and phosphorus intake. It was observed that phosphorus nutrition was reinforced when mycorrhizal arbuscular fungi were inculcated into plants, which have direct impact on metabolism of nitrogen, integrity of vacuolar membrane, production of antioxidants, and distribution of ions driving the growth of plants. Therefore, plants' interaction with AMF minimizes the harmful effect due to elevated concentration of salts. Furthermore, arbuscular mycorrhizal fungi can sustain Na+/K+ proportions through exacerbated intake of K or potassium and impede sodium assimilation resulting in improved adaptation towards stress conditions (Porcel et al. 2012).

18.3.7 *Trichoderma* Species

It's appropriately recorded that preparations made from species of *Trichoderma* have become quite frequently employed to upgrade propagation and productivity of plants during cultivation. These species were competent to colonize in the rhizosphere with roots of plants. Good colonization with roots enhances better absorption of nutrients. *Trichoderma* impacts upgrowth and advancement of plants by using varied contrivances like mobilization and improved intake of mineral nutrients, production of hormones promoting plant growth, and repression of phytopathogens in the soil (Ram and Singh 2018). It was reported that secondary metabolites produced from the species of *Trichoderma* have multiple uses in agricultural, cosmetic, pharmaceutical, beverage, and other related areas (Keswani et al. 2014; Ram et al. 2016). Among *Trichoderma* species, *Trichoderma harzianum* was proved in hydrolyzing a lot of mineral composites like rock phosphate, MnO_2, and metallic zinc. In different crops, *Trichoderma* spp. were observed to mobilize various forms of phosphate that resulted in increased phosphorus nutrition to plants (Li et al. 2015). Furthermore, these species have also been reported for the improvement of potassium absorption in chickpea grains and leaves (Bidyarani et al. 2016). One more strain of *Trichoderma* referred to as T34 *T. asperellum* was assessed in terms of activity in micronutrient assimilation in wheat cultured on a calcitic medium. Furthermore, it was noticed that plants infected with strain T34 displayed enhanced iron (Fe) concentration in iron inadequate medium (de Santiago et al. 2011).

18.3.8 Prominence of Certain Microorganisms as Biofertilizers and Phytostimulators

Sinorhizobium, *Bradyrhizobium*, and *Rhizobium* are certain examples of various bacterial genera used as biological fertilizers which promote augmentation of plants (Perret et al. 2000; Jones et al. 2007; Franche et al. 2009). These organisms interact cooperatively with leguminous crop plants through synergetic mode and supply accessible form of nitrogen to host. Biofertilizers were produced through IMPACT (Interactions between Microbial inoculants and resident Populations in the rhizosphere of Agronomically important Crops in Typical soils) program via genetic modification of microbial organisms which are persuasive in establishing symbiotic relation with host plants and minimize the requirement of fertilizers. Bacteria, *Azospirillum*, can be approved as one of the effective phytostimulators which secrete growth-enhancing compounds that boost proper root development and cause better absorption of nitrogen and water by plants that finally result in encouraged plant growth. Genetic engineering has produced strains which are capable of generating substantial levels of growth-stimulating substances with a prospect to raise the crop yield and safeguard the environment from harmful inorganic chemical fertilizers. In the IMPACT program, in addition to checking their efficiency in hiking the agricultural yields, the effect of genetically engineered phytostimulators and biofertilizers on the naturally established population of microbes was also assessed (Walsh et al. 1999).

Other than carbon dioxide (acquired from the atmosphere), plants absorb most of nutrients from the soil that need to be supplemented with nutrients obtained from renewable sources in compliance with the current policy of sustainable agriculture. The excellent example to illustrate such concept represents a biologic way of fixing nitrogen within Leguminosae group of plants in which huge N source (nitrate or ammonia) discharged into surface water bodies is evaded. Bacteria competent in fixing nitrogen may be explored as an independent accelerating origin for supplying nitrogen to plants, where lamentably all seedlings (such as maize, rice and wheat) cannot initiate a symbiotic kind of relationship with nitrogen-fixing bacteria even though they exist in enormous numbers at their roots.

Until advanced techniques are established to prompt symbiotic associations between the agronomically essential crops and microorganisms involved in nitrogen fixation, the agricultural yields depend primarily on inorganic fertilizer sources. To augment nitrogen level, agrochemicals are furnished to soil in the form of nitrates which are extremely portable in nature; owing to this reason, abundant quantities of fertilizers (containing nitrogen) are supplied to soil to sustain the ideal development of plants. At present, to get more yields, about 450 kg N/ha are utilized in paddy fields, of which merely around 200–250 kg N/ha is being used for plant cultivation. Thus, greater than half of nitrogen furnished is drained (budgetary loss) into the atmosphere with significant atmospheric consequences (environmental adulteration) (Santi et al. 2013).

Distinct systems have been developed to enhance the fertilizer intake by roots that constitute use of alternative fertilizer formulations such as regulated and delayed releasing fertilizers and usage of PGPR (rhizobacteria stimulating growth of plants). These PGPRs function precisely in the production or obliquely in the removal of devastating deleterious pathogens and microorganisms as biocontrol agents (Glick 1995).

18.4 Biopesticides or Microbial Pesticides

In recent times, biopesticides have earned much attention in controlling plant diseases. The main marketed biopesticides with high demand in Indian market are *P. fluorescens*, *T. viride*, *B. thuringiensis*, and *T. harzianum.* These are under the regulation of CIB (Central Insecticide Board) Faridabad, India. CIBRAC (Central Insecticides Board and Registration Committee), India (http://cibrc.nic.in/bpr.doc), has reported approximately 970 number of biopesticides derived from microorganisms in accordance with articles 9(3) and 9(3b) included in Insecticide Act (1968), Govt. of India (Fig. 18.3). Further, CIBRC also cataloged 568 numbers of fungal derived substances with biopesticide nature under 9 (3B) sections on provisional basis. These are *T. viride* (270), *Metarhizium anisopliae* (37), *Beauveria bassiana* (95), *Verticillium lecanii* (106), *V. chlamydosporium* (5), and *T. harzianum* (55). Similarly, products related to bacterial origin such as *B. thuringiensis* (58), *B. sphaericus* (3), *B. subtilis* (5), and *Pseudomonas fluorescens* (157) were also registered. Additionally, 68 numbers of biopesticide-related products were matriculated by different companies under section 9(3).

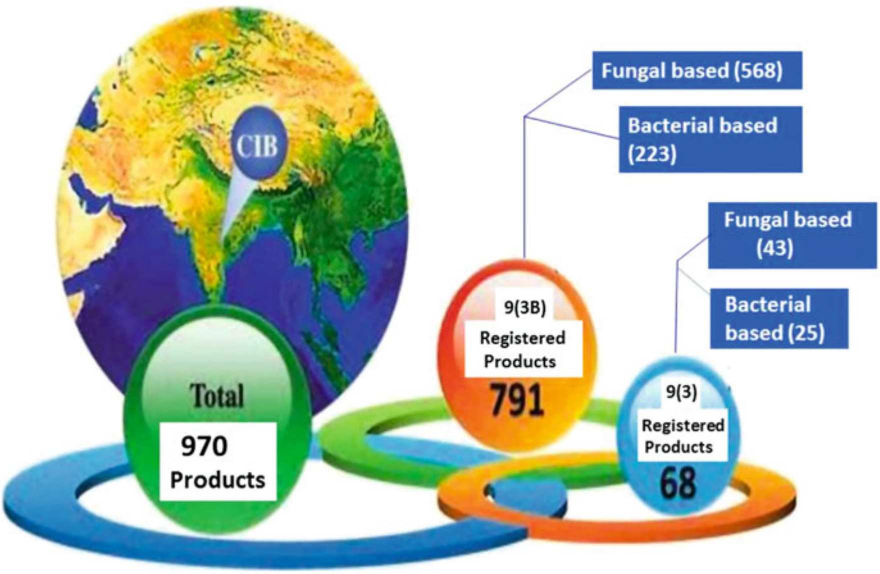

Fig. 18.3 Biopesticides registered with CIBRC at commercial level (Source: www.cibrc.nic.in/G_biopesticides.doc)

18.4.1 Bacillus thuringiensis

B. thuringiensis (Bt) is the most extensively employed biopesticide in the world. This is a facultative gram-positive aerobic soil bacterium that has insecticidal activity. At the time of sporulation, these bacteria generate a set of proteins called as Cry proteins or δ-endotoxins with insecticidal function. When they are ingested by larvae, these proteins become functional in alkaline pH of the midgut and induce gut cell lysis. The versatility of Bt and its greater performance towards coleopteran and lepidopteran larval stages make it universally prominent and account in excess of 60% of biopesticide trading (Sanchis and Bourguet 2008). Origins concerning *B. thuringiensis* preparations derive primarily with strains related to *galeriae*, *kurstaki*, and *dendrolimukurstaki* subspecies. Sprays of *B. thuringiensis* were employed in order to constrain caterpillars that destroy fruits and vegetables and are also exercised in controlling *Ostrinia nubilalis* (European corn borer) and gypsy moth larvae. Bt-based formulations were proved to be productive on different crops, viz., maize, cotton, and soybean, where impedance to non-natural inorganic insecticides is troublesome. Moreover, transgenic Bt plants are also developed using *Bacillus thuringiensis* (Bt) toxins through genetic engineering strategy to defend against insects (Chandler et al. 2011).

18.4.2 Entomopathogenic Microorganisms as Biopesticides

Diverse forms of insecticides from biological origin were prepared relying on insect killing properties of certain fungal groups and Baculoviridae viruses. Baculoviruses are specific to host and can attack a wide array of insects belonging to *Lepidoptera* including butterflies and moths, *Diptera* like flies, and *Hymenoptera* (ants, wasps, and bees) orders. These viruses specifically target larvae of *Lepidoptera* pests, i.e., caterpillar stage. For instance, in the USA, during the cultivation of apple crop, CpGV (*Cydia pomonella granulovirus*) is exercised extensively as a persuasive biological pesticide in combating codling moth (Chandler et al. 2011). Similarly, in the case of Brazil, NPV (nuclear polyhedrosis virus) was employed to control *Anticarsia gemmatalis* caterpillars within soybean crop (Moscardi 1999). Next, derivatives of fungal species with entomopathogenic activity were also utilized as biological pesticides. Best examples of such fungi are *Metarhizium anisopliae* and *Beauveria bassiana* which infiltrate into cuticle tissues of insect host and harness the available nutrients present in the hemocoel and finally assassinate the insect by releasing toxic substances. Insecticide called as "Boverin" prepared from *B. bassiana* was identified to control *Cydia pomella* L. and lowered the dosage of chemical insecticide trichlorfon application (Ferron 1971). Nevertheless, more favorable and quicker results were noticed when *B. bassiana* was *sprayed* in combination with imidacloprid in minute quantity (Ambethgar 2009). Additionally, biopesticides formulated from *M. anisopliae* were administered against spittle bugs in Brazil for maximum sugarcane production (Li et al. 2010). Furthermore, this fungus was commended under FAO (Food and Agriculture Organization), United

Nations, in 2007 to control locust pest. In a similar approach, *M. anisopliae* (*Metarhizium anisopliae*), previously presented as *Entomophthora anisopliae*, was also exploited to manage adult mosquitoes of *Aedes albopictus* and *Aedes aegypti* (Scholte et al. 2007).

18.4.3 Alternative Diverse Microorganisms

Alternatively, species of *Trichoderma*, *Mycorrhizae*, and *Pseudomonas* are also employed as biopesticides. Application of *Trichoderma* is appropriate to control soil-borne pathogens like *Rhizoctonia*, *Fusarium*, and *Pythium* in dryland crops like green gram, groundnut, chickpea, and black gram. It liberates a broad array of secondary metabolites (diffusible, non-volatile, and volatile) that could impede pathogen expansion and protect them against attack (Waghunde et al. 2016). Next to *Trichoderma*, bacterial species of *Pseudomonas* are well examined in perspective of their potent rhizospheric colonization and wide spectrum of their antagonistic property. This particular group of bacteria symphonize a broad range of bioactive molecules like 2,4-DAPG (2,4-diacetylphloroglucinol), pyoluteorin, gluconic acid, quinolones, siderophores, hydrogen cyanide (HCN), phenazines, pyrrolnitrin, 2,5-dialkylresorcinol, lipopeptides, and rhamnolipids to prevent the growth of pathogens in plants (Raaijmakers and Mazzola 2012). It was documented substantially that *Pseudomonas fluorescens* species can properly adjust within the soil and settle in the roots of more than one plant species, which is making them distinct from other pathogens (Couillerot et al. 2009). Further, mycorrhizae amply mask plant roots by developing a mat-like structure called fungal mat that acts like an extrinsic restraint to protect plants from trespassing pathogens like roundworms, fungi, bacteria, and insects (Harrier and Watson 2004). Additionally, mycorrhizae also escalate the competence of nutrient intake by the plants that make them more strong and healthy towards pathogens causing disease (Ortas et al. 2017).

18.4.4 Use of Non-infectious Microorganisms

Employment of non-infectious organisms to inhibit soil-borne pathogens is attaining importance in the contexture of biostimulants. These organisms control pathogenic organisms by contending with them for nutrients as well as for sites of infection for colonization. Furthermore, plants induced memory protection system against non-infectious determinants result in rapid and robust elicitation of necessary forbearance mechanisms after exposing to pathogens (Vaishnav et al. 2018). It was stated that nonpathogenic species of *Pseudomonas* and *Fusarium oxysporum* are capable of restraining *Fusarium* wilt disease (Alabouvette 1999). These particular groups of organisms contend for iron and carbon sources. It was proved that non-infectious strains of *Fusarium* Fo47 were responsible for controlling *Fusarium* wilt disease in the case of suppressive type of soils (Alabouvette et al. 1979). Moreover, strain Fo47 is identified to subdue *Pythium ultimum* pathogen causing cucumber disease through the process of antibiosis and mycoparasitism (Benhamou et al. 2002).

18.5 Microbial Organisms Used in Abiotic Stress Alleviation

Certain microorganisms that are actively participated in the extenuation of various abiotic stress conditions are *Pseudomonas syringae*, *Bacillus*, *Azospirillum* sp., and *Pseudomonas fluorescens* for salinity; *Pseudomonas putida*, species of *Azospirillum*, and species of *Bacillus* for drought; and *Bacillus polymyxa* and *Pseudomonas alcaligenes* for nutrient deficiency. Besides these bacteria, some lenient *Trichoderma* species are also noticed in the mitigation of abiotic stresses. Further, some arbuscular mycorrhiza fungal species, viz., *Glomus fasciculatum*, *G. mosseae*, *G. intraradices*, *G. coronotum*, *G. etunicatum*, and *G. macrocarpum*, support for reduction related to abiotic stress situations among different crop plants through enhancing assimilation of nutrients and accumulation of osmolytes.

Microbial-based approach of stress alleviation in agriculture is more favored than traditional strategies and is a growing concern with plants (Nadeem et al. 2014; Souza et al. 2015; Vaishnav et al. 2018). Rhizospheric microorganisms belonging to various genera represent complicated operation towards promotion of plant upswing and alleviating the effects connected with various abiotic stress situations, i.e., *Azospirillum* (Omar et al. 2009), *Pseudomonas* (Sorty et al. 2016; Vaishnav et al. 2015, 2016a, b), *Burkholderia* and *Pantoea* (Sorty et al. 2016), *Bacillus* (Tiwari et al. 2011; Kumari et al. 2015), *Methylobacterium* (Meena et al. 2012), *Trichoderma* spp. (Ahmad et al. 2015), *Rhizobium* (Sorty et al. 2016), *Bradyrhizobium* spp. (Swaine et al. 2007; Panlada et al. 2013), *Cyanobacterium* (Singh et al. 2011), *Azotobacter* (Sahoo et al. 2014a, b), and *Enterobacter* (Sorty et al. 2016). Discrete groups of microorganisms that can mitigate the problem of various abiotic stressful conditions are represented in Table 18.2. Thus, employment of these microorganisms expedites reduction resulting from stressful situations in cultivation by initiating a pioneering path to perform multiple functions.

18.6 Contribution of Biotechnology to Improve Crop Yields in Agriculture

Biotechnology has started reforming agriculture with stratagies of cultivating various new crop varieties with enhanced yield, embellished content of nutrients, and survival skills during unpropitious situations with minimal application of fertilizers and pesticides. In GMOs or in genetically modified organisms, sequence of genome has been changed or altered by genetic engineering to produce transgenic living organisms with required traits (Key et al. 2008). Furthermore, diverse microbial strains proficient in reducing the emergence of disease and stimulating the plant growth tend to restrict the application of pesticides, fungicides, and fertilizers and open up new avenues to avoid significant crop losses that cannot even today be

Table 18.2 List of microorganisms employed to handle various abiotic stress circumstances

S. No	Microbes	Plants	Stress	Tolerance mechanism	References
1	*P. mendocina* and *Glomus intraradices*	Lettuce	Drought	Induce antioxidant machinery in plant	Kohler et al. (2008)
2.	*Pseudomonas polymyxa* and *Rhizobium tropici*	Common bean	Drought	Regulate stomatal conductance and hormonal balance in plant	Figueiredo et al. (2008)
3	*Rhizobium* spp.	Sunflower	Drought	Bacterial EPS induces soil aggregation that enhances survival of plant	Alami et al. (2000)
4	*Variovorax paradoxus*	Pea	Drought	Reduce ethylene level in plant	Dodd et al. (2005)
5	*Pseudomonas* spp.	Pea	Drought	Bacterial ACC deaminase reduces deleterious ethylene level in plant	Arshad et al. (2008)
6	*Paraphaeosphaeria quadriseptata*	*Arabidopsis*	Drought	Upregulation of heat shock proteins in plants	McLellan et al. (2007)
7	*Rhizobium* spp.	Wheat	Drought	Bacterial inoculation improved water source and sink relation	Creus et al. (2004)
8	AM fungi	Sorghum	Drought	AM fungus improves water relations in plant	Cho et al. (2006)
9	*Bacillus thuringiensis* AZP2	Wheat	Drought	Bacterial-mediated VOCs promote plant growth and tolerance	Timmusk et al. (2014)
10	*Bacillus licheniformis* strain K11	Capsicum	Drought	Bacterial inoculation induces stress related genes and proteins in plants	Lim and Kim (2013)
11	*Burkholderia phytofirmans* and *Enterobacter* sp. FD17	Maize	Drought	Bacterial inoculation affects plant physiology including photosynthesis	Naveed et al. (2014)
12	*Bacillus cereus* AR156, *B. subtilis* SM21, and *Serratia* sp. XY21	Cucumber	Drought	Bacterial inoculation induces accumulation of osmolytes and antioxidants	Wang et al. (2012)
13	*Pseudomonas chlororaphis* O6	Arabidopsis	Drought	Volatile compound 2R, 3R-butanediol induces plant growth and tolerance	Cho et al. (2008)

14	*Rhizobium* and *Pseudomonas*	Mung bean	Salinity	Bacterial ACC deaminase activity enhances plant growth and metabolism in plant	Ahmad et al. (2011)
15	*Pseudomonas* and *Enterobacter*	Maize	Salinity	Decrease negative effect of ethylene and increase nutrient content in plants	Nadeem et al. (2009)
16	*Brachybacterium saurashtrense* (JG-06), *Brevibacterium casei* (JG-08), and *Haererohalobacter* (JG-11)	Groundnut	Salinity	Enhance phosphate and nitrogen content and reduce Na + content in plant	Shukla et al. (2012a, b)
17	*Pseudomonas pseudoalcaligenes* and *Bacillus pumilus*	Maize, rice GJ-17	Salinity	Bacterial inoculation induces antioxidants that lowers toxicity of ROS	Jha and Subramanian (2014)
18	*Pseudomonas putida*	Canola and maize	Salinity	Bacterial ACC deaminase activity modulates plant protein expression	Cheng et al. (2012)
19	*Bacillus amyloliquefaciens*NBRISN13 (SN13)	Rice	Salinity	Modulate expression of stress-responsive gene expression	Nautiyal et al. (2000)
20	*Azospirillum* sp.	Wheat	Salinity	Regulate plant physiology	Nia et al. (2012)
21	*Azospirillum* sp.	Lettuce seeds	Salinity	Induce antioxidant compound in plant	Fasciglione et al. (2015)
22	*Pseudomonas* sp., *Serratia* sp.	Wheat	Salinity	Bacterial ACC deaminase activity enhances plant growth and metabolism in plant	Zahir et al. (2009)
23	*Bacillus thuringiensis* GDB-1	Alnus	Cu, Ni, As, Zn, and Pb toxicity	Bacterial PGP activities improved phytoremediation efficiency	Babu et al. (2013)
24	*Enterobacter* sp. JYX7 and *Klebsiella* sp. JYX10	Drooping knotweed	Cd, Zn, and Pb toxicity	Bacterial pGP activities improved phytoremediation efficiency	Jing et al. (2014)
25	*Pseudomonas* spp. Lk9	European nightshade	Cu, Zn, and Cd toxicity	Induce siderophore and organic acid production in soil that reduces toxicity	Chen et al. (2014)
26	*Bacillus polymyxa*, *Mycobacterium phlei*, *Pseudomonas alcaligenes*	Maize	Nutrient deficiency	Increase N, P, and K content in plant	Egamberdiyeva (2007)

(continued)

Table 18.2 (continued)

S. No	Microbes	Plants	Stress	Tolerance mechanism	References
27	*Burkholderia phytofirmans*	Grapevine	Temperature	Bacterial inoculation induces osmolyte contents in plant	Barka et al. (2006)
28	*Photobacterium* spp.	Common reed	Hg toxicity	Bacterial inoculation induces mercury reductase activity in plant	Mathew et al. (2015)
29	*Pseudomonas aeruginosa*	Wheat	Zn toxicity	Increase nutrient content in plant leading to high biomass and protein content	Islam et al. (2014)
30	*Bacillus subtilis*, *Bacillus megaterium*, *Bacillus* sp.	Rice	Fe toxicity	Bacterial siderophore reduces toxic concentrations of Fe	Asch and Padham (2005) and Terre et al. (2007)

controlled by available agrochemicals. Thus, it is visualized that biotechnology can serve as a credible source for achieving environmental-friendly sustainable food production.

18.6.1 Production of Potent Microbial Strains to Generate Effective Biofertilizers Via Genetic Engineering Approach

The attributes of the diversified processions studied in relation to the functioning of PGPR along with the possibility of modifying the specific microbial strain genome as an effective strategic plan to encourage plants multiplication, production, and output suggest harnessing of genetically manipulated transgenic microbial organisms as biofertilizers for exploiting diverse prospects for execution in the future.

The majority of chemical fertilizers used by farmers nowadays are industrially developed through the Haber-Bosch process (Appl 2006; Appl 1982) that is affordable in well-developed countries which can bear the expenses of that process, but these are unbearable in poor countries where such costs are not tolerable. Furthermore, fossil fuels are burned during this process to produce ammonia from nitrogen gas (molecular nitrogen), which absorbs around 5% of the total extracted global natural gas. Thus, the use of in situ mode of functioning transgenic diazotrophs produced by genetic engineering through gene modification could mitigate environmental pollution problems and decrease the shipping costs compared to Haber-Bosch-derived fertilizers. In addition, they ameliorate the nutrient assimilation and promote their availability to the plants to improve growth and development which can resolve present problems related to discharge of agricultural leftovers (Barney et al. 2016).

The basic procedures pivotal for microbial prompted proliferation and progression pertaining to plants were at the initial level and required to be interpreted at different intents of molecular level. This knowledge was used for the alteration of microorganism's genetic material to develop better microbial strains by genetic engineering. There are many such examples: PGPB transformation with ACC or 1-aminocyclopropane-1-carboxylate deaminase gene in decreasing plants' ethylene concentrations (Glick 2014), development of potent strains of microbes (*Azospirillum*) which could synthesize IAA (indole-3-acetic acid) at elevated levels (Bashan 2010), and generation of genetically altered microbial strains capable of producing fixed ammonium (Van Dommelen et al. 2009), where significant progress is being made in exploiting the ability of microorganisms as plant growth inducers.

Environmental safety assessment with respect to exploitation of these developed strains requires proper understanding of the mechanisms inducing the growth of plants. For example, lateral gene transfer of available ACC deaminase genes had been recommended within rhizospheric bacteria (Hontzeas et al. 2005).

18.6.2 Development of Genetically Engineered Transgenic *Azotobacter vinelandii* as an Important Biofertilizer of Diazotrophs

Genetically altered *Azotobacter vinelandii* (*A. vinelandii*) has been produced that release substantial amounts of nitrogenous compounds such as ammonia or urea when compared to their wild forms. This type of end products produced during metabolism of microorganisms could be efficiently exploited as biofertilizers. Different from the majority of nitrogen-producing bacteria that only function under anaerobic conditions, *A. vinelandii* operates in aerobic conditions, making it as an excellent organic or biological forge that supports the growth of routine crops in agriculture or growth of algae in production (http://www.license.umn.edu › technologies › 20140348_biofertilizer-from-genetical).

18.6.3 Development of Genetically Engineered Strains of *Azospirillum* to Secrete Higher Levels of Phytohormones

Phytostimulation is a well-studied case to explain the direct action of PGPR in stimulating plant growth. Bacteria, *Azospirillum*, can be approved as one of the effective phytostimulators, which colonize in plant roots and provide plant growth-enhancing factors (cytokinins, auxins, etc.) required to support raise and refinement of plants and also ensure better nutriment uptake and possible water absorption that result in increased crop yield.

Bacterial strains of *Azospirillum* (containing non-antibiotic resistance genes as marker genes) were constructed through gene alteration by IMPACT program to evaluate their activity on yield of sorghum grains. In the IMPACT group's translation scheme, multiple trials were performed in fields by research and industry associates on a commercial scale. The capacity of colonization, survival, and endurance of genetically engineered bacteria on sorghum roots and their effect on sorghum grain yields and endemic microflora were determined (Walsh et al. 1999). Consequently, to verify the effect of microbial inoculants, sorghum was cultivated in three separate plots enriched with different concentrations of nitrogen. *Azospirillum*'s two strains, specifically *Azospirillum brasilense* Sp6, which secrete ordinary levels of plant growth-regulating factor, especially IAA, and other strain *Azospirillum brasilense* Sp6IAA++, which produce elevated levels of IAA, were being examined in compliance with the regulations set out in EU Directive 90/220.

IMPACT's three main functions for solving the issue are:

1. To procure knowledge on biochemistry and genetics corresponding to the generation of IAA (indole-3-acetic acid) using *Azospirillum* bacteria to secrete.
2. To produce genetically engineered *Azospirillum* strains which are capable of producing accepted quantities of IAA (IAA attenuated, IAA minus, and IAA over-producers).

3. To examine the impact of those bacteria developed by genetic modification on different plant growth parameters (taking up nutrients and nitrogen, promoting growth) and on the habitat or ecosystem (mutual association with inhabitant microbial flora, survival, and transmission) under the circumstances of field.

These efforts were exercised in research program involving genetic alteration of bacterial genomes and evaluation of different physiological processes succeeded by their screening finally under field conditions.

Developing a method to promote plant root induction and to improve nitrogen absorption using *Azospirillum* strains requires adequate knowledge about controlling mechanisms and regulated conditions that allow these bacteria to secrete phytohormones. It's also important to have a good understanding of plant-bacterial association. Synthesis of indole-3-acetic acid (IAA) by *Azospirillum* strains seems to be intricate and intervened by not less than three pathways. Improved production of IAA was achieved by modifying the *ipdC* gene regulating an enzyme responsible for indole-3-pyruvic pathway (major biosynthetic pathway). These bacterial strains were developed with some marker genes which enables their easy detection in soil while conducting field trials. The two genes chosen for use as markers are *lue* and *gfp*, where *lue* gene expression elicits bioluminescence which renders the bacterial cells to glow and the gene *gfp* encodes for green fluorescent protein that causes bacterial cell to fluoresce (Walsh et al. 1999).

Predicated on the conducted investigations, it has become revealed that as far as the roots were adequately colonizing with genetically modified microorganisms, their density appears to be more after 15–20 days of post-inoculation. Further, colonization was observed to be reduced, and finally cell density was low (9×10^3 cells/g soil dry weight) during harvesting time (Walsh et al. 1999). In addition, seed inoculation with these genetically modified transgenic strains did not disrupt the native microbial population unique to that region.

Finally, it was identified that *Azospirillum* inoculum improved root growth of sorghum (Fig. 18.4) and increased the production of grains which demonstrates the production of comparable grain yields with other crops also through the administration of *Azospirillum* inoculants together with seeds by minimizing the application of nitrogenous fertilizers (Walsh et al. 1999). In addition, they also facilitate in better intake of nitrogen that decreases its remnants in soil and dramatically minimizes the potential for groundwater contamination (Fig. 18.5).

Azospirillum strains are currently available with core features. But thorough and precise experimental research under controlled conditions is mandatory before they are exposed for field trials. Nowadays, in IMPACT, much attention is focused on the impact of genetically modified *Azospirillum* strains on the endemic population of microbes, efficiency of plants to absorb nitrogen from soil, and growth parameters of plants. These experiments are performed in glass house and growth cabinets to gain critical knowledge on the action of GM strains under the conditions of the field (Walsh et al. 1999). IMPACT consortium transitional partnership assists in undertaking experiments with various groups of crops in different types of soil under varying climatic conditions.

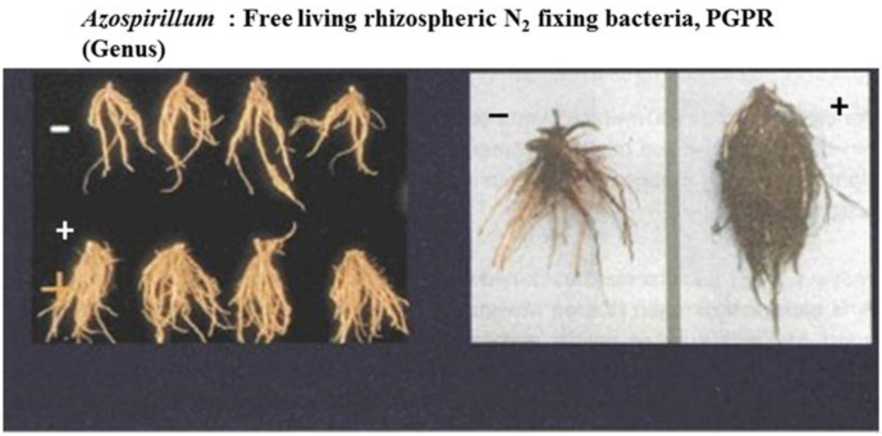

Fig. 18.4 Improved root growth with the use of *Azospirillum* inoculum (− Non-inoculated + Inoculated) (Source: Harnessing the potential of genetically modified microorganisms and plants, European commission community research)

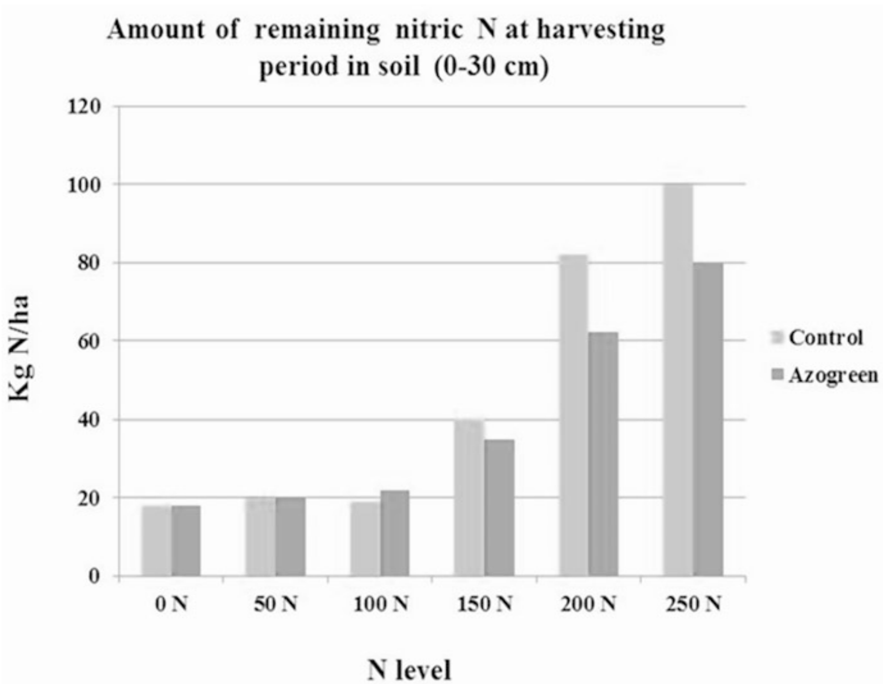

Fig. 18.5 Declined nitrogen levels of soil treated with *Azospirillum* inoculum (Source: Results of Azogreen-m field experiment 1997)

18.6.4 Development of Genetically Altered *Pseudomonas fluorescens* Strains with Binary Biocontrol Functions

Phl or 2,4-diacetylphloroglucinol is an antibiotic secreted in a wide variety of commonly existing *Pseudomonas fluorescens* spp. Among these, specific strain referred to as *fluorescent Pseudomonas* F113 defends pea crop against *Pythium ultimum* (fungal pathogen) attack via curtailing the development of lacerations upon roots of plants along with simultaneous deduction in the development of plants. The genes regulating the synthesis of Phl were identified to be conserved in the Q2–87 and F113 strains of wild-type *Pseudomonas*. The genes coding for Phl were isolated from the F113 strain (Phl-producing *P. fluorescens* strain) and introduced into SBW25 *EeZY6KX* strain (non-Phl-producing *P. fluorescens* strain). The insertion of genes controlling Phl biosynthesis from F113 into the SBW25 chromosome resulted in the development of transformant producing phl (Pa21 strain) capable of suppressing *P. ultimum* via antibiotic synthesis (Bainton et al. 2004). Further, the strain developed is competent in biologically controlling the invading fungal pathogen *Pythium ultimum* via competitive exclusion due to its strong colonizing ability in the rhizosphere (Bainton et al. 2004). In this way, both antifungal Phl and competitive exclusion systems were integrated into one strain of *Pseudomonas*.

Growth of the transformed strains (Pa21) inoculated into pea plants was normal with no negative effect on plant development. When these genetically modified strains are used to inoculate pea roots, the density of the native microbial population inhabiting the rhizosphere was significantly decreased compared to wild-type that indicates their persistent colonizing nature (Bainton et al. 2004). So, the genetically manipulated Pa21 strains comprise properties required to furnish productive amalgamated biocontrol by containing genes for both Phl production and competitive exclusion. Nevertheless, these produced strains have less survival potential in comparison with their wild-type that can avoid the unnecessary spread of these organisms in rhizospheric soil. So, this feature makes them ecological and can be investigated further for possible exploitation in the future.

18.6.5 Improvement of *Rhizobium* Bacterial Strains through Genetic Manipulation to Enhance their Competency

Crop production of legumes (beans, clover, peas, etc.) can be improved by utilizing highly proficient bacteria effectual in fixing nitrogen present in the atmosphere during the time of seed inoculation. However, inoculation of leguminous plants is typically ineffective because of the existence of native microbes which are incompetent in nitrogen fixation and can be addressed with extrinsic or introduced strains to initiate root nodule formation. The capability to induce nodule formation is defined as competitiveness that is very important for the promising use of rhizobacteria as inoculants (Toro 1996). It is therefore desirable to alter the strain that is being used as an inoculant by genetic modification that facilitates sufficient

Table 18.3 Efficiency of genetically engineered *Rhizobium* strains in nodule tenancy in reference to wild-type organisms (Source: Harnessing the potential of genetically modified microorganisms and plants, European commission community research)

S.No.	*Rhizobium* strains	Nodule occupancy in co-inoculation (%)
1	2011	5
2	2011-GM	95
3	L5.30	22
4	L5.30-GM	78
5	GR0–13	7
6	GR0–13-GM	93
7	Rm41	13
8	Rm41-GM	87

number of root nodule formation to sustain increased nitrogen-fixing ability of host plants.

It has been established that the nodule-forming ability of different *Sinorhizobium meliloti* bacterial strains acquired from various geographical regions can be improved through the strategy of gene modification. Strains can be developed by enabling the expression of *nifA* gene, which plays a crucial role in controlling majority of genes (*nif* genes) involved in nitrogen fixation process. It was revealed that in an experimental analysis conducted using mixed inocula compared to wild-type, genetically altered strains of *S. meliloti* inhabited greater number of root nodules in alfalfa roots (Table 18.3). The genetic basis for this development is not precisely understood, but it has been hypothecated that gene *nifA* modulates the functioning of other pertinent genes apart of *nif* genes. It was believed that the altered expression of these genes would promote and encourage the development of root nodules (Walsh et al. 1999).

Along with gene expression, another essential factor important in nodule development process is the effective recognition of plant roots by *Rhizobium* bacterial strains. It was hypothesized that the use of microbial inoculants which are especially captivated by desired plant roots may allow more efficient inoculation, thus reducing the demand for bacterial strains as inocula (Walsh et al. 1999). The role of bacterial movement toward roots has been assessed using the competitive ability of genetically engineered *Rhizobium leguminosarum* bacterial strains containing a reporter gene, *ß-glucuronidase* (gusA gene), to enable their easy identification in root nodules. Based on the tests, the induction percentage of root nodules was proved to be greater in those bacterial strains labelled through *gusA* collated to immotile bacterial strains deficient in flagella. Compared with the flagella-deficient non-motile strain (a flagellum is a whip-like structure responsible for propelling the bacterium through water), gusA-labelled strain developed a high percentage of nodules (Walsh et al. 1999). In this way, it has been shown that functional flagellum is required in the formation of nodules for adequate competition.

Rhizobium's initial behavior against host plant roots is detection of substances secreted by plant roots. These secreted compounds are recognized by a particular type of proteins called methyl-accepting chemotaxis proteins (Mcp) located on the cell wall of *Rhizobium* bacterium and stimulate them to move towards host roots. Genes such as *Mcp* have been discovered in *R. leguminosarum* bacteria, and it was investigated that their protein products are involved in the detection of substances released by the plants (Walsh et al. 1999). This will ensure useful information about the course of root affinity permitting *Rhizobium* bacterial growth with increased nodulation competence and hastened host specificity.

18.6.6 Influence of Genetically Engineered *Rhizobium* Bacterial Strains on Arbuscular Mycorrhizal (AM) Fungi

As discussed above, AM fungi are a critical community of fungi which ascertain the synergistic mode of coalition with plants. Important part of an IMPACT project is to examine whether genetically designed strains of *Rhizobium* have an effect on the potential of mycorrhizal fungi in establishing beneficial symbiotic association with plant root system.

In a series of experiments carried out in the glass house and growth space, it was identified that the strain *Sinorhizobium meliloti*, produced by genetic modification with meliorated nodule developing potential, has not messed with any such prospect appertaining to mycorrhiza inception in characteristic arbuscular mycorrhizal fungal spp. (*Glomus mosseae*). Certainly, genetically engineered strains of *S. meliloti* elevated the number of AM fungi colonization units and capacity of accumulating nutrients compared to wild isolates. The development of symbiotic relationship also triggered changes in root morphology, particularly in the plants infected with genetically manipulated strains of *S. meliloti*, where the number of lateral roots and extent of branching were noticed to be greater (Walsh et al. 1999).

18.6.7 Exploitation of Genetically Modified *Rhizobium* for Field Experiments

Genetically modified *Rhizobium leguminosarum* bv. viciae strains with *HgCb* resistance (*mer* genes) and *lacZ* genes were developed by IMPACT association, and their impact on plants under field conditions was assessed. *R. leguminosarum* bv. viciae 1003 (wild-type) and its derivatives strain 1110, strain 1111, and strain 1112 were utilized for trials under field conditions. Strain 1110 comprising pDG3 plasmid that has resistant genes for HgCb or mer and *lacZ* genes who has the function that is regulated by lac-lacO system, next one is strain 1111, consisting of pDG4 plasmid where *lacZ* gene is constitutionally expounded in higher standards, continued by another strain 1112 having a set of genes ciphering mer with a controlled lacZ gene sequence incorporated within the part of chromosome.

These strains were identified by lacZ/mer reporter system and were screened using MPN (most probable number) method. Furthermore, the presence of microbes in soil has been measured by testing the levels of CO_2, which provide evidence for

Table 18.4 Microbial density of control and genetically manipulated strains of *R. leguminosarum* bv. viciae in rhizospheric soil after 10 days of seeding pea plants (Source: Harnessing the potential of genetically modified microorganisms and plants, European commission community research)

S.No.	Strain	*Rhizobium leguminosarum* bv. viciae		
		Total	Genetically modified	Revertants
1	1003	1.9×10^4	–	–
2	1110	3.4×10^5	3.4×10^5	$<10^2$
3	1111	1.3×10^5	9.9×10^4	2.6×10^4
4	1112	2.9×10^4	2.9×10^4	$<10^2$

metabolic activity in soil and the release of N_2O to assess the transformation of nitrogen (Walsh et al. 1999).

Existence of inoculant microbial strains within soil rhizosphere of pea plants had been detected after 10 days of sowing. It was noted that most of the strains were colonized to the same degree in the rhizosphere. When these bacterial strains were examined for stability assays, revertants from 1110 and 1112 (both strains are engineered to have regulated or controlled gene expression) were not detected by plate count, whereas substantial instability was identified in strain 1111 because of constitutive *lacZ* gene expression (Table 18.4).

In the case of uninoculated plants, the release of CO_2 in non-rhizosphere soil tends to be considerably lower than in rhizosphere soil. Non-rhizosphere soil had a decreased rate of respiration relative to inoculated plants in the rhizosphere portion of the field. In addition, the levels of CO_2 produced in soil inoculated with non-GM and GM strains are identical. These studies suggest that although plants' subsistence had an appreciable influence towards decomposition of carbon within the ground, influence of genetically altered microbial strains of *Rhizobium* is negligible as compared to wild-type strains. Concerning N_2O production, soil without plants had a substantially different N_2O emission system compared to soil with plants. However, the production of N_2O was not significantly distinguishable between uninoculated and inoculated plants or between unmodified and GM strains. These results are consistent with those of the production of CO_2 and imply that effect derived from the plant regarding the functionality of microorganisms is remarkably higher corresponding to that of genetically modified microbial inoculants when compared to wild strains (Walsh et al. 1999).

In this way, microorganisms perform a crucial job in controlling the active decomposition of organic constituents and accessibility of nourishing elements, i.e., N, P, and K, to the plants. It is well understood that microbial inoculants are one among the major constituents in amalgamated nutriment management system that precedes to sustainable method of agriculture. Furthermore, microbial inoculants can be utilized as a cost-effective strategy to intensify the crop productivity by harvesting more nutrients from soil with reduced dosage of agrochemicals.

18.7 Conclusion

Rapidly expanding worldwide human population demands more food production. The current strategy of conventional agricultural system uses huge quantities of agrochemicals as fertilizing substances and pesticides with a view to combat increasing food demand. But, it has a significant negative impact on the nature contributing to environmental pollution and global warming followed by degradation of natural ecosystems. The use of naturally occurring agriculturally important microorganisms as plant excrescence boosting microorganisms to elevate the crop productivity with nutrient-deficient ecosystems is an appealing, environmental-friendly, inexpensive, and safe alternate to the chemical pesticides and fertilizers. These beneficial microorganisms portray an indispensible part in integrated nutrimental coordination system and upgrade crop output by minimizing the effect from various forms of biotic and abiotic stress conditions. Such accents serve as primary obstacles to crop nobility, food hygiene, and overall food sufficiency. One of such important alternatives to those constraints is the development of viable microbial tools and methods which can encourage effective interaction between plants and microorganisms. Symbiotic and non-symbiotic microbial associations have several advantageous mechanisms. A wide number of microorganisms are identified to associate with plants through various plant-microbe interactions such as PGPR, arbuscular mycorrhiza-plant mutual relationship, bacteria fixing atmospheric nitrogen, bacteria producing siderophores, and fungi. These microbial interactions have multifold useful properties like fixation of atmospheric nitrogen, phosphate mobilization, acting like biological control agents, and secreting plant surge stimulating essentialities. Because of these beneficial characteristic features, improvement of microbial strains can be a novel and constructive strategy for sustainable development. The numbers of naturally occurring and established microbes are limited in soil. This problem can be resolved by creating pilot−/ large-scale microbial inoculum and microbial consortia for inoculum development to balance the nutrient level of soil. Moreover, the established transgenic technology that has emerged recently also encourages the development of productive genetically engineered strains of microorganisms to act as effective biofertilizers and biopesticides. Despite that, a systematic approach based on omics strategy is expected to recognize and develop innovative microbial strains and interpret their mechanism of action. We have already entered a critical stage where novel microorganisms generated under stressful environmental conditions need to be extracted and established. Based on the previous investigations, it was clear that advances in microbial development to date have displayed propitious imminence for revivification of nutritional constituents and the management of soil-borne diseases. But, an additional comprehensive analysis by exploiting metabolomics-derived methodology could assist to uncover concealed keys for agricultural benefits that portray pivotal function in plant microbial reciprocal relationship.

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References

Abaid-Ullah M, Hassan MN, Jamil M, Brader G, Shah MK, Sessitsch A, Hafeez FY (2015) Plant growth promoting rhizobacteria: an alternate way to improve yield and quality of wheat (*Triticum aestivum*). Int J Agri Biol 17:51–60

Adesemoye A, Torbert H, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929. https://doi.org/10.1007/s00248-009-9531-y

Ahanger MA, Hashem A, Abd Allah EF, Ahmad P (2014) Arbuscular mycorrhiza in crop improvement under environmental stress. In: Ahmad P, Rasool S. (Eds.). Emerging Technologies and Management of Crop Stress Tolerance 2:69–95

Ahmad M, Zahir ZA, Asghar HN, Asghar M (2011) Inducing salt tolerance in mung bean through co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase. Can J Microbiol 57:578–589

Ahmad P, Hashem A, Abd-Allah EF, Alqarawi AA, John R, Egamberdieva D (2015) Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. Front Plant Sci 6:868. https://doi.org/10.3389/fpls.2015.00868

Ahmad M, Nadeem SM, Naveed M, Zahir ZA (2016) Potassium-solubilizing Bacteria and their application in agriculture. In: Meena V, Maurya B, Verma J, Meena R. (eds) Potassium Solubilizing Microorganisms for Sustainable Agriculture. Springer, New Delhi. doi:https://doi.org/10.1007/978-81-322-2776-2_21

Ahmad M, Pataczek L, Hilger TH (2018) Perspectives of microbial inoculation for sustainable development and environmental management. Front Microbiol 9:2992. https://doi.org/10.3389/fmicb.2018.02992

Alabouvette C (1999) Fusarium wilt suppressive soils: an example of disease-suppressive soils. Australas Plant Pathol 28:57–64. https://doi.org/10.1071/AP99008

Alabouvette C, Rouxel F, Louvet J (1979) Characteristics of fusarium-wilt suppressive soils and prospects for their utilization in biological control. In: Schippers B, Gams W: & dquo; soil borne plant pathogens & dquo;. Academic press, 165-182; 686 p

Alami Y, Achouak W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing rhizobium sp. strain isolated from sunflower roots. Appl Environ Microbiol 66(8):3393–3398. https://doi.org/10.1128/aem.66.8.3393-3398

Ambethgar V (2009) Potential of entomopathogenic fungi in insecticide resistance management (IRM): a review. J Biopest 2(2):177–193

Appl M (1982) The Haber–Bosch process and the development of chemical engineering. A Century of Chemical Engineering New York: Plenum Press pp 29–54. ISBN 978-0-306-40895-3

Appl M (2006) Ammonia. Ullmann's Encyclopedia of Industrial ChemistryWeinheim: Wiley-VCH. https://doi.org/10.1002/14356007.a02_143.pub2

Arshad M, Shaharoona B, Mahmood T (2008) Inoculation with *Pseudomonas* spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.). Pedosphere 18(5):611–620

Asch F, Padham JL (2005) Root associated bacterial suppress symptoms of iron toxicity in lowland rice. In: Tielkes E, Hulsebusch C, Hauser I, Deininger A, Becker K (eds) The global food and product chain-dynamics, innovations, conflicts, strategies. MDD GmbH Stuttgart, p 276

Avis TJ, Gravel V, Antoun H, Tweddell RJ (2008) Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biol Biochem 40:1733–1740

Babu AG, Kim JD, Oh BT (2013) Enhancement of heavy metal phytoremediation by Alnus firma with endophytic Bacillus thuringiensis GDB-1. J Hazard Mater 250-251:477–483. https://doi.org/10.1016/j.jhazmat.2013.02.014

Bainton N, Lynch J, Naseby D, Way J (2004) Survival and ecological fitness of Pseudomonas fluorescens genetically engineered with dual biocontrol mechanisms. Microb Ecol 48 (3):349–357. http://www.jstor.org/stable/25153117

Barka EA, Nowak J, Clément C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, Burkholderia phytofirmans strain PsJN. Appl Environ Microbiol 72(11):7246–7252. https://doi.org/10.1128/AEM.01047-06

Barney B, Knutson CM, Plunkett M (2016) Biofertilizer from genetically-modified *Azotobacter vinelandii*, US patent US9796957B2, assigned to regents of the University of Minnesota

Bashan Y and de-Bashan LE (2010) How the plant growth-promoting bacterium *Azospirillum* promotes plant growth-a critical assessment. Adv Agron, 108, 77–136, doi:https://doi.org/10.1016/S0065-2113(10)08002-8

Benhamou N, Garand C, Goulet A (2002) Ability of nonpathogenic Fusarium oxysporum strain Fo47 to induce resistance against Pythium ultimum infection in cucumber. Appl Environ Microbiol 68(8):4044–4060. https://doi.org/10.1128/aem.68.8.4044-4060.2002

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Berman-Frank I, Lundgren P, Falkowski PG (2003) Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. Res Microbiol 154:157–164

Bhattacharyya P, Jha D (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Bhogal A, Nicholson F, Rollett A, Taylor M, Litterick A, Whittingham MJ, Williams JR (2018) Improvements in the quality of agricultural soils following organic material additions depend on both the quantity and quality of the materials applied. Front Sustain Food Syst 2:9

Bidyarani N, Prasanna R, Babu S, Hossain F, Saxena AK (2016) Enhancement of plant growth and yields in chickpea (Cicer arietinum L.) through novel cyanobacterial and biofilmed inoculants. Microbiol Res 188-189:97–105

Biswas DR, Narayanasamy G (2006) Rock phosphate enriched compost: an approach to improve low-grade Indian rock phosphate. Bioresour Technol 97(18):2243–2251. https://doi.org/10.1016/j.biortech.2006.02.004

Bruinsma (2017) World agriculture: towards 2015/2030: an FAO study. Routledge, Abingdon

Carvalho TLG, Balesmao-Pires E, Saraiva RM, Ferreira PCG, Hemerly AS (2014) Nitrogen signaling in plant interactions with associative and endophytic diazotrophic bacteria. J Exp Bot 65:5631–5642

Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP (2011) The development, regulation and use of biopesticides for integrated pest management. Philosophical Transactions of the Royal Society B: Biological Sciences 366:1987–1998

Chen L, Luo S, Li X, Wan Y, Chen J, Liu C (2014) Interaction of cd hyperaccumulator Solanum nigrum L. and functional endophyte Pseudomonas sp. Lk9 on soil heavy metals uptake. Soil biol. Biochemist 68:300–308. https://doi.org/10.1016/j.soilbio.2013.10.021

Cheng Z, Woody OZ, McConkey BJ, Glick BR (2012) Combined effects of the plant growth-promoting bacterium *Pseudomonas putida* UW4 and salinity stress on the *Brassica napus* proteome. Appl Soil Ecol 61:255–263. https://doi.org/10.1016/j.apsoil.2011.10.006

Cho K, Toler H, Lee J, Ownley B, Stutz JC, Moore JL, Auge RM (2006) Mycorrhizal symbiosis and response of sorghum plants to combined drought and salinity stresses. J Plant Physiol 163:517–528

Cho SM, Kang BR, Han SH, Anderson AJ, Park JY, Lee YH (2008) 2R, 3Rbutanediol, a bacterial volatile produced by Pseudomonas chlororaphis O6, is involved in induction of systemic tolerance to drought in Arabidopsis thaliana. Mol Plant-Microbe Interact 21:1067–1075. https://doi.org/10.1094/mpmi-21-8-1067

Choudhary DK, Kasotia A, Jain S, Vaishnav A, Kumari S, Sharma KP, Varma A (2016) Bacterial-mediated tolerance and resistance to plants under abiotic and biotic stresses. J Plant Growth Regul 35:276–300. https://doi.org/10.1007/s00344-015-9521-x

Couillerot O, Prigent-Combaret C, Caballero-Mellado J, Moënne-Loccoz Y (2009) Pseudomonas fluorescens and closely-related fluorescent pseudomonads as biocontrol agents of soil-borne phytopathogens. Lett Appl Microbiol 48(5):505–512. https://doi.org/10.1111/j.1472-765X.2009.02566.x

Creus CM, Sueldo RJ, Barassi CA (2004) Water relations and yield in Azospirillum-inoculated wheat exposed to drought in the field. Can J Bot 82:273–281

Datta A, Shrestha S, Ferdous Z, Win CC (2015) Strategies for enhancing phosphorus efficiency in crop production systems. In: Rakshit A, Singh HB, Sen A (eds) Nutrient use efficiency: from basics to advances. Springer, New Delhi, India

Dodd IC, Belimov AA, Sobeih WY, Safronova VI, Grierson D, Davies WJ (2005) Will modifying plant ethylene status improve plant productivity in water-limited environments? 4th international crop science congress

Egamberdiyeva D (2007) The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl Soil Ecol 36(2):184–189. https://doi.org/10.1016/j.apsoil.2007.02.005

Fasciglione G, Casanovas EM, Quillehauquy V, Yommi AK, Goñi MG, Roura SI, Barassi CA (2015) Azospirillum inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. Sci Hortic 195:154–162

Ferron P (1971) Modification of the development of *Beauveria tenella* mycosis in *Melolontha melolontha* larvae, by means of reduced doses of organophosphorus insecticides. Entomol gia Experimetalis et Applicata 14:457–466

Figueiredo MVB, Martinez CR, Burity HA, Chanway CP (2008) Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). World J Microbiol Biotechnol 24:1187–1193. https://doi.org/10.1007/s11274-007-9591-4

Franche C, Lindstrom K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39. https://doi.org/10.1016/j.micres.2013.09.009

Gopalakrishnan S, Sathya A, Vijayabharathi R, Srinivas V (2016) Formulations of plant growth-promoting microbes for field applications. In: Microbial inoculants in sustainable agricultural productivity. Springer, New Delhi, pp 239–251. isbn:978-81-322-2642-0

Harrier LA, Watson CA (2004) The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. Pest Manag Sci 60(2):149–157. https://doi.org/10.1002/ps.820

Hontzeas N, Richardson A, Belimov AA, Safranova VI, Abu-Omar MM, Glick BR (2005) Evidence for horizontal gene transfer (HGT) of ACC deaminase genes. Appl Environ Microbiol 71:7556–7558

Imran (2017) Climate change is a real fact confronting to agricultural productivity. Int J Environ Sci Nat Res 3(3):555613. https://doi.org/10.19080/IJESNR.2017.03.555613

Imran AK, Inamullah AF (2016) Yield and yield attributes of Mungbean (*Vigna radiate* L.) cultivars as affected by phosphorous levels under different tillage systems. Cogent Food Agric 2:1151982

Imran AAK, Khan IU, Shahida N (2016) Weeds density and late sown maize productivity influenced by compost application and seed rates under temperate environment. Pak J Weed Sci Res 22(1):169–181

Insecticide Act (1986). http://cibrc.nic.in

Islam F, Yasmeen T, Ali Q, Ali S, Arif MS, Hussain S, Rizvi H (2014) Influence of Pseudomonas aeruginosa as PGPR on oxidative stress tolerance in wheat under Zn stress. Ecotoxicol Environ Saf 104:285–293. https://doi.org/10.1016/j.ecoenv.2014.03.008

Jha Y, Subramanian RB (2014) PGPR regulate caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. Physiol Mol Biol Plants 20(2):201–207. https://doi.org/10.1007/s12298-014-0224-8

Jing YX, Yan JL, He HD, Yang DJ, Xiao L, Zhong T, Yuan M, Cai XD, Li SB (2014) Characterization of bacteria in the rhizosphere soils of Polygonum pubescens and their potential in promoting growth and cd, Pb, Zn uptake by Brassica napus. Int J Phytoremediation 16 (4):321–333

Jnawali AD, Ojha RB, Marahatta S (2015) Role of *Azotobacter* in soil fertility and sustainability – a review. Adv Plants Agric Res 2:1–5

Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC (2007) How symbionts invade plants: the Sinorhizobium-Medicago model. Nat Rev Microbiol 5:619–633

Keswani C, Mishra S, Sarma BK, Singh SP, Singh HB (2014) Unraveling the efficient applications of secondary metabolites of various Trichoderma spp. Appl Microbiol Biotechnol 98 (2):533–544. https://doi.org/10.1007/s00253-013-5344-5

Key S, Ma JK, Drake PM (2008) Genetically modified plants and human health. J R Soc Med 101 (6):290–298. https://doi.org/10.1258/jrsm.2008.070372

Khan MS, Zaidi A, Ahmad E (2014) Mechanism of phosphate Solubilization and physiological functions of phosphate-solubilizing microorganisms in: phosphate solubilizing microbes for crop improvement. Springer International Publishing, Berlin, pp 31–62

Khan A, Singh P, Srivastava A (2018) Synthesis, nature and utility of universal iron chelator-siderophore: a review. Microbiol Res 212–213:103–111

Kohler J, Hernández JA, Caravaca F, Roldán A (2008) Plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water-stressed plants. Funct Plant Biol 35(2):141–151

Kumar VV (2018) Biofertilizers and biopesticides in sustainable agriculture. In: role of rhizospheric microbes in soil. Springer, Singapore

Kumari S, Vaishnav A, Jain S, Varma A, Choudary DK (2015) Bacterial-mediated induction of systemic tolerance to salinity with expression of stress alleviating enzymes in soybean (*Glycine max* L. Merrill). J Plant Growth Regul 34:558–573. https://doi.org/10.1007/s00344-015-9490-0

Li Z, Alves SB, Roberts DW, Fan M, Delalibera I, Tang J, Lopes RB, Faria M, Rangel DEN (2010) Biological control of insects in Brazil and China: history, current programs and reasons for their successes using entomopathogenic fungi. Biocontrol Sci Tech 20(2):117–136

Li RX, Cai F, Pang G, Shen QR, Li R, Chen W (2015) Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. PLoS One 10(6):e0130081. https://doi.org/10.1371/journal.pone.0130081

Lim JH, Kim SD (2013) Induction of drought stress resistance by multi-functional PGPR Bacillus licheniformis K11 in pepper. Plant Pathol J 29(2):201–208. https://doi.org/10.5423/PPJ.SI.02.2013.0021

Loaces I, Ferrando L, Fernández Scavino A (2011) Dynamics, diversity and function of endophytic Siderophore-producing Bacteria in Rice. Microb Ecol 61:606–618. https://doi.org/10.1007/s00248-010-9780-9

Lone R, Shuab R, Khan S, Ahmad J, Koul KK (2017) Arbuscular mycorrhizal fungi for sustainable agriculture. In: Kumar V, Kumar M, Sharma S, Prasad R (eds) Probiotics and Plant Health. Springer, Singapore. doi:https://doi.org/10.1007/978-981-10-3473-2_25

Martinez-Viveros O, Jorquera M, Crowley DE, Gajardo G, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10(3):293–319

Mathew DC, Ho YN, Gicana RG, Mathew GM, Chien MC, Huang CC (2015) A rhizosphere-associated symbiont, Photobacterium spp. strain MELD1, and its targeted synergistic activity for phytoprotection against mercury. PLoS One 10(3):e0121178. doi:https://doi.org/10.1371/journal.pone.0121178

McLellan CA, Turbyville TJ, Wijeratne EM, Kerschen A, Vierling E, Queitsch C, Whitesell L, Gunatilaka AA (2007) A rhizosphere fungus enhances Arabidopsis thermotolerance through production of an HSP90 inhibitor. Plant Physiol 145(1):174–182. https://doi.org/10.1104/pp.107.101808

Meena KK, Kumar M, Kalyuzhnaya MG, Yandigeri MS, Singh DP, Saxena AK (2012) Epiphytic pink-pigmented methylotrophic bacteria enhance germination and seedling growth of wheat (Triticum aestivum) by producing phytohormone. Antonie Van Leeuwenhoek 101(4):777–786. https://doi.org/10.1007/s10482-011-9692-9

Moscardi F (1999) Assessment of the application of baculoviruses for control of Lepidoptera. Annu Rev Entomol 44:257–289. https://doi.org/10.1146/annurev.ento.44.1.257

Nadeem SM, Zahir ZA, Naveed M, Arshad M (2009) Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt affected fields. Can J Microbiol 55(11):1302–1309. https://doi.org/10.1139/w09-092

Nadeem SM, Ahmad M, Zahir ZA, Javaid A, Ashraf M (2014) The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol Adv 32(2):429–448

Nautiyal CS, Bhadauria S, Kumar P, Lal H, Mondal R, Verma D (2000) Stress induced phosphate solubilization in bacteria isolated from alkaline soils. FEMS Microbiol Lett 182(2):291–296. https://doi.org/10.1111/j.1574-6968.2000.tb08910.x

Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A (2014) Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp FD17. Environ Exp Bot 97:30–39. https://doi.org/10.1016/j.envexpbot.2013.09.014

Nia SH, Zarea MJ, Rejali F, Varma A (2012) Yield and yield components of wheat as affected by salinity and inoculation with Azospirillum strains from saline or non-saline soil. J Saudi Soc Agric Sci 11:113–121

Omar MNA, Osman MEH, Kasim WA and Abd El-Daim IA (2009) Improvement of salt tolerance mechanisms of barley cultivated under salt stress using Azospirillum brasilense. In: Ashraf M, Ozturk M, Athar H (eds) Salinity and water stress. Tasks for vegetation sciences, vol 44. Springer, Dordrecht. doi:https://doi.org/10.1007/978-1-4020-9065-3_15

Ortas I, Rafique M, Ahmed IAM (2017) Application of arbuscular mycorrhizal fungi into agriculture. In: Wu QS (ed) Arbuscular mycorrhizas and stress tolerance of plants. Springer Nature Singapore Pte Ltd., Singapore, pp 305–327

Panlada T, Piromyou P, Longtonglang A, Noisa-Ngiam R, Boonkerd N, Teaumroong N (2013) Alleviation of the effect of environmental stresses using co-inoculation of mungbean by *Bradyrhizobium* and rhizobacteria containing stress-induced ACC deaminase enzyme. Soil Sci Plant Nutr 59(4):559–571. https://doi.org/10.1080/00380768.2013.804391

Perret X, Staehelin C, Broughton WJ (2000) Molecular basis of symbiotic promiscuity. Microbiol Mol Biol Rev 64:180–201

Porcel R, Aroca R, Ruiz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi. A review Agronomy for Sustainable Development 32:181–200

Raaijmakers JM, Mazzola M (2012) Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annu Rev Phytopathol 50:403–424. https://doi.org/10.1146/annurev-phyto-081211-172908

Ram RM, Singh HB (2018) *Trichoderma spp*: Nature's gift to mankind. In Chaurasiya HK, Mishra DP (eds) plant systematics and biotechnology: challenges and opportunities. Today's and Tomorrow's printers and publishers, New Delhi, pp. 133–141

Ram RM, Keswani C, Mishra S, Tripathi R, Ray S, Singh SP, Singh HB (2016) Trichoderma secondary metabolites: applications and future prospects. In: Vaish SS (ed) Plant diseases and their sustainable management. Biotech books, New Delhi, pp 113–127

Reddy C, Saravanan RS (2013) Polymicrobial multi-functional approach for enhancement of crop productivity. Adv Appl Microbiol 82: 53-113

Rillig MC, Sosa-Hernández MA, Roy J, Aguilar-Trigueros CA, Vályi K, Lehmann A (2016) Towards an integrated mycorrhizal technology: harnessing mycorrhiza for sustainable intensification in agriculture. Front Plant Sci 7:1625. https://doi.org/10.3389/fpls.2016.01625

Rodriguez A, Sanders IR (2015) The role of community and population ecology in applying mycorrhizal fungi for improved food security. ISME J 9(5):1053

Sahoo RK, Ansari MW, Pradhan M, Dangar TK, Mohanty S, Tuteja N (2014a) Phenotypic and molecular characterization of native Azospirillum strains from rice fields to improve crop productivity. Protoplasma 251(4):943–953. https://doi.org/10.1007/s00709-013-0607-7

Sahoo RK, Ansari MW, Pradhan M, Dangar TK, Mohanty S, Tuteja N (2014b) A novel Azotobacter vinelandii (SRIAz3) functions in salinity stress tolerance in rice. Plant Signal Behav 9(7): e29377. https://doi.org/10.4161/psb.29377

Sanchis V, Bourguet D (2008) *Bacillus thuringiensis*: applications in agriculture and insect resistance management. A review. Agron Sustain Dev 28:11–20

Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. Ann Bot 111(5):743–767. https://doi.org/10.1093/aob/mct048

de Santiago A, Quintero JM, Avilés M, Delgado A (2011) Effect of *Trichoderma asperellum* strain T34 on iron, copper, manganese, and zinc uptake by wheat grown on a calcareous medium. Plant Soil 342:97–104. https://doi.org/10.1007/s11104-010-0670-1

Scholte EJ, Takken W, Knols BG (2007) Infection of adult Aedes aegypti and ae. Albopictus mosquitoes with the entomopathogenic fungus Metarhizium anisopliae. Acta Trop 102 (3):151–158. https://doi.org/10.1016/j.actatropica.2007.04.011

Schwarzott D, Walker C, Schüssler A (2001) Glomus, the largest genus of the arbuscular mycorrhizal fungi (Glomales), is nonmonophyletic. Mol Phylogenet Evol 21(2):190–197. https://doi.org/10.1006/mpev.2001.1007

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2(1):587

Shelake RM, Waghunde RR, Verma PP, Singh C, Kim JY (2019) Carbon sequestration for soil fertility management: microbiological perspective. In: Soil fertility management for sustainable development. Springer, Singapore, pp 25–42

Shukla N, Awasthi RP, Rawat L, Kumar J (2012a) Biochemical and physiological responses of rice (Oryza sativa L.) as influenced by Trichoderma harzianum under drought stress. Plant Physiol Biochem 54:78–88. https://doi.org/10.1016/j.plaphy.2012.02.001

Shukla PS, Agarwal PK, Jha B (2012b) Improved salinity tolerance of Arachis hypogaea (L.) by the interaction of halotolerant plant-growth-promoting rhizobacteria. J. Plant Growth Regul 31:95–206

Singh HB (2014) Management of plant pathogens with microorganisms. Proc Natl Acad Sci 80:443–454

Singh G, Biswas DR and Marwaha TS (2010) Mobilization of potassium from waste mica by plant growth promoting rhizobacteria and its assimilation by maize (zea mays) and wheat (triticum aestivum l.): a hydroponics study under phytotron growth chamber. J Plant Nutr 33:1236–1251. doi:10.1080/01904161003765760

Singh DP, Prabha R, Yandigeri MS and Arora DK (2011) Cyanobacteria mediated phenyl propanoids and phytohormones in rice (*Oryza sativa*) enhance plant growth and stress tolerance Antoon Leeuw 100: 557-568

Singh HB, Singh BN, Singh SP, Sarma BK (2012) Exploring different avenues of Trichoderma as potent biofungicidal and plant growth promoting candidate—an overview. Rev plant Pathol 109:315–426

Sorty AM, Meena KK, Choudhary K, Bitla UM, Minhas PS, Krishnani KK (2016) Effect of plant growth promoting Bacteria associated with halophytic weed (Psoralea corylifolia L) on germination and seedling growth of wheat under saline conditions. Appl Biochem Biotechnol 180 (5):872–882. https://doi.org/10.1007/s12010-016-2139-z

Souza RD, Ambrosini A, Passaglia LM (2015) Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38(4):401–419. https://doi.org/10.1590/S1415-475738420150053

Srivastava PC, Rawat D, Pachauri SP, Shrivastava M (2015) Strategies for enhancing zinc efficiency in crop plants. In: Rakshit A, Singh HB, Sen A (eds) Nutrient use efficiency: from basics to advances. Springer, New Delhi, pp 87–10

Steenhoudt O, Vanderleyden J (2000) Azospirillum, a free living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24:487–506

Swaine EK, Swaine MD, Killham K (2007) Effects of drought on isolates of *Bradyrhizobium elkanii* cultured from *Albizia adianthifolia* seedlings of different provenances. Agrofor Syst 69:135–145. https://doi.org/10.1007/s10457-006-9025-6

Terre S, Asch F, Padham J, Sikora RA, Becker M (2007) Influence of root zone bacteria on root iron plaque formation in rice subjected iron toxicity. In: Tielkes E (ed) utilization of diversity in land use systems: sustainable and organic approaches to meet human needs. Tropentag, Witzenhausen, p 446

Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Kännaste A, Behers L, Nevo E, Seisenbaeva G, Stenström E, Niinemets Ü (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PloSone 9(5):e96086. https://doi.org/10.1371/journal.pone.0096086

Tiwari S, Singh P, Tiwari R, Meena KK, Yanadegeri M, Singh DP (2011) Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*Triticum aestivum*) and chemical diversity in rhizosphere enhance plant growth. Biol Fertil Soils 47:907. https://doi.org/10.1007/s00374-011-0598-5

Toro A (1996) Nodulation competitiveness in the rhizobium-legume symbiosis. World J Microbiol Biotechnol 12(2):157–162. https://doi.org/10.1007/BF00364680

Trivedi P, Schenk PM, Wallenstein MD, Singh BK (2017) Tiny microbes, big yields: enhancing food crop production with biological solutions. Microb Biotechnol 10(5):999–1003. https://doi.org/10.1111/1751-7915.12804

Vaid SK, Kumar B, Sharma A, Shukla AK, Srivastava PC (2014) Effect of zinc solubilizing bacteria on growth promotion and zinc nutrition of rice. J Soil Sci Plant Nutr 14:889–910

Vaishnav A, Kumari S, Jain S, Varma A, Choudhary DK (2015) Putative bacterial volatile-mediated growth in soybean (Glycine max L. Merrill) and expression of induced proteins under salt stress. J Appl Microbiol 119(2):539–551. https://doi.org/10.1111/jam.12866

Vaishnav A, Kumari S, Jain S, Varma A, Tuteja N, Choudhary DK (2016a) PGPR-mediated expression of salt tolerance gene in soybean through volatiles under sodium nitroprusside. J Basic Microbiol 56:1274–1288. https://doi.org/10.1002/jobm.201600188

Vaishnav A, Varma A, Tuteja N and Choudhary DK (2016b) PGPR mediated amelioration of crops under salt stress. In: Choudhary D, Varma A, Tuteja N (eds) Plant-microbe interaction: an approach to sustainable agriculture. Springer, Singapore

Vaishnav A, Hansen AP, Agarwal PK, Verma A and Chowdary DK (2017a) A biotechnological perspectives of legume-rhizobium symbiosis. In rhizobium biology and biotechnology, soil biology, Vol. 50, springer, Cham

Vaishnav A, Varma A, Tuteja N, Choudhary DK (2017b) Characterization of bacterial volatiles and their impact on plant health under abiotic stress. In: Choudhary DK et al (eds) Volatiles and food security. Springer, Singapore

Vaishnav A, Sharma SK, Choudhary DK, Sharma KP, Ahmad E, Sharma MP, Ramesh A, Saxena AK (2018) Nitric oxide as a signaling molecule in plant-bacterial interactions. In: Egamberdieva D, Ahmad P (eds) Plant microbiome: stress response, Microorganisms for sustainability. Springer Nature, Singapore, pp 183–199

Van Dommelen A, Croonenborghs A, Spaepen S, Vanderleyden J (2009) Wheat growth promotion through inoculation with an ammonium-excreting mutant of *Azospirillum brasilense*. Biol Fertil Soils 45:549–553. https://doi.org/10.1007/s00374-009-0357-z

Vassilev N, Vassilev M, Nikolaeva I (2006) Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl Microbiol Biotechnol 71(2):137–144. https://doi.org/10.1007/s00253-006-0380-z

Waghunde RR, Shelake RM, Sabalpara AN (2016) Trichoderma: a significant fungus for agriculture and environment. Afr J Agric Res 11:1952–1965

Walsh U, O'Gara F, Economidis I and Hogan S (1999) Harnessing the potential of genetically modified microorganisms and plants, ISBN 92-894-0295-4

Wang CJ, Yang W, Wang C, Gu C, Niu DD, Liu HX, Wang HP, Gua HP (2012) Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. PLoS One 7(12):e52565. https://doi.org/10.1371/journal.pone.0052565

Wang H, Li H, Zhang M, Song Y, Huang J, Huang H, Shao M, Liu Y, Kang Z (2018) Carbon dots enhance the nitrogen fixation activity of *Azotobacter chroococcum* ACS Appl mater. Interfaces 10:16308–16314

Winkelmann G (2007) Ecology of siderophores with special reference to the fungi. Biometals 20:379. https://doi.org/10.1007/s10534-006-9076-1

Yakhin OI, Lubyanov AA, Yakhinn IA, Brown PH (2017) Biostimulants in plant science: a global perspective. Front Plant Sci 7:2049. https://doi.org/10.3389/fpls.2016.02049

Zahir ZA, Ghani U, Naveed M, Nadeem SM and Asghar HN (2009) Comparative effectiveness of Pseudomonas and Serratia sp. containing ACC-deaminase for improving growth and yield of wheat (Triticum aestivum L.) under salt-stressed conditions. Arch. Microbiol.,191(5): 415-424

Zhang F, Huo Y, Cobb AB, Luo G, Zhou J, Yang G, Wilson GWT, Zhang Y (2018) *Trichoderma* biofertilizer links to altered soil chemistry, altered microbial communities, and improved grassland biomass. Front Microbiol 9:848. https://doi.org/10.3389/fmicb.2018.oo848

Application of Microbes in Bioremediation of Pesticides

19

Naveen Patel, Vinod Kumar Chaudhary, Akansha Patel, Anurag Singh, Arun Lal Srivastav, and Dhananjai Rai

Abstract

With rapid increase in population around the globe, demand for agricultural food products had been increased at much faster rate. In order to enhance the yield of the crop various types of pesticides had been started to be applied in the agricultural fields. Most of the pesticides are known for their detrimental impact on environment and human health and moreover these are highly persistent in nature. Hence it became highly important to remove these contaminants from the environment. Initially, treatment of pesticides was totally done by landfill and incineration, but these methods lead to development of secondary pollutants in the environment. In recent decades, bioremediation had gained immense importance because of its efficiency and ability to degrade pollutants into non-toxic substances. Wide range of microbes can be employed in the process of

N. Patel (✉)
Department of Civil Engineering, IET, Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India

V. K. Chaudhary · A. Patel
Department of Environmental Sciences, Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India

A. Singh
Department of Mechanical Engineering, IET, Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India

A. L. Srivastav
Chitkara University School of Engineering and Technology, Chitkara University, Chandigarh, Himachal Pradesh, India

D. Rai
Department of Civil Engineering, BIET, Jhansi, Uttar Pradesh, India

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bioremediation and hence proving one of the best methods for the removal of pesticides from water.

Keywords

Microbes · Bioremediation · Pesticides · Algae · Bacterial · Fungi

19.1 Introduction

Recently, it has been noticed that substantial improvements have been made in the agro-industries to obtain better productivity, yield and quality of the crops (Fahad et al. 2017). There has been an increase in the utilisation of chemically synthesised products in agricultural practices including herbicides, insecticides, and fungicides to prevent the loss of the crop from the insects, nematodes, weeds, rats, and other diseases related to plant (Mattah et al. 2015; Patel et al. 2020a). A huge quantity of pesticide application has been increased globally for agricultural and household activities (Grube et al. 2011; Spina et al. 2018; Srivastav 2020). Pesticides, although being one of the most toxic compounds, are considered to be one of the most effective means to prevent and protect the crops (Fenner et al. 2013; Verma et al. 2014;Özkara et al. 2016). These are creating a lot of pressure on the environment by posing a serious threat to natural resources and poisoning to plants and animals. Pesticides affect humans indirectly through the food chain, because of their complexity in chemical structure and persistency in the environment (Sun et al. 2018; Balomajumder 2019). These pesticide particles reach the food chain mainly due to bio-magnification (Kolpin et al. 1998; Lefrancq et al. 2013). People around the world had been exposed to various categories of pesticides including organophosphates, carbamates, organochlorine, dinitrophenols, organosulfur, thiocarbamates, and triazines (Anjum et al. 2017). Carbamates, because of low bioaccumulative and non-persistence in nature, are one of the most widely used pesticides in various fields, i.e. agriculture, forestry, gardening, and in therapeutic pharmaceuticals too (Ecobichon 2001; Dias et al. 2015). But, carbamate pesticides had been reported for their life-threatening effects on plants, aquatic organisms, and other non-target animals (Gupta 2006; Dias et al. 2015). Moreover, these chemical materials (pesticides and fertilisers) with each passing year are also posing serious quality issues on soil health and water (both surface and groundwater) too. Through the reports, it had been found that about 98% of the pesticides that are being utilised are having a detrimental effect on fishes and crustaceans (Klemick and Lichtenberg 2008; Lushchak et al. 2018), while the presence of phosphorus in fertilisers in huge quantity may lead to eutrophication (Carpenter 2008). These chemical materials are needed to removed or treated, in order to minimise the hazardous effects of these on various living organisms, after its application from the environment. The water flowing out from the agricultural fields after rainfall contains enormous amount of chemical contaminants. In order to treat these chemically contaminated water from water resources various physicochemical techniques have been utilised (Li and Yang

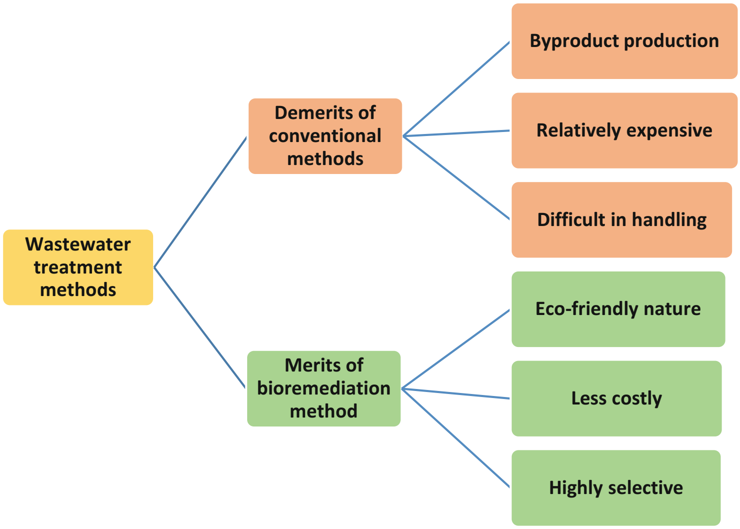

Fig. 19.1 A comparison of merits and demerits of traditional methods of treatment and bioremediation

2018; Mandal et al. 2014). These techniques have some demerits such as generation of secondary pollutants, costly in nature, and complexity. Biological treatment methods have gained enormous importance as compared to various other conventional treatment technologies mainly because of their environmentally friendly nature, cost-effectiveness, and higher selectivity (Aresta et al. 2015; Saez et al. 2015; Gupta et al. 2016; Rizwan et al. 2018; Patel et al. 2019a). A comparative representation of the merits and demerits is given in Fig. 19.1.

'Bioremediation' is breakdown or removal of recalcitrant pollutants such as pesticides, phenols present in the environment by using microorganisms (Hussaini et al. 2013; Ojuederie and Babalola 2017; Huang et al. 2018; Gupta et al. 2019). Broad range of microorganisms had been found to have the potential to eliminate these toxic pollutants (Gupta et al. 2017; Rathour et al. 2018). But, biological degradation of these pesticides had certain limitations including complex pesticide structure, fragile conditions related to environment, and screening of potent microorganisms (Vikrant et al. 2018; Liu et al. 2019). In recent decades, wide range of biological approaches had been used for remediation of pollutants but still enormous work is needed to be done for its utilisation on wide scale. This chapter deals with the bioremediation of pesticides from the environment.

19.2 Microorganisms Utilised for Removal and Degradation of Pesticides

Biological treatment, because of its nature friendly technology and cost-effectiveness, has been used for the treatment of various different types of pollutants or contaminants present in the environment (Gupta et al. 2016). Still there is need of more research to be done in the areas of bioremediation as it is still facing a lot number of challenges in the real field of application. Degradation of pesticides with the help of microbes is a natural phenomenon generally performed by wide range of indigenous species and hence helping in maintain the crops and fields ecology (Geed et al. 2018). However, pesticide because of its recalcitrant nature and chemical structure requires longer time for its natural degradation and hence remains persistence in soil for longer duration (Huang et al. 2018; Pérez-Lucas et al. 2018). Table 19.1 shows various types of pesticides. In order to improve the present condition, there is a need of isolation and development of potent microbial strains with the help of modern biotechnological methods (Ahmad et al. 2018). For removal of organic or inorganic pollutants, microbes such as fungi, bacteria, or algae can be employed (Megharaj and Naidu 2017). These are the microbial species utilised for treating various toxic contaminants that are either in its original or genetically modified form. These microbes can treat the pollutants completely or help in altering their chemical structure for its possible degradation and hence making the environment free from these pollutants (Endeshaw et al. 2017; Gupta et al. 2019; Rathour et al. 2018). A wide range of chemical materials including heavy metals, organic complexes, and pesticides, etc. had been eliminated using these microbes. In case of pesticide treatment, microbes utilise pesticides as sole source of carbon and nutrient (Huang et al. 2018). Through the reports, it had been found that soil sample of 1 g contains bacteria more than 100 million that includes inimitable strains in the range of 5000–7000 along with 10,000 colonies of fungi (Kalevitch and Kefeli 2007; Anjum et al. 2012). Utilisation of indigenous microbes or natural attenuation had proved to an effective technique for the removal of recalcitrant pollutants from the environment (Mrozik and Piotrowska-Seget 2010; Endeshaw et al. 2017).

19.3 Bioremediation

Production and utilisation of pesticides had been increasing at an alarming rate. The waste generated from the pesticide industries and pesticide residues after its application in various agricultural activities leads to environmental pollution and that ultimately affects the health of human being (Hussain et al. 2007; Yao et al. 2015; Ojuederie and Babalola 2017). Because of these problems, it is one of the prime importance to treat these pollutants either naturally or artificially. As compared to various physicochemical methods utilised for remediation, bioremediation, because of its cost-effectiveness and environmental friendly nature is considered one of the best methods (Song and Bartha 1990; Baker and Herson 1994; Matsumoto et al.

Table 19.1 Types of pesticides

Types of pesticides	Target organism	Name of pesticide	References
Insecticide	Insects	Acephate, Azadirachtin, Aldicarb, aldrin, azinphos-methyl, benzoylphenylUreas, bromophos, benzoylphenylurea, carbaryl, chlordimeform, coumaphos, chlordane, carbofuran, carbosulfan, cartap, Cypermethrin, DDT, chlorfenvinphos, diflubenzuron, chlorpyrifos, dieldrin, deltamethrin, dicofol, dioxathion, endrin, endosulfan, fenvalerate, fipronil, fipronil, fenitrothion, flumethrin, fenitrooxon, glyphosate, γ-BHC, lindane, malathion, hexachlorocyclohexane, heptachlor, ivermectin, parathion, mathamidophos, methoxyfenozide, permethrin, profenofos, phorate, phosmet, phosphothion, trichloffon, trichlorfon, pyriproxyfen, pyriproxyfen, tebufenozide, γ-spinosad	Beeman and Matsumura (1973), Hajjar and Casida (1978), Mohamed et al. (1987), Awumbila and Bokuma (1994), Maloney (2001), Sagar and Singh (2011), Hai et al. (2012), Chowdhury et al. (2013), Liu et al. (2013), Chaussonnerie et al. (2016), Bhandari (2017), Upadhyay and Dutt (2017)
Acaricides	Mites	Menthol, amitraz, formic acid, coumaphos, thymol, dimethoatet, fenpyroximate, tau-fluvalinate	Singh (2008), Boncristiani et al. (2012), Ye et al. (2018)
Herbicide	Weeds	2,4,5-T, 2,4-D, alachlor, barban, chlorophenoxy, chlorbromuron, dalapon, diuron, glyphosate, linuron, monuron, neburon, pentachlorophenol, propham, salted iron phosphorus, pendimethalin, swep	Maloney (2001), Ngowi et al. (2007), Hai et al. (2012), Liu et al. (2013), Prabha et al. (2017), Nour et al. (2017), Tang (2018)
Bactericide	Bacteria	Chlorothalonil, bayleton, blue copper, oxychloride, copper hydrochloride, dithiocarbamates, copper, copper sulphate, metalaxyl, dithane, thiovit, , rice blast net, methyl phosphorus, impact, polytrin, ridomil, triazoles, thiocarbamates, mancozeb	Ngowi et al. (2007), Martins et al. (2017), Prabha et al. (2017), Tang (2018), Jiang and Li (2018)

2009; Patel et al. 2020b). In bioremediation, we use bacteria, plant, fungi and their derivatives, i.e. enzymes to remove pollutants from the environment.

19.3.1 In Situ Bioremediation

In situ bioremediation involves treatment of pollutant at its original place. It is the treatment technique in which direct contact is made between the microorganisms and the pollutants for its degradation (Alcalde et al. 2006). This technique had been found to be suitable for remediation of cultivatable lands and groundwater because treatment can be done, at subsurface or surface of the contaminated, directly (Campbell 2009). In situ bioremediation is of two types, i.e. intrinsic and extrinsic in situ bioremediation. In intrinsic type in situ bioremediation, microorganisms are added in natural form or without modification of any conditions or adding any supplement for treatment of pollutants. Intrinsic in situ bioremediation or natural attenuation plays important role in remediation of groundwater contaminated with pesticides and solid wastes having toxic wastes (Pedro et al. 2015). It also can be used for remediation of soil contaminated with oil (Hinchee 1998). This technique of bioremediation requires free carbon source, aerobic condition, suitable pH and temperature, etc. for maintaining its efficiency (Azubuike et al. 2016). This technique also had one more disadvantage, i.e. time consuming.

Whereas when the native microorganisms lack the degradation ability, there is a need of addition of genetically engineered microbial strain or modification of certain conditions including temperature, ventilation, and pH. This type of bioremediation is known as extrinsic in situ bioremediation. Compared to natural attenuation, it is considered to be more effective in pesticide remediation and moreover does not generate any kind of secondary pollutants. It has been further classified into bioaugmentation and biostimulation. In bioaugmentation, suitable microbes were primarily cultured under optimised laboratory condition and further utilised at the contaminated site for the economical, faster, and efficient removal of pesticides (Alvarez and Illman 2005). Single strain or combined strains had been used in this technique (Ulrich and Edwards 2003; Brown and Jaffé 2001; Wilson and Lindow 1993; Kane et al. 2001). This techniques had been used for the degradation of pesticides including atrazine, organophosphates, DDT (dichlorodiphenyltrichloroethane), carbendazim, carbamate, organochlorine, endosulfan, etc. (Ramanathan and Lalithakumari 1996; Feakin et al. 1995; Mulbry and Kearney 1991; Ghadiri et al. 1995; Aislabie et al. 1997; Singh et al. 2003; Tomlin 2003, 1997).

For efficient and eco-friendly removal of residues of pesticides from contaminated areas introduction of constructed plasmids with genes having pesticide degrading ability along with natural or cultures microbes is needed to be done (Carlos et al. 2017). Whereas in biostimulation, stimulation of natural potential of treatment of pollutants by indigenous microbes is done by maintaining the required aerobic conditions, free carbon source, pH, temperature, and other stimulants (Morgan and Atlas 1989; Margesin and Schinner 1999).

19.3.2 Ex Situ Bioremediation

As the depth of sites that had to be remediated increases, microbial concentration decreases, therefore decreasing the efficiency of in situ bioremediation, hence ex situ bioremediation had been suggested by various researchers. In this technique, polluted sample of water or soil was taken out from their original site and then treated in laboratory having suitable equipment and favourable conditions were maintained (Kuppusamy et al. 2016). This technique is highly recommended for various pollutants that are present in higher concentration. Bioremediation by ex situ bioremediation can be enhanced by regular monitoring of microbial culture, water, oxygen, pH, temperature, nutrient along with several other parameters (Whelan et al. 2015a, b; Dias et al. 2015; Barr et al. 2002). Ex situ technique of bioremediation had been used for remediation of carbofuran, petroleum, and various hydrocarbon pollutants (Coulon et al. 2010; Plangklang and Reungsang 2010; Chikere et al. 2016). Ex situ technique besides being costly due to labour involvement, transportation, utilisation of bioreactor, and other equipment had been utilised on large scale as compared to in situ bioremediation techniques, mainly due to effective and fast remediation of contaminant (Tomei and Daugulis 2013).

19.4 Microorganisms Assisted Degradation of Pesticides

Microbes play an important role in the remediation of pesticides. Microbial degradation of various organic pesticides done by employing several species of microbes helps in remediation of soil and water, hence improving both environment and agricultural field productivity (Gupta et al. 2016; Geed et al. 2018). Slow degradation rate of contaminants is the main advantage of this techniques and this is mainly due to pesticides persistency in environment and complex structure (Huang et al. 2018; Pérez-Lucas et al. 2018). Bacteria, algae, and fungi had been efficiently employed for treatment of both inorganic and organic pollutants from soil (Megharaj and Naidu 2017). These species can be used either in natural or modified form for the treatment of pesticides. These microbial strains help in remediating the pollutants completely or change their structure chemically and hence making environment free from its toxic effects (Endeshaw et al. 2017; Rathour et al. 2018; Gupta et al. 2019). Some examples of microorganisms are presented in Fig. 19.2 which have been employed in the biodegradation of pesticides.

19.4.1 Bacterial-Assisted Biodegradation

Flavobacterium, Burkholderia, Azotobacter, Arthrobacter, and Pseudomonas are the genus of bacteria with pesticides degrading ability (Glazer and Nikaido 2007). The degradation of pesticide with the bacteria's involves oxidisation of organic compound into less toxic compounds, water, or carbon dioxide (Endeshaw et al. 2017; Doolotkeldieva et al. 2018). Degradation of pesticide by using bacteria is dependent

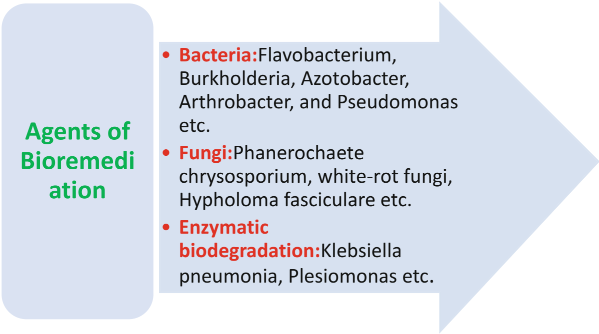

Fig. 19.2 Microorganisms employed in the biodegradation of pesticides

on nutrients, pH, temperature, and also on the availability of anionic species (Julia et al. 2001; Doolotkeldieva et al. 2018).

Organophosphorus compounds and neonicotinoids had been easily degraded by using Pseudomonas sp. and Klebsiella pneumoniae (Pathak 2018). Due to unfavourable environmental conditions sometimes incomplete degradation of pesticides occurs and this leads to deposition of metabolites into the soil and toxicity of these metabolites is more than that of its original compounds (Foght et al. 2001). Therefore optimisation of environmental conditions is needed to be done to eliminate these problems.

19.4.2 Fungi-Assisted Biodegradation

Degradation of pesticides is dependent on its chemical structure, type of microbes, and environmental conditions. Fungi causes transformation of pollutants into other non-harmful compounds and after that is eliminated completely by the help of bacteria (Ortiz-Hernández et al. 2013; Gianfreda and Rao 2004). Several group of pesticides including atrazine, phenylamide, chlorinated, organophosphorus compounds, DDT, diuron, atrazine, dicarboximide, heptachlor, triazine, dieldrin, phenylurea, Lindens, etc. can be degraded by Phanerochaete chrysosporium, white-rot fungi, Hypholoma fasciculare, Agrocybesemiorbicularis, Avatha discolor, Coriolus versicolor, Dichomitus squalens, Auricularia auricular, etc. (Bending et al. 2002; Singh 2017; Pathak 2018;).

19.4.3 Enzymatic-Assisted Biodegradation

Various crop plants and microbes lead to production of enzymes and these play an important role in the remediation of pesticides from the environment (Rao et al. 2010). It helps in the remediation of both soil and water in much more complex environment. Laccase and peroxides are the fungal enzymes and these are applied for the remediation of poly-aromatic hydrocarbons from the environment (Balaji et al. 2014; Patel et al. 2019b). *Klebsiella pneumonia, Plesiomonas*, *Arthrobacter*, *Ralstonia, Sphingobium*, *Flavobacterium*, *Burkholderia, Agrobacterium* based enzymes had been used for the degradation of pesticides (Weir et al. 2006; Latifi et al. 2012; Nandavaram et al. 2016).

19.5 Advanced Mechanism and Biotechnological Approach for Pesticide Bioremediation

There is a need of development of new more advanced methods like biotechnology, microbiology, and bioinformatics for complete elimination of pesticides from soil (Ahmad et al. 2018). The new approaches will not only help in complete remediation but also helps in the speeding up the whole process of bioremediation (Huang et al. 2018). Some of the modern approaches are explained below.

19.5.1 Genetic Engineering

Microbial strains act differently for their survival in the polluted environment and utilise pollutants as source of energy and hence leading to the complete degradation of pollutants (Parales and Haddock 2004; Cavicchioli et al. 2019). Still, several pesticides are persistent in the environment because of their chemical structure and hence the degradation of these toxic elements is very difficult by employing microbes (Parrilli et al. 2010). The process of degradation of pollutants by employing these microbes can be enhanced by using methods of genetic engineering (Paul et al. 2005). By application of genetic engineering methods like recombinant DNA technology had led to several improvement in the microbial strain including increased energy generation, increased redox, elimination of limiting pathways, and disruption or amplification of the genes responsible for the metabolic pathways (Cases and De Lorenzo 2005; Megharaj et al. 2011; Perpetuo et al. 2011). Through the reports from various researchers it had been found that the microorganisms modified genetically were capable of the degradation of pesticides and other chemical contaminants including chlorobenzene, phenol, mercury, polycyclic aromatic hydrocarbon, indole, etc. (Wilson and Lindow 1993; Fujita et al. 1994; Chen and Mulchandani 1998; Lange et al. 1998; Dejonghe et al. 2000; Watanabe et al. 2002; Ningfeng et al. 2004; Fu et al. 2004; Yu et al. 2009; Wang et al. 2012; Shen et al. 2010).

19.5.2 Metagenomic Approach

The various contaminated sites have varying environmental conditions like pH, soil structure, biotic activity, water content, etc. and these lead to complexity in microbial diversity (Liu et al. 2019). Through the research it had been found that more than

99% of microbes present in the environment were not suitable for research as even they were difficult to culture in the laboratory (Zhou et al. 2010). But, in recent years, the study on these microbes had become easier because of development of more easy methods related to isolation of these microbial strains (Jeffries et al. 2018). Development of metagenomics approach had led to development of mixed genomes microbes strains for its application in the wide areas of biotechnology (Kumar et al. 2015). This approach had helped in eliminating the previous demerits of the microbes and hence making them more efficient for its application in the field of removal of pesticides from soil and water (Jeffries et al. 2018).

19.5.3 Functional Genomics

Functional genomics includes traditional methods of molecular genetics and other biological methods that study the changes caused within the microorganisms genome due to gene knockins or mutagenesis. In recent years, because of the utilisation of bioinformatics based on more significant developed techniques, for the assessment of wide genome, had gained immense attention (Zhao and Poh 2008; Jaiswal et al. 2019). The main importance of the approach of functional genomics is based on the expansion of the areas of biological research (Rayu et al. 2012). Biological function can be evaluated directly by determining the specific conditions at which the gene is either complemented or disrupted along with other gene. With the development of genomic based tools the limitation related to nutrient availability, oxygen shortage and various other limiting factors can be eliminated more easily (Ruuskanen et al. 2020). Hence, by proper observation of the microbial structure and function, the degradation of pesticides with the help of microbes can be done easily (Maphosa et al. 2012).

19.6 Conclusions and Prospects

With increase in population and globalisation, there has been an immense increase in the utilisation of pesticides in agricultural related activities and other chemicals as food preservatives in the food based industries. These chemicals being persistent in nature had created enormous pressure on environment and human health. Bioremediation technologies by employing microbes had been found to be one of the best and efficient ways of treating such toxic pesticides and other chemicals from the environment in most eco-friendly manner. Enormous range of microbes had been utilised for the degradation of pesticides into non-toxic substances. In order to increase the efficiency of degradation of pesticides combination of bioremediation technology along with other conventional technologies can be done. The biodegradation of pollutants by microbes is dependent on the physical and chemical properties of the contaminated sites. Hence there is need of proper research in order to establish a proper understanding of degradation pathways of microbes and its interaction with the pollutants and surrounding environmental conditions. There is a need of development of new more adaptable, robust, and advanced microbial strains by employing advanced and modern biotechnological approach, for bioremediation of pesticides. There is need of interdisciplinary research between the researchers of several areas, i.e. biotechnology, biochemistry, environmental engineering, genetic engineering, and microbiology in order to overcome the present limitation of various bioremediation approaches.

References

Ahmad M, Pataczek L, Hilger TH, Zahir ZA, Hussain A, Rasche F, Solberg S (2018) Perspectives of microbial inoculation for sustainable development and environmental management. Front Microbiol 9:2992

Aislabie JM, Richards NK, Boul HL (1997) Microbial degradation of DDT and its residues—a review. N Z J Agric Res 40(2):269–282

Alcalde M, Ferrer M, Plou FJ, Ballesteros A (2006) Environmental biocatalysis: from remediation with enzymes to novel green processes. Trends Biotechnol 24(6):281–287

Alvarez, P. J., & Illman, W. A. (2005). Bioremediation and natural attenuation: process fundamentals and mathematical models (Vol. 27). Wiley, New York

Anjum R, Rahman M, Masood F, Malik A (2012) Bioremediation of pesticides from soil and wastewater. In: Environmental protection strategies for sustainable development. Springer, Dordrecht, pp 295–328

Anjum MM, Ali N, Iqbal S (2017) Pesticides and environmental health: A review. Toxicol Environ Chem 5:555671

Aresta A, Marzano CN, Lopane C, Corriero G, Longo C, Zambonin C, Stabili L (2015) Analytical investigations on the lindane bioremediation capability of the demosponge Hymeniacidon perlevis. Mar Pollut Bull 90(1–2):143–149

Awumbila B, Bokuma E (1994) Survey of pesticides used in the control of ectoparasites of farm animals in Ghana. Trop Anim Health Prod 26(1):7–12

Azubuike CC, Chikere CB, Okpokwasili GC (2016) Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. World J Microbiol Biotechnol 32(11):1–18

Baker KH, Herson DS (1994) Bioremediation. McGraw-Hill, New York

Balaji V, Arulazhagan P, Ebenezer P (2014) Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. J Environ Biol 35(3):521–529

Balomajumder C (2019) Simultaneous biodegradation of mixture of carbamates by newly isolated Ascochyta sp. CBS 237.37. Ecotoxicol Environ Saf 169:590–599

Barr D, Finnamore JR, Bardos RP, Weeks JM, Nathanail CP (2002). Biological methods for assessment and remediation of contaminated land: case studies. CIRIA

Beeman RW, Matsumura F (1973) Chlordimeform: a pesticide acting upon amine regulatory mechanisms. Nature 242(5395):273–274

Bending GD, Friloux M, Walker A (2002) Degradation of contrasting pesticides by white rot fungi and its relationship with ligninolytic potential. FEMS Microbiol Lett 212(1):59–63

Bhandari G (2017) Mycoremediation: an eco-friendly approach for degradation of pesticides. In: Mycoremediation and environmental sustainability. Springer, Cham, pp 119–131

Boncristiani H, Underwood R, Schwarz R, Evans JD, Pettis J (2012) Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera*. J Insect Physiol 58 (5):613–620

Brown DG, Jaffé PR (2001) Effects of nonionic surfactants on bacterial transport through porous media. Environ Sci Technol 35(19):3877–3883

Campbell K (2009) Radionuclides in surface water and groundwater. Handbook of Water Purity and Quality 10:213–236

Carpenter SR (2008) Phosphorus control is critical to mitigating eutrophication. Proc Natl Acad Sci U S A 105(32):11039–11040

Cases I, De Lorenzo V (2005) Promoters in the environment: transcriptional regulation in its natural context. Nat Rev Microbiol 3(2):105–118

Carlos G, Olatz G, Lur E, Elisabeth G, Itziar A (2017) Plasmid-mediated bioaugmentation for the bioremediation of contaminated soils. Front Microbiol. https://doi.org/10.3389/fmicb.2017.01966

Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M et al (2019) Scientists' warning to humanity: microorganisms and climate change. Nat Rev Microbiol 17(9):569–586

Chaussonnerie S, Saaidi PL, Ugarte E, Barbance A, Fossey A, Barbe V et al (2016) Microbial degradation of a recalcitrant pesticide: chlordecone. Front Microbiol 7:2025

Chen W, Mulchandani A (1998) The use of live biocatalysts for pesticide detoxification. Trends Biotechnol 16(2):71–76

Chikere CB, Okoye AU, Okpokwasili GC (2016) Microbial community profiling of active oleophilic bacteria involved in bioreactor-based crude-oil polluted sediment treatment. J Appl Environ Microbiol 4(1):1–20

Chowdhury MAZ, Fakhruddin ANM, Islam MN, Moniruzzaman M, Gan SH, Alam MK (2013) Detection of the residues of nineteen pesticides in fresh vegetable samples using gas chromatography–mass spectrometry. Food Control 34(2):457–465

Coulon F, Al Awadi M, Cowie W, Mardlin D, Pollard S, Cunningham C et al (2010) When is a soil remediated? Comparison of biopiled and windrowed soils contaminated with bunker-fuel in a full-scale trial. Environ Pollut 158(10):3032–3040

Dejonghe W, Goris J, El Fantroussi S, Höfte M, De Vos P, Verstraete W, Top EM (2000) Effect of dissemination of 2, 4-dichlorophenoxyacetic acid (2, 4-D) degradation plasmids on 2, 4-D degradation and on bacterial community structure in two different soil horizons. Appl Environ Microbiol 66(8):3297–3304

Dias RL, Ruberto L, Calabró A, Balbo AL, Del Panno MT, Mac Cormack WP (2015) Hydrocarbon removal and bacterial community structure in on-site biostimulated biopile systems designed for bioremediation of diesel-contaminated Antarctic soil. Polar Biol 38(5):677–687

Doolotkeldieva T, Konurbaeva M, Bobusheva S (2018) Microbial communities in pesticide-contaminated soils in Kyrgyzstan and bioremediation possibilities. Environ Sci Pollut Res 25 (32):31848–31862

Ecobichon DJ (2001) Carbamate insecticides. In: Handbook of pesticide toxicology. Academic, New York, pp 1087–1106

Endeshaw A, Birhanu G, Zerihun T, Misganaw W (2017) Application of microorganisms in bioremediation-review. J Environ Microb 1(1):2–9

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A et al (2017) Crop production under drought and heat stress: plant responses and management options. Front Plant Sci 8:1147

Feakin SJ, Blackburn E, Burns RG (1995) Inoculation of granular activated carbon in a fixed bed with s-triazine-degrading bacteria as a water treatment process. Water Res 29(3):819–825

Fenner K, Canonica S, Wackett LP, Elsner M (2013) Evaluating pesticide degradation in the environment: blind spots and emerging opportunities. Science *341*(6147):752–758

Foght J, April T, Biggar K, Aislabie J (2001) Bioremediation of DDT-contaminated soils: a review. Biorem J 5(3):225–246

Fu G, Cui Z, Huang T, Li S (2004) Expression, purification, and characterization of a novel methyl parathion hydrolase. Protein Expr Purif 36(2):170–176

Fujita M, Ike M, Uesugi K (1994) Operation parameters affecting the survival of genetically engineered microorganisms in activated sludge processes. Water Res 28(7):1667–1672

Geed SR, Prasad S, Kureel MK, Singh RS, Rai BN (2018) Biodegradation of wastewater in alternating aerobic-anoxic lab scale pilot plant by Alcaligenes sp. S3 isolated from agricultural field. J Environ Manag 214:408–415

Ghadiri H, Rose CW, Connell DW (1995) Degradation of organochlorine pesticides in soils under controlled environment and outdoor conditions. J Environ Manag 43(2):141–151
Gianfreda L, Rao MA (2004) Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzym Microb Technol 35(4):339–354
Glazer AN, Nikaido H (2007) Microbial biotechnology: fundamentals of applied microbiology. Cambridge University Press, Cambridge
Grube A, Donaldson D, Kiely T, Wu L (2011) Pesticides industry sales and usage. US EPA, Washington, DC
Gupta RC (2006) Classification and uses of organophosphates and carbamates. In: Toxicology of organophosphate & carbamate compounds. Academic, New York, pp 5–24
Gupta M, Mathur S, Sharma TK, Rana M, Gairola A, Navani NK, Pathania R (2016) A study on metabolic prowess of Pseudomonas sp. RPT 52 to degrade imidacloprid, endosulfan and coragen. J Hazard Mater 301:250–258
Gupta J, Rathour R, Kumar M, Thakur IS (2017) Metagenomic analysis of microbial diversity in landfill lysimeter soil of Ghazipur landfill site, New Delhi, India. Genome Announc 5(42)
Gupta J, Rathour R, Singh R, Thakur IS (2019) Production and characterization of extracellular polymeric substances (EPS) generated by a carbofuran degrading strain Cupriavidus sp. ISTL7. Bioresour Technol 282:417–424
Hai FI, Modin O, Yamamoto K, Fukushi K, Nakajima F, Nghiem LD (2012) Pesticide removal by a mixed culture of bacteria and white-rot fungi. J Taiwan Inst Chem Eng 43(3):459–462
Hajjar NP, Casida JE (1978) Insecticidal benzoylphenyl ureas: structure-activity relationships as chitin synthesis inhibitors. Science 200(4349):1499–1500
Hinchee RE (1998) In situ bioremediation: practices and challenges. Biotechnology for soil remediation. Scientific bases and practical applications. R. Serra. CIPASrl, Milan, Italy, 17–20
Huang Y, Xiao L, Li F, Xiao M, Lin D, Long X, Wu Z (2018) Microbial degradation of pesticide residues and an emphasis on the degradation of cypermethrin and 3-phenoxy benzoic acid: a review. Molecules 23(9):2313
Hussain S, Arshad M, Saleem M, Khalid A (2007) Biodegradation of α-and β-endosulfan by soil bacteria. Biodegradation 18(6):731–740
Hussaini SZ, Shaker M, Iqbal MA (2013) Isolation of bacterial for degradation of selected pesticides. AdvBiores 4(3):82–85
Jaiswal S, Singh DK, Shukla P (2019) Gene editing and systems biology tools for pesticide bioremediation: a review. Front Microbiol 10:87
Jeffries TC, Rayu S, Nielsen UN, Lai K, Ijaz A, Nazaries L, Singh BK (2018) Metagenomic functional potential predicts degradation rates of a model organophosphorus xenobiotic in pesticide contaminated soils. Front Microbiol 9:147
Jiang J, Li S (2018) Microbial degradation of chemical pesticides and bioremediation of pesticide-contaminated sites in China. In: Twenty years of research and development on soil pollution and remediation in China. Springer, Singapore, pp 655–670
Julia Ng SC, Zainal-Zahari Z, Adam N (2001) Wallows and wallow utilization of the Sumatran rhinoceros (Dicerorhinus sumatrensis) in a natural enclosure in Sungai Dusun Wildlife Reserve, Selangor, Malaysia. J Wildl Parks 19(2001):7–12
Kalevitch MV, Kefeli VI (2007) Study of bacterial activity in fabricated soils. Int J Environ Pollut 29(4):412–423
Kane SR, Beller HR, Legler TC, Koester CJ, Pinkart HC, Halden RU, Happel AM (2001) Aerobic biodegradation of methyltert-butyl ether by aquifer bacteria from leaking underground storage tank sites. Appl Environ Microbiol 67(12):5824–5829
Klemick H, Lichtenberg E (2008) Pesticide use and fish harvests in Vietnamese rice agroecosystems. Am J Agric Econ 90(1):1–14
Kolpin DW, Barbash JE, Gilliom RJ (1998) Occurrence of pesticides in shallow groundwater of the United States: initial results from the National Water-Quality Assessment Program. Environ Sci Technol 32(5):558–566

Kumar S, Krishnani KK, Bhushan B, Brahmane MP (2015) Metagenomics: retrospect and prospects in high throughput age. Biotechnol Res Int 2015

Kuppusamy S, Palanisami T, Megharaj M, Venkateswarlu K, Naidu R (2016) Ex-situ remediation technologies for environmental pollutants: a critical perspective. In Reviews of environmental contamination and toxicology. Springer, Cham, 236: 117–192

Lange CC, Wackett LP, Minton KW, Daly MJ (1998) Engineering a recombinant Deinococcus radiodurans for organopollutant degradation in radioactive mixed waste environments. Nat Biotechnol 16(10):929–933

Latifi AM, Khodi S, Mirzaei M, Miresmaeili M, Babavalian H (2012) Isolation and characterization of five chlorpyrifos degrading bacteria. Afr J Biotechnol 11(13):3140–3146

Lefrancq M, Imfeld G, Payraudeau S, Millet M (2013) Kresoxim methyl deposition, drift and runoff in a vineyard catchment. Sci Total Environ 442:503–508

Li Z, Yang P (2018) Review on physicochemical, chemical, and biological processes for pharmaceutical wastewater. In IOP Conference Series: Earth and Environmental Science (Vol. 113, p. 012185)

Liu B, Zhou P, Liu X, Sun X, Li H, Lin M (2013) Detection of pesticides in fruits by surface-enhanced Raman spectroscopy coupled with gold nanostructures. Food Bioprocess Technol 6 (3):710–718

Liu M, Sui X, Hu Y, Feng F (2019) Microbial community structure and the relationship with soil carbon and nitrogen in an original Korean pine forest of Changbai Mountain, China. BMC Microbiol 19(1):218

Lushchak VI, Matviishyn TM, Husak VV, Storey JM, Storey KB (2018) Pesticide toxicity: a mechanistic approach. EXCLI J 17:1101

Maloney SE (2001) Pesticide degradation in Fungi in bioremediation by GM Gadd.

Mandal K, Singh B, Jariyal M, Gupta VK (2014) Bioremediation of fipronil by a Bacillus firmus isolate from soil. Chemosphere 101:55–60

Maphosa F, Lieten SH, Dinkla I, Stams AJ, Smidt H, Fennell DE (2012) Ecogenomics of microbial communities in bioremediation of chlorinated contaminated sites. Front Microbiol 3:351

Margesin R, Schinner F (1999) Biological decontamination of oil spills in cold environments. J Chem Technol Biotechnol 74(5):381–389

Martins MR, Santos C, Pereira P, Cruz-Morais J, Lima N (2017) Metalaxyl degradation by mucorales strains Gongronella sp. and Rhizopus oryzae. Molecules 22(12):2225

Matsumoto E, Kawanaka Y, Yun SJ, Oyaizu H (2009) Bioremediation of the organochlorine pesticides, dieldrin and endrin, and their occurrence in the environment. Appl Microbiol Biotechnol 84(2):205–216

Mattah MM, Mattah PA, Futagbi G (2015) Pesticide application among farmers in the catchment of Ashaiman irrigation scheme of Ghana: health implications. J Environ Public Health 2015

Megharaj M, Naidu R (2017) Soil and brownfield bioremediation. Microb Biotechnol 10 (5):1244–1249

Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R (2011) Bioremediation approaches for organic pollutants: a critical perspective. Environ Int 37(8):1362–1375

Mohamed AK, Pratt JP, Nelson FR (1987) Compatability of Metarhizium anisopliae var. anisopliae with chemical pesticides. Mycopathologia 99(2):99–105

Morgan P, Atlas RM (1989) Hydrocarbon degradation in soils and methods for soil biotreatment. Crit Rev Biotechnol 8(4):305–333

Mrozik A, Piotrowska-Seget Z (2010) Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. Microbiol Res 165(5):363–375

Mulbry W, Kearney PC (1991) Degradation of pesticides by micro-organisms and the potential for genetic manipulation. Crop Prot 10(5):334–346

Nandavaram A, Sagar AL, Madikonda AK, Siddavattam D (2016) Proteomics of Sphingobium indicum B90A for a deeper understanding of hexachlorocyclohexane (HCH) bioremediation. Rev Environ Health 31(1):57–61

Ngowi AV, Maeda DW, Partanen TJ (2007) Knowledge, attitudes and practices (KAP) among agricultural extension workers concerning the reduction of the adverse impact in agricultural areas in Tanzania. Crop Prot 26(11):1617–1624
Ningfeng W, Minjie D, Guoyi L, Xiaoyu C, Bin Y, Yunliu F (2004) Cloning and expression of ophc2, a new organphosphorus hydrolase gene. Chin Sci Bull 49(12):1245–1249
Nour EH, Elsayed TR, Springael D, Smalla K (2017) Comparable dynamics of linuron catabolic genes and IncP-1 plasmids in biopurification systems (BPSs) as a response to linuron spiking. Appl Microbiol Biotechnol 101(11):4815–4825
Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14(12):1504
Ortiz-Hernández ML, Sánchez-Salinas E, Dantán-González E, Castrejón-Godínez ML (2013) Pesticide biodegradation: mechanisms, genetics and strategies to enhance the process. Biodegradation-life of Science:251–287
Özkara A, Akyıl D, Konuk M (2016) Pesticides, environmental pollution, and health. In Environmental health risk-hazardous factors to living species. IntechOpen
Parales RE, Haddock JD (2004) Biocatalytic degradation of pollutants. Curr Opin Biotechnol 15 (4):374–379
Parrilli E, Papa R, Tutino ML, Sannia G (2010) Engineering of a psychrophilic bacterium for the bioremediation of aromatic compounds. Bioengineered bugs 1(3):213–216
Patel N, Rai D, Shahane S, Mishra U (2019a) Lipases: sources, production, purification, and applications. Recent Pat Biotechnol 13(1):45–56
Patel N, Shahane S, Majumdar R, Mishra U (2019b) Mode of action, properties, production, and application of laccase: a review. Recent Pat Biotechnol 13(1):19–32
Patel N, Khan M, Shahane S, Rai D, Chauhan D, Kant C, Chaudhary V (2020a) Emerging pollutants in aquatic environment: source, effect, and challenges in biomonitoring and bioremediation- a review. Pollution 6(1):99–113. https://doi.org/10.22059/poll.2019.285116.646
Patel N, Pathak P, Rai D, Chaudhary VK (2020b) Biosensors used for monitoring of environmental contaminants. In Nanosensor technologies for environmental monitoring (pp. 69-83). Springer, Cham
Pathak VM (2018). Handbook of research on microbial tools for environmental waste management. IGI Global
Paul D, Pandey G, Pandey J, Jain RK (2005) Accessing microbial diversity for bioremediation and environmental restoration. Trends Biotechnol 23(3):135–142
Pedro-Cedillo S, Méndez-Novelo RI, Rojas-Valencia MN, Barceló-Quintal M, Castillo-Borges ER, Sauri-Riancho MR, Marrufo-Gómez JM (2015) Evaluation of adsorption and Fenton-adsorption processes for landfill leachate treatment. Revista mexicana de ingeniería química 14(3):745–755
Pérez-Lucas G, Vela N, El Aatik A, Navarro S (2018). Environmental risk of groundwater pollution by pesticide leaching through the soil profile. In Pesticides-use and misuse and their impact in the environment. IntechOpen
Perpetuo EA, Souza CB, Nascimento CAO (2011) Engineering bacteria for bioremediation. In: Progress in molecular and environmental bioengineering-from analysis and modeling to technology applications
Plangklang P, Reungsang A (2010) Bioaugmentation of carbofuran by Burkholderia cepacia PCL3 in a bioslurry phase sequencing batch reactor. Process Biochem 45(2):230–238
Prabha R, Singh DP, Verma MK (2017) Microbial interactions and perspectives for bioremediation of pesticides in the soils. In Plant-microbe interactions in agro-ecological perspectives. Springer, Singapore, pp. 649–671
Ramanathan MP, Lalithakumari D (1996) Methylparathion degradation by Pseudomonas sp. A3 immobilized in sodium alginate beads. World J Microbiol Biotechnol 12(1):107–108
Rao MA, Scelza R, Scotti R, Gianfreda L (2010) Role of enzymes in the remediation of polluted environments. J Soil Sci Plant Nutr 10(3):333–353

Rathour R, Gupta J, Tyagi B, Kumari T, Thakur IS (2018) Biodegradation of pyrene in soil microcosm by Shewanella sp. ISTPL2, a psychrophilic, alkalophilic and halophilic bacterium. Bioresource Technology Reports 4:129–136

Rayu S, Karpouzas DG, Singh BK (2012) Emerging technologies in bioremediation: constraints and opportunities. Biodegradation 23(6):917–926

Rizwan M, Ali S, ur Rehman MZ, Rinklebe J, Tsang DC, Bashir A et al (2018) Cadmium phytoremediation potential of Brassica crop species: a review. Sci Total Environ 631:1175–1191

Ruuskanen MO, Colby G, St. Pierre KA, St. Louis VL, Aris-Brosou S, Poulain AJ (2020) Microbial genomes retrieved from high Arctic lake sediments encode for adaptation to cold and oligotrophic environments. Limnol Oceanogr 65:S233–S247

Saez JM, Aparicio JD, Amoroso MJ, Benimeli CS (2015) Effect of the acclimation of a Streptomyces consortium on lindane biodegradation by free and immobilized cells. Process Biochem 50 (11):1923–1933

Sagar V, Singh DP (2011) Biodegradation of lindane pesticide by non white-rots soil fungus Fusarium sp. World J Microbiol Biotechnol 27(8):1747–1754

Shen YJ, Lu P, Mei H, Yu HJ, Hong Q, Li SP (2010) Isolation of a methyl parathion-degrading strain Stenotrophomonas sp. SMSP-1 and cloning of the ophc2 gene. Biodegradation 21 (5):785–792

Singh DK (2008) Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments. Indian J Microbiol 48(1):35–40

Singh RL (ed) (2017) Principles and applications of environmental biotechnology for a sustainable future. Springer, Singapore

Singh BK, Walker A, Morgan JAW, Wright DJ (2003) Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Appl Environ Microbiol 69 (9):5198–5206

Song HG, Bartha R (1990) Effects of jet fuel spills on the microbial community of soil. Appl Environ Microbiol 56(3):646–651

Spina F, Cecchi G, Landinez-Torres A, Pecoraro L, Russo F, Wu B et al (2018) Fungi as a toolbox for sustainable bioremediation of pesticides in soil and water. Plant Biosyst 152(3):474–488

Srivastav AL (2020) Chemical fertilizers and pesticides: role in groundwater contamination in: agrochemicals detection, treatment and remediation (Elsevier), pp. 143-159

Sun S, Sidhu V, Rong Y, Zheng Y (2018) Pesticide pollution in agricultural soils and sustainable remediation methods: a review. Curr Pollut Rep 4(3):240–250

Tang W, Ji H, Hou X (2018) Research progress of microbial degradation of organophosphorus pesticides. Prog Appl Microbiol 1:29–35

Tomei MC, Daugulis AJ (2013) Ex situ bioremediation of contaminated soils: an overview of conventional and innovative technologies. Crit Rev Environ Sci Technol 43(20):2107–2139

Tomlin CDS (1997) The pesticide manual: A world compendium 11th edn BCPC. Farrham, survey, UK, 1011-1013

Tomlin CDS (2003) The pesticide manual, 13th edn, British crop protection council, Alton. Hampshire, 555-556

Ulrich AC, Edwards EA (2003) Physiological and molecular characterization of anaerobic benzene-degrading mixed cultures. Environ Microbiol 5(2):92–102

Upadhyay LS, Dutt A (2017) Microbial detoxification of residual organophosphate pesticides in agricultural practices. In Microbial biotechnology. Springer, Singapore, pp. 225–242

Verma JP, Jaiswal DK, Sagar R (2014) Pesticide relevance and their microbial degradation: a-state-of-art. Rev Environ Sci Biotechnol 13(4):429–466

Vikrant K, Giri BS, Raza N, Roy K, Kim KH, Rai BN, Singh RS (2018) Recent advancements in bioremediation of dye: current status and challenges. Bioresour Technol 253:355–367

Wang XX, Chi Z, Ru SG, Chi ZM (2012) Genetic surface-display of methyl parathion hydrolase on Yarrowia lipolytica for removal of methyl parathion in water. Biodegradation 23(5):763–774

Watanabe K, Teramoto M, Harayama S (2002) Stable augmentation of activated sludge with foreign catabolic genes harboured by an indigenous dominant bacterium. Environ Microbiol 4 (10):577–583

Weir KM, Sutherland TD, Horne I, Russell RJ, Oakeshott JG (2006) A single monooxygenase, ese, is involved in the metabolism of the organochlorides endosulfan and endosulfate in an Arthrobacter sp. Appl Environ Microbiol 72(5):3524–3530

Whelan MJ, Coulon F, Hince G, Rayner J, McWatters R, Spedding T, Snape I (2015a) Fate and transport of petroleum hydrocarbons in engineered biopiles in polar regions. Chemosphere 131:232–240

Whelan MJ, Coulon F, Hince G, Rayner J, McWatters R, Spedding T, Snape I (2015b) Fate and transport of petroleum hydrocarbons in engineered biopiles in polar regions. Chemosphere 131:232–240

Wilson Á, Lindow SE (1993) Release of recombinant microorganisms. Annu Rev Microbiol 47 (1):913–944

Yao ZT, Ji XS, Sarker PK, Tang JH, Ge LQ, Xia MS, Xi YQ (2015) A comprehensive review on the applications of coal fly ash. Earth Sci Rev 141:105–121

Ye X, Dong F, Lei X (2018) Microbial resources and ecology-microbial degradation of pesticides. Nat Resour Conserv Res 1(1)

Yu H, Yan X, Shen W, Hong Q, Zhang J, Shen Y, Li S (2009) Expression of methyl parathion hydrolase in Pichia pastoris. Curr Microbiol 59(6):573

Zhao B, Poh CL (2008) Insights into environmental bioremediation by microorganisms through functional genomics and proteomics. Proteomics 8(4):874–881

Zhou J, He Z, Van Nostrand JD, Wu L, Deng Y (2010) Applying GeoChip analysis to disparate microbial communities. Microbe 5(2):60–65

Application of Microbes in Vaccine Production

20

Arka Bagchi, Partha Saha, Arunima Biswas, and Sk Manirul Islam

Abstract

Vaccination is closely associated with the use of microbes in various different ways. The disease-causing microbes are either attenuated or heat/chemically treated to be used for vaccines. The generation of DNA vaccines also relies on the genetic material of the microorganisms for the vaccination. Moreover, microorganisms are also used as vectors for vaccination to have increased immune response and memory against the disease-causing pathogen. This chapter deals in a nutshell the roles of microbes in vaccination: how microorganisms have been exploited for the generation of vaccines, from the first generation of vaccines to the present generation of DNA vaccines and subunit or conjugate vaccines.

Keywords

Vaccine · Live attenuated vaccines · Killed whole organism vaccines · Vectored vaccines · Nucleic acid vaccines · Pandemics · Strategies to combat pandemic

A. Bagchi · P. Saha · A. Biswas (✉)
Molecular Cell Biology Laboratory, Department of Zoology, University of Kalyani, Kalyani, India
e-mail: arunima10@klyuniv.ac.in

S. M. Islam (✉)
Department of Chemistry, University of Kalyani, Kalyani, India

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20.1 Introduction

The world population has increased many times in recent years, and as a result, living habits of people have changed drastically. High population density and extreme mobility of people all over the world make them easy target for infectious diseases and also favor the spread of any pathogen very quickly. Frequent pandemic outbreaks in the last few decades have shown the great pandemic risk. There are also other influencers such as climate change to make things worse, as these environmental factors are mostly helping these pathogens or their host vector to survive and proliferate. Therefore, it is of extreme importance that scientists come up with a permanent solution that can completely remove the threat of pandemic.

Vaccination is one of the greatest achievements of medical science to improve public health. It can be stated undoubtedly that vaccination programs have played a key role in the reduction of mortality due to infectious diseases and increasing the average life span of people all over the world. Though the first vaccine was discovered more than 200 years ago, the importance of vaccines has not decreased a bit; rather, it has increased drastically due to high population density and change in living habit and travel habit of people all over the world. Vaccine not only provides direct protection to the individual having it but also provides indirect protection to the whole community by inducing herd immunity, thereby slowing down the progress of the infectious disease (Dubé et al. 2013). The World Health Organization (WHO) has designated top seven priority pathogens in their 2017 Annual review of diseases prioritized under the Research and Development Blueprint. They have also mentioned that these pathogens have the capability of causing a major outbreak as there is no effective medical treatment present. These pathogens are the Crimean-Congo hemorrhagic fever virus (CCHFV), Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), Ebola and Marburg virus (EBOV), Rift Valley fever virus (RVFV), and Nipah virus (NiV). In recent days, development of vaccines of these priority pathogens including some other pathogens such as chikungunya virus and Zika virus is of prime importance to the scientists so that an uncontrolled pandemic can be avoided in the recent future.

20.2 History of Vaccine Development

Development of vaccines has a history of more than two centuries. Over this large span of time, the techniques of vaccine development have modified frequently according to the requirement and complexity of infectious diseases. These modifications have led to the development of different generations of vaccines, but it is important to note that none of these generations can claim absolute supremacy in terms of efficacy and safety. It has been observed in the case of many diseases that the old school vaccines are more effective and safe, whereas in many other diseases, the newer and more sophisticated vaccines are more effective. Although the process of vaccine development has modified over centuries, the importance of microbes in developing such vaccines has not decreased. Microbes are utilized in many ways to develop effective vaccines; sometimes, it is used as the vaccine itself, sometimes as carriers of important genes, or sometimes as the vector of the vaccine.

Depending upon the principle of development of vaccine, all the vaccines are classified in several broad classes that are described below in brief.

20.2.1 Live Attenuated Vaccines

The idea of vaccination probably originated from the procedure of variolation, which is the administration of small amount of poison or toxin into the body of an individual to make him immune to the toxic effects (Plotkin 2014). In other words, it was a way to induce memory of the immune system of an individual, though the idea of immune memory was not established at that time period. Edward Jenner, the pioneer of vaccine development, used the same idea and administered animal pox virus into the human body to prevent small pox in humans. The concept behind this application was that the agent virulent for animals might be non-pathogenic for human but can induce significant immune response (Baxby 1999). It was declared in 1980 that small pox has completely been eradicated, making vaccinia arguably one of the most successful vaccines. Another alarming human disease, poliomyelitis, has also been nearly eradicated (Minor 2012) by the use of a live attenuated vaccine developed by Albert Sabin through a worldwide program initiated by WHO. There are many other infectious diseases such as yellow fever, measles, mumps, and rotavirus that are countered with the application of live attenuated vaccines (Collins and Barrett 2017; Tangy and Naim 2005; Bernstein 2006). The vaccine for tuberculosis, a live attenuated vaccine developed by Calmette and Guerin, is not made with the contagious strain of *Mycobacterium tuberculosis*; instead, it is an attenuated strain of *M. tuberculosis* which was named as BCG (Bacillus Calmette-Guerin), an avirulent strain effective enough to cause immune response enough to protect against tuberculosis (Luca and Mihaescu 2013). The BCG strain was derived by empirical procedures during prolonged in vitro culture in laboratories in a media slightly different from its original culture media leading to unidentified mutation. Sterne's *Bacillus anthracis* spore vaccine is a strain, incapable of synthesizing the polypeptide capsule, which is very important as a determinant, and consequent manifestations of avirulence (Hudson et al. 2008). Live attenuated thyroid vaccine (Ty21a) was developed in the early 1970s containing lyophilized Ty21a, a mutant strain of *Salmonella enterica* serovar *Typhi* (*S. Typhi*) (Pennington et al. 2016). But there are several other types of thyroid vaccine available like the conjugate polysaccharide vaccine, monovalent typhoid vaccine, and multivalent combination vaccine. Attenuated vaccines have shown significant advantages over killed vaccines. For example, attenuated *Salmonella* vaccines prevent the propagation of the parasite in the liver and spleen where the killed vaccines are not sufficiently effective (Galen et al. 2016).

While attenuated vaccines have been proven to be very successful against a variety of human diseases, there are a lot of issues that also speak against their actual benefits. Questions were raised against the safety and efficacy of certain vaccines such as mumps vaccine, the first rotavirus vaccine (Minor 2015). It is important to note that the use of live attenuated form of wild-type virulent virus as a vaccine requires extensive knowledge of the pathogenesis of the virus as the attenuated form can change to pathogenic form, risking lives of the individuals who have taken the vaccine. For example, some strains experimentally considered for prospective use as live vaccines against *Salmonella* might have an unacceptable degree of virulence that is achieved either due to reversion or by mutation. One classic example of the reversion of virulence was in the case of outbreak of paralytic polio in children vaccinated by oral polio vaccine (OPV) (Famulare et al. 2016). As virulence was returned with mutations in the attenuated strains, inactivated polio vaccine (IPV) of Stalk was also considered for treating polio. Presently, a sequential schedule of OPV and IPV is used as a vaccination method for polio (Baicus 2012). Some live vaccines also have instances of displaying short persistence of immunity and at times incomplete immunity.

The important live attenuated vaccines used till date are listed below (Griesenauer and Kinch 2017; Research 2020):

1. Small pox (1798).
2. Rabies (1885).
3. Tuberculosis/BCG (1927).
4. Yellow fever (1935).
5. Oral polio vaccine (1963).
6. Measles (1963).
7. Rubella (1969).
8. Mumps (1967).
9. Typhoid (1989).
10. Adenovirus (1980).
11. Rotavirus reassortants (1999).
12. Varicella (1995).
13. Rotavirus (attenuated and new reassortants) (2006).
14. Cholera (1994).
15. Cold-adapted influenza (1999).
16. Zoster (2006).

20.2.2 Killed but Metabolically Active Whole Organism Vaccines

Another popular method of vaccination is the use of killed but metabolically active microbes. These are whole microbes that are inactivated by genetic engineering or other defined methods in such a way that they are incapable of growth and pathogenesis but retain sufficient metabolic activities to elicit immune response in the human body (Brockstedt et al. 2005). The advantage of this type of vaccines over live attenuated vaccines is that there is no chance of recurrence of pathogenesis of the pathogen used as vaccine. There are two broad ways of developing this type of vaccines, one of which is to genetically engineer attenuated strains of intracellular bacterium *Listeria monocytogenes* in such a way that it expresses specific antigens derived from pathogens of infectious diseases and the other one is the use of killed attenuated form of virulent pathogens (Dubensky et al. 2012). In both the cases, these microbes are modified to introduce an absolute block to their DNA replication, eliminating the possibility of their growth and pathogenesis. There are several other examples where inactivated whole organisms were successfully used as vaccine. Killed cholera bacteria with presence or absence of the B subunit of cholera toxin were used as an orally administered vaccine (Holmgren et al. 1992). There is also evidence of use of formalin-inactivated whole cell pertussis vaccine (Madsen 1933). Important examples of killed whole organism vaccines are listed below (Griesenauer and Kinch 2017; Research 2020):

1. Typhoid (1896).
2. Cholera (1896).
3. Plague (1897).
4. Pertussis (1926).
5. Influenza (1936).
6. Rickettsia (1938).
7. Polio (injected) (1955).
8. Rabies (1980).
9. Tick-borne encephalitis (1981).
10. Cholera (WC-rBS) (1991).
11. Japanese encephalitis (mouse brain) (1992).
12. Hepatitis A (1996).
13. Meningococcal conjugate (group c) (1999).
14. Japanese encephalitis (vero cell) (2009).
15. Cholera (WC only) (2009).

20.2.3 Purified Proteins and Polysaccharides Vaccine

The main idea behind the development of these kinds of vaccines is to provide a target to the host immune system that can induce sufficient immunogenicity, without the incorporation of the organism itself within the host body. Advances in morphological and chemical studies in bacteriology revealed that most of them are surrounded by a polysaccharide capsule (Reckseidler-Zenteno 2012), which serves as a key recognition element for the host immune system. These capsules can serve as antigen for host immune system, but they are not responsible for causing pathogenesis in the host body. Artenstein and group (Gotschlich et al. 1969) first developed a polysaccharide vaccine for meningococcus, where they used the polysaccharide capsule of meningococcus, which raised significant immune response in the host body, and most importantly, this capsule was unable to cause pathogenesis. After this, there were several other polysaccharide vaccines that were introduced, such as typhoid, pneumococcus, and influenza vaccine (Daniels et al. 2016; Gilchrist et al. 2012; Ni et al. 2017). Despite having immense usefulness, these vaccines also have some drawbacks as polysaccharide vaccine works on the principle of producing B-cell-mediated immune response and polysaccharide alone cannot induce B-cell response in infants. In 1980, Schneerson and group (Schneerson et al. 1980) developed a new vaccine for *Haemophilus influenzae*, where they conjugated the polysaccharide capsule with a protein subunit, making them more immunogenic and effective. Later scientists utilized this idea to develop several other more effective vaccines such as for pneumococcus and meningococcus. On the other hand, scientists developed protein-based vaccines in the form of toxoids. Toxoids are basically inactivated toxin elements from both bacterial and viral origin that are capable of eliciting immune response but are incapable of causing pathogenicity (Yadav et al. 2014). Such toxoid vaccine was developed against diphtheria by inactivating the toxin in such a way that it retains its ability to induce antibody production against the toxin within the host body (Glenny and Hopkins 1923; Ramon 1923). Toxoid vaccine against tetanus is another successful vaccine which is used extensively till date. In recent years, protein-based vaccines have been adopted against different diseases. One such example is pertussis vaccine. Sato and group (Sato and Sato 1999) created an acellular pertussis vaccine consisting of key protein components of the microbe that can elicit immune response. Similar type of strategy was taken for influenza virus, where the virus was artificially developed and digested to purify the key protein components that can serve as immunogen to the host body (Cate et al. 1977). There were several previously produced vaccines that were modified later on to bring more efficacy and safety, reducing the chance of pathogenesis caused by the whole organism. The list given below depicts the protein-based and polysaccharide (ps)-based vaccines developed in the course of time (Griesenauer and Kinch 2017; Research 2020):

1. Diphtheria toxoid (1923).
2. Tetanus toxoid (1926).
3. Anthrax proteins (1970).
4. Meningococcus ps (1974).
5. Pneumococcus ps (1977).
6. *H. influenzae* (B) ps (1985).
7. Typhoid ps (1994).
8. Pertussis (1996).
9. Hepatitis B (1981).
10. *H. influenzae* (B) conjugate (1987).
11. Pneumococcal conjugate (2000).
12. Meningococcal conjugate (2005).

20.2.4 Genetically Engineered and Vectored Vaccine

Genetically engineered vaccines not only include the genetic modification of pathogens but also include the production of pathogen recognition antigens within other organisms that can be administered within the host body independent of the pathogen. Advancement in the field of genetic engineering in the twentieth century has brought this new regime of vaccine development to the scientists. The first stable and successful genetically engineered vaccine was developed against hepatitis B in the year 1982 (Valenzuela et al. 1982), where the DNA sequence for the pathogen's surface antigen was inserted into a yeast cell to produce multiple copies of the antigen. These antigens were capable of inducing sufficient immune response within the host body and therefore could be used as vaccine. Moreover, in some cases, the pathogen itself was engineered in such a way that it was unable to cause pathogenesis. Germanier and Füer (1975) genetically engineered a strain of typhoid in such a way that they were unable to produce any enzyme required for them to cause pathogenesis but retain their recognition elements and immunogenicity. Scientists have also developed vaccines against other infectious diseases such as Lyme disease (1998), cholera (1993), human papillomavirus (quadrivalent/bivalent) (2006/2009), meningococcal proteins (2013), etc.

On the other hand, introduction of genetic engineering in vaccine development gave rise to another kind of vaccine that may be termed as vectored vaccines. These vaccines constitute a carrier virus, in most cases adenovirus or pox virus, that carries particular genes of the pathogen of interest. These carriers are non-virulent but are capable of expressing those genes of interest in large number. Once these vectors express those inserted genes, protective immunity and T-cell response against those antigens are generated (Minor 2015). The adenovirus-based vaccines are constructed by altering the E1A and E1B region of their genetic material so that the virus loses its ability to replicate (Wold and Toth 2013), but due to their innate character, the host cells are made to express the adenoviral receptors on their surface allowing the immune system to identify it (Lee et al. 2017). They are capable of expressing inserts of up to 8 kb (Lauer et al. 2017). Even the measles virus has been utilized as a vector after introducing several mutations, rendering them replication deficient and non-virulent (Zuniga et al. 2007).

Studies have proved that vector-based genetically modified vaccines are in the pipeline for the release in the market and two vectors are most successful experimentally for this kind of vaccines: pox virus and adenovirus (Ramezanpour et al. 2016). Some of the bacterial vectors and DNA vectors have been studied for this purpose as the only problem of using the viral vector is the fact that viral vectors might have pre-existing immunity. This might be considered as a very important drawback for the use of adenoviral vectors, although scientists have developed several strategies to overcome this problem (Antrobus et al. 2014; Dicks et al. 2012; Nébié et al. 2014). These vectored vaccines have immense possibilities, but large-scale production of such vaccines to meet the global requirement is still a concern.

20.3 Recent Strategies of Vaccine Production to Combat Emerging Diseases

Despite having successful conventional vaccine-producing methods and significant restriction of many infectious diseases, multiple pandemic outbreaks in the last few decades have alarmed the scientists to prepare more efficient, cost-effective, and full-proof methods. In the recent past, outbreaks like influenza A, Zika virus, severe acute respiratory syndrome (SARS), dengue, etc. have been a great threat to the mankind. Among parasitic diseases, malaria is also a worldwide menace which requires immediate effective vaccines. But, the complexity and evasive mechanisms of the parasite make malaria vaccine development a very difficult task. Some progress has been made in this field as the RTS,S/AS01 candidate vaccine has completed its phase 3 trial. But the use of the parasitic microorganism for the vaccine development is consistent. A recent study published in *Nature* showed that immunization with upregulated in infective sporozoites gene 3 (*uis3*)-deficient sporozoites showed to produce complete protection against infectious sporozoite challenge in a rodent malaria model (Mueller et al. 2005).

Influenza A viruses have caused severe pandemic more than once in the recent past, starting from “Spanish flu” in the 1920s to “swine flu” (H1N1pdm09) in the early 2000s. In 2009, WHO declared phase 6 pandemic alert for swine flu, which was a mild symptomatic flu, whereas Spanish flu caused millions of deaths in the 1920s (Johnson and Mueller 2002). The high genome variability of influenza A virus and its ability to infect a large variety of hosts make this virus more dangerous. This high variability can develop a new pathogenic condition with completely different properties in a very short time, but to predict its pathogenicity or infectivity is next to impossible.

Vector-borne diseases like dengue, Zika, and chikungunya are very common outbreaks in different parts of the world, specifically in Asia and America. These diseases are basically associated with high fever and joint pains, but in the recent past, they have caused severe clinical manifestations such as congenital abnormalities and Guillain-Barré syndrome, caused by Zika virus (Rauch et al. 2018). Another important outbreak that occurred in the 1970s is of Ebola virus, characterized with hemorrhagic fever and very high mortality rate. Vaccine development against this virus is still in very preliminary phase.

The recent most outbreak that literally shook the whole world with its high infectivity is COVID-19, which is a form of SARS and is caused by a novel corona virus. SARS was first reported in China in the year 2002, and it is likely to be originated in bats (Drosten et al. 2003; Fouchier et al. 2003). In 2012, a new corona virus outbreak was recorded in Saudi Arabia, termed as Middle East respiratory syndrome (MERS). Both these outbreaks were believed to be managed efficiently without much damage to the mankind. But, in 2019, another corona virus (SARS-CoV2) appeared and spread all over the world infecting more than 260 million people around the world and causing death of almost a million people till date. Although the mortality rate is near about 4%, the high infectivity and the ability of the virus to mutate to different forms make it very dangerous.

Scientists have developed various successful methods for vaccine development, but in outbreak situations however, most of these conventional vaccine development methods do not even stand a chance. For example, using the live attenuated vaccines has been of great success against various diseases as described earlier, but using such attenuated forms in an outbreak situation can cause severe issues as these forms can revert back to virulence. Moreover, most of these recent outbreak-causing pathogens are very poorly understood. For example, scientists have not yet confirmed about the numbers and characters of different strains of SARS-CoV2, where the development of a vaccine is of utmost importance.

Scientists are now doing extensive research on developing new methods of vaccine production and also modifying some pre-existing methods. Use of modified vectored vaccine is one of the promising approaches that are currently studied by researchers extensively. Till date, there are a large number of viral vectors available, and scientists have also managed to gather a vast amount of knowledge about their manipulation and function as immunogen is also vast. The biggest advantage with viral vectors might be the surety of expression of target antigen within the host body, their ability to mimic the condition that occurred during natural infection, and their ability to induce both cellular and humoral immunity against the target antigen.

Another new regime of vaccine development is the production of nucleic acid vaccine. These are simply the antigen coding DNA or RNA that are introduced within the host. After the host cell uptakes these nucleic acid sequences, they express those antigens on the surface of the cell, making them easy target for both cellular and humoral immune response. There are several ways of insertion of such DNA or RNA vaccines within the host body such as conventional intramuscular or intradermal injections. DNA vaccines are, however, less immunogenic when administered via such conventional methods. The reason behind this may be the fact that DNA need to enter the nucleus crossing two membranes, plasma membrane and nuclear membrane. Only then DNA can be transcripted to RNA and then translated to the desired antigen. On the other hand, RNA vaccine can work efficiently after crossing only the plasma membrane where it can be utilized by the endoplasmic reticulum to synthesize desired protein. To overcome this limitation with DNA vaccines, scientists have developed many other ways of vaccine administration such as use of gene gun, jet injection, electroporation, etc. (Lambricht et al. 2016; Sardesai and Weiner 2011).

Nucleic acid vaccines are presently being tested for a wide array of pandemic-causing pathogens such as HIV, MERS, SARS, and Ebola, which is mainly due to their high level of versatility, target specificity, and safety. The first effective vaccine developed against Ebola virus is a DNA vaccine, consisting of viral glycoprotein and nucleoprotein coding sequences that are believed to be capable of inducing both cellular and humoral immunity (Vanderzanden et al. 1998). After the outbreak of H1N1 influenza in the last decade as mentioned earlier, a vaccine was opted for clinical trial, which consists of DNA encoding hemagglutinin protein of H1N1pdm09 (Crank et al. 2015). DNA vaccines are also being developed against Zika virus that encodes precursor membrane and envelop proteins which serve as the target for protective antibodies (Muthumani et al. 2016). mRNA vaccines are also being used against several pathogens, among which mRNA vaccine encoding HIV-1 clade C envelop glycoprotein induced immune response in non-human primates (179, V2). Another RNA replicon encoding Lassa virus glycoprotein complex also has been shown to elicit sufficient immune response (Wang et al. 2018). Scientists are also trying to develop RNA vaccines against Zika virus, Ebola virus, etc.

20.4 Conclusion

The development of vaccines is undoubtedly one of the most important advancements of medical science; however, modifications and improvements in this field with the emerging and upcoming pandemic threats raised by known or unknown pathogens are of utmost importance. It is quite evident that the production of vaccine is very closely associated with the utilization of different microbes in several ways. Starting from the preliminary vaccines like live attenuated vaccines or killed whole organism vaccines to the modern vectored vaccine or nucleic acid vaccine, the necessity of microbes had never been questioned. Experience from the recent past has taught us that pandemic outbreaks cannot be predicted by any means and even the very well-known pathogen can cause unexpected damage to the whole mankind due to a very small mutation. Therefore, it is extremely important for scientists to have profound knowledge about the characteristics of the microbes so that they can be utilized to the best of their ability at the time of outbreaks. Furthermore, the microbes should be studied thoroughly to eliminate the chance of unfavorable and adverse effects on the host organism over a long period of time. It has to be made sure that these microbes should never cause pathogenesis itself or should never interfere with the pre-existing immunity of the individual. The basic needs of a vaccination program at the time of an outbreak are that the vaccines should be safe, efficient, well-targeted, cost-effective, easy to develop, and use, so that they can be produced at large amount meeting the global requirement and applied to people all over the pandemic-stricken areas within a minimum amount of time. It is impossible for any of the single methods of vaccination to provide solution for every future infectious disease or pandemic situation. It will be really very foolish to eliminate conventional methods just because they are age old and new strategies are coming forward. Therefore, it is important to understand that the combination of our present knowledge, ongoing researches, and future developments is extremely necessary to combat the upcoming pandemic situations more efficiently.

References

Antrobus RD, Coughlan L, Berthoud TK, Dicks MD, Hill AV, Lambe T, Gilbert SC (2014) Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved influenza a antigens. Mol Ther J Am Soc Gene Ther 22:668–674. https://doi.org/10.1038/mt.2013.284

Baicus A (2012) History of polio vaccination. World J Virol 1:108–114. https://doi.org/10.5501/wjv.v1.i4.108
Baxby D (1999) Edward Jenner's inquiry after 200 years. BMJ 318:390
Bernstein DI (2006) Live attenuated human rotavirus vaccine. Rotarix Semin Pediatr Infect Dis 17:188–194. https://doi.org/10.1053/j.spid.2006.08.006
Brockstedt DG, Bahjat KS, Giedlin MA, Liu W, Leong M, Luckett W, Gao Y, Schnupf P, Kapadia D, Castro G, Lim JYH, Sampson-Johannes A, Herskovits AA, Stassinopoulos A, Bouwer HGA, Hearst JE, Portnoy DA, Cook DN, Dubensky TW (2005) Killed but metabolically active microbes: a new vaccine paradigm for eliciting effector T-cell responses and protective immunity. Nat Med 11:853–860. https://doi.org/10.1038/nm1276
Cate TR, Couch RB, Kasel JA, Six HR (1977) Clinical trials of monovalent influenza a/New Jersey/76 virus vaccines in adults: reactogenicity, antibody response, and antibody persistence. J Infect Dis 136(Suppl):S450–S455. https://doi.org/10.1093/infdis/136.supplement_3.s450
Collins ND, Barrett ADT (2017) Live attenuated yellow fever 17D vaccine: a legacy vaccine still controlling outbreaks in modern day. Curr Infect Dis Rep 19:14. https://doi.org/10.1007/s11908-017-0566-9
Crank MC, Gordon IJ, Yamshchikov GV, Sitar S, Hu Z, Enama ME, Holman LA, Bailer RT, Pearce MB, Koup RA, Mascola JR, Nabel GJ, Tumpey TM, Schwartz RM, Graham BS, Ledgerwood JE, VRC 308 Study Team (2015) Phase 1 study of pandemic H1 DNA vaccine in healthy adults. PLoS One 10:e0123969. https://doi.org/10.1371/journal.pone.0123969
Daniels CC, Rogers PD, Shelton CM (2016) A review of pneumococcal vaccines: current polysaccharide vaccine recommendations and future protein antigens. J Pediatr Pharmacol 21:27–35. https://doi.org/10.5863/1551-6776-21.1.27
Dicks MDJ, Spencer AJ, Edwards NJ, Wadell G, Bojang K, Gilbert SC, Hill AVS, Cottingham MG (2012) A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. PLoS One 7:e40385. https://doi.org/10.1371/journal.pone.0040385
Drosten C, Günther S, Preiser W, van der Werf S, Brodt H-R, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RAM, Berger A, Burguière A-M, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra J-C, Müller S, Rickerts V, Stürmer M, Vieth S, Klenk H-D, Osterhaus ADME, Schmitz H, Doerr HW (2003) Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348:1967–1976. https://doi.org/10.1056/NEJMoa030747
Dubé E, Laberge C, Guay M, Bramadat P, Roy R, Bettinger JA (2013) Vaccine hesitancy. Hum Vaccines Immunother 9:1763–1773. https://doi.org/10.4161/hv.24657
Dubensky TW, Skoble J, Lauer P, Brockstedt DG (2012) Killed but metabolically active vaccines. Curr Opin Biotechnol 23:917–923. https://doi.org/10.1016/j.copbio.2012.04.005
Famulare M, Chang S, Iber J, Zhao K, Adeniji JA, Bukbuk D, Baba M, Behrend M, Burns CC, Oberste MS (2016) Sabin vaccine reversion in the field: a comprehensive analysis of Sabin-like poliovirus isolates in Nigeria. J Virol 90:317. https://doi.org/10.1128/JVI.01532-15
Fouchier RAM, Kuiken T, Schutten M, van Amerongen G, van Doornum GJJ, van den Hoogen BG, Peiris M, Lim W, Stöhr K, Osterhaus ADME (2003) Aetiology: Koch's postulates fulfilled for SARS virus. Nature 423:240. https://doi.org/10.1038/423240a
Galen JE, Buskirk AD, Tennant SM, Pasetti MF (2016) Live Attenuated Human Salmonella Vaccine Candidates: tracking the pathogen in natural infection and stimulation of host immunity. EcoSal Plus:7. https://doi.org/10.1128/ecosalplus.ESP-0010-2016
Germanier R, Füer E (1975) Isolation and characterization of gal E mutant ty 21a of Salmonella typhi: a candidate strain for a live, oral typhoid vaccine. J Infect Dis 131:553–558. https://doi.org/10.1093/infdis/131.5.553
Gilchrist SAN, Nanni A, Levine O (2012) Benefits and effectiveness of administering pneumococcal polysaccharide vaccine with seasonal influenza vaccine: an approach for policymakers. Am. J. Public Health 102:596–605. https://doi.org/10.2105/AJPH.2011.300512

Glenny AT, Hopkins BE (1923) Diphtheria toxoid as an Immunising agent. Br J Exp Pathol 4:283–288

Gotschlich EC, Liu TY, Artenstein MS (1969) Human immunity to the meningococcus. 3. Preparation and immunochemical properties of the group a, group B, and group C meningococcal polysaccharides. J Exp Med 129:1349–1365. https://doi.org/10.1084/jem.129.6.1349

Griesenauer RH, Kinch MS (2017) An overview of FDA-approved vaccines & their innovators. Expert Rev Vaccines 16:1253–1266. https://doi.org/10.1080/14760584.2017.1383159

Holmgren J, Svennerholm AM, Jertborn M, Clemens J, Sack DA, Salenstedt R, Wigzell H (1992) An oral B subunit: whole cell vaccine against cholera. Vaccine 10:911–914. https://doi.org/10.1016/0264-410x(92)90324-d

Hudson MJ, Beyer W, Böhm R, Fasanella A, Garofolo G, Golinski R, Goossens PL, Hahn U, Hallis B, King A, Mock M, Montecucco C, Ozin A, Tonello F, Kaufmann SHE (2008) *Bacillus anthracis*: balancing innocent research with dual-use potential. Int J Med Microbiol 298:345–364. https://doi.org/10.1016/j.ijmm.2007.09.007

Johnson NPAS, Mueller J (2002) Updating the accounts: global mortality of the 1918-1920 "Spanish" influenza pandemic. Bull Hist Med 76:105–115. https://doi.org/10.1353/bhm.2002.0022

Lambricht L, Lopes A, Kos S, Sersa G, Préat V, Vandermeulen G (2016) Clinical potential of electroporation for gene therapy and DNA vaccine delivery. Expert Opin Drug Deliv 13:295–310. https://doi.org/10.1517/17425247.2016.1121990

Lauer KB, Borrow R, Blanchard TJ (2017) Multivalent and multipathogen viral vector vaccines. Clin Vaccine Immunol 24. https://doi.org/10.1128/CVI.00298-16

Lee CS, Bishop ES, Zhang R, Yu X, Farina EM, Yan S, Zhao C, Zheng Z, Shu Y, Wu X, Lei J, Li Y, Zhang W, Yang C, Wu K, Wu Y, Ho S, Athiviraham A, Lee MJ, Wolf JM, Reid RR, He T-C (2017) Adenovirus-mediated gene delivery: potential applications for gene and Cell-based therapies in the new era of personalized medicine. Genes Dis 4:43–63. https://doi.org/10.1016/j.gendis.2017.04.001

Luca S, Mihaescu T (2013) History of BCG vaccine. Maedica 8:53–58

Madsen T (1933) Vaccination against whooping cough. J Am Med Assoc 101:187–188. https://doi.org/10.1001/jama.1933.02740280007003

Minor PD (2012) The polio-eradication programme and issues of the end game. J Gen Virol 93:457–474. https://doi.org/10.1099/vir.0.036988-0

Minor PD (2015) Live attenuated vaccines: historical successes and current challenges. Virology 479–480:379–392. https://doi.org/10.1016/j.virol.2015.03.032

Mueller A-K, Labaied M, Kappe SHI, Matuschewski K (2005) Genetically modified Plasmodium parasites as a protective experimental malaria vaccine. Nature 433:164–167. https://doi.org/10.1038/nature03188

Muthumani K, Griffin BD, Agarwal S, Kudchodkar SB, Reuschel EL, Choi H, Kraynyak KA, Duperret EK, Keaton AA, Chung C, Kim YK, Booth SA, Racine T, Yan J, Morrow MP, Jiang J, Lee B, Ramos S, Broderick KE, Reed CC, Khan AS, Humeau L, Ugen KE, Park YK, Maslow JN, Sardesai NY, Joseph Kim J, Kobinger GP, Weiner DB (2016) In vivo protection against ZIKV infection and pathogenesis through passive antibody transfer and active immunisation with a prMEnv DNA vaccine. NPJ Vaccines 1:16021. https://doi.org/10.1038/npjvaccines.2016.21

Nébié I, Edwards NJ, Tiono AB, Ewer KJ, Sanou GS, Soulama I, Sanon S, Diarra A, Yaro JB, Kangoye D, Imoukhuede EB, Hill AVS, Sirima SB (2014) Assessment of chimpanzee adenovirus serotype 63 neutralizing antibodies prior to evaluation of a candidate malaria vaccine regimen based on viral vectors. Clin Vaccine Immunol 21:901–903. https://doi.org/10.1128/CVI.00723-13

Ni Y, Springer MJ, Guo J, Finger-Baker I, Wilson JP, Cobb RR, Turner D, Tizard I (2017) Development of a synthetic vi polysaccharide vaccine for typhoid fever. Vaccine 35:7121–7126. https://doi.org/10.1016/j.vaccine.2017.10.081

Pennington SH, Thompson AL, Wright AKA, Ferreira DM, Jambo KC, Wright AD, Faragher B, Gilmour JW, Gordon SB, Gordon MA (2016) Oral typhoid vaccination with live-attenuated Salmonella Typhi strain Ty21a generates Ty21a-responsive and heterologous influenza virus-responsive CD4+ and CD8+ T cells at the human intestinal mucosa. J Infect Dis 213:1809–1819. https://doi.org/10.1093/infdis/jiw030

Plotkin S (2014) History of vaccination. Proc Natl Acad Sci U S A 111:12283–12287. https://doi.org/10.1073/pnas.1400472111

Ramezanpour B, Haan I, Osterhaus A, Claassen E (2016) Vector-based genetically modified vaccines: exploiting Jenner's legacy. Vaccine 34:6436–6448. https://doi.org/10.1016/j.vaccine.2016.06.059

Ramon G (1923) Sur le pouvoir floculant et sur les proprietes immunisantes d'une toxin diphterique rendu anatoxique (anatosine). C R Acad Sci Paris 177:1338–1340

Rauch S, Jasny E, Schmidt KE, Petsch B (2018) New vaccine technologies to combat outbreak situations. Front Immunol 9. https://doi.org/10.3389/fimmu.2018.01963

Reckseidler-Zenteno SL (2012) Capsular polysaccharides produced by the bacterial pathogen Burkholderia pseudomallei. Complex World Polysacch. https://doi.org/10.5772/50116

Research C (2020) Vaccines licensed for use in the United States. FDA

Sardesai NY, Weiner DB (2011) Electroporation delivery of DNA vaccines: prospects for success. Curr Opin Immunol 23:421–429. https://doi.org/10.1016/j.coi.2011.03.008

Sato Y, Sato H (1999) Development of acellular pertussis vaccines. Biol J Int Assoc Biol Stand 27:61–69. https://doi.org/10.1006/biol.1999.0181

Schneerson R, Barrera O, Sutton A, Robbins JB (1980) Preparation, characterization, and immunogenicity of *Haemophilus influenzae* type b polysaccharide-protein conjugates. J Exp Med 152:361–376. https://doi.org/10.1084/jem.152.2.361

Tangy F, Naim HY (2005) Live attenuated measles vaccine as a potential multivalent pediatric vaccination vector. Viral Immunol 18:317–326. https://doi.org/10.1089/vim.2005.18.317

Valenzuela P, Medina A, Rutter WJ, Ammerer G, Hall BD (1982) Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. Nature 298:347–350. https://doi.org/10.1038/298347a0

Vanderzanden L, Bray M, Fuller D, Roberts T, Custer D, Spik K, Jahrling P, Huggins J, Schmaljohn A, Schmaljohn C (1998) DNA vaccines expressing either the GP or NP genes of Ebola virus protect mice from lethal challenge. Virology 246:134–144. https://doi.org/10.1006/viro.1998.9176

Wang M, Jokinen J, Tretyakova I, Pushko P, Lukashevich IS (2018) Alphavirus vector-based replicon particles expressing multivalent cross-protective Lassa virus glycoproteins. Vaccine 36:683–690. https://doi.org/10.1016/j.vaccine.2017.12.046

Wold WSM, Toth K (2013) Adenovirus vectors for gene therapy. Vaccination and Cancer Gene Therapy Curr Gene Ther 13:421–433

Yadav DK, Yadav N, Khurana SMP (2014) Chapter 26 - vaccines: present status and applications. In: Verma AS, Singh A (eds) Animal biotechnology. Academic Press, San Diego, pp 491–508. https://doi.org/10.1016/B978-0-12-416002-6.00026-2

Zuniga A, Wang Z, Liniger M, Hangartner L, Caballero M, Pavlovic J, Wild P, Viret JF, Glueck R, Billeter MA, Naim HY (2007) Attenuated measles virus as a vaccine vector. Vaccine 25:2974–2983. https://doi.org/10.1016/j.vaccine.2007.01.064

21 Applications of Microbes in Municipal Solid Waste Treatment

Ouahid El Asri, Soufiane Fadlaoui, and Mohamed Elamin Afilal

Abstract

The production of municipal solid waste continues to grow every year, which has received great reflection. This daily production without recycling, treatment, and valorization generates environmental crises, health impacts, and economic costs on several municipalities in the world. Currently, microbes in the treatment and recovery processes of municipal solid waste have become a common practice. We will present in this chapter two essential microbial practices (composting and anaerobic digestion) which used microbial inoculation for improved and increased performances.

In the composting process, the application of microbes plays several roles: improvement of humification, secretion of catabolism enzymes, minimize the initial lag time, and reduce the process treatment and odorous emissions. So, the microbial inoculation in the composting way of municipal solid waste provides good compost, increasing soil fertilization, and improving agriculture. In anaerobic digestion technology, the application of microbes in this process has become mandatory. It increases the methane production and hydrolysis rate of municipal solid waste and shortens the start-up time to get the high energy quickly. So, the microbial application for municipal solid waste treatment is an eco-friendly tool, less expensive, and efficient for a zero-waste economy.

O. El Asri (✉)
Plant Technology Laboratory, Ibn Zohr University, Agadir, Morocco

S. Fadlaoui
Ecology, Water and Environment Laboratory, Mohamed First University, Oujda, Morocco

M. E. Afilal
LBBS, Faculty of sciences, Mohamed First University, Oujda, Morocco

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Anaerobic digestion · Composting · Inoculation · Microbial enzymes · Waste

21.1 Introduction

Currently, all the world countries are looking for suitable strategies for the management and treatment of the waste resulting from daily activities. The production of municipal solid waste (MSW) continues to grow every year, which has received significant attention. For example, Morocco generates 5.3 million tons in urban cities and 1.5 million tons in a rural towns in 2018 (Ouigmane et al. 2018). More than 2400 and 96 million tons of nonhazardous and hazardous wastes, respectively, have been produced by the European Union in 2015 (Scarlat et al. 2019). In the United States, the annual MSW production increased from 88 in 1960 to 267 million tons in 2017 (Dogaris et al. 2020). China's yearly MSW production is expected to grow from about 190 million tons (2004) to over 480 million tons (2030) (Minghua et al. 2009). So, the increase of MSW in each country is linked to several socioeconomic and cultural factors of each nation, such as economic growth, the rapid expansion of the cities, and massive migration of population from rural to urban centers (Awasthi et al. 2014; Mian et al. 2017).

Without recycling, treatment, and valorization, the MSW production generates environmental crises, health impacts, and economic costs on several municipalities. Munawar et al. (2018) declared that one- to two-thirds of MSW produced is not collected and transported to the treatment units (Munawar et al. 2018). These wastes contaminate aquatic ecosystems and groundwater via leachate (Elasri and Afilal 2014; Raghab et al. 2013), the soil by direct contact or leachate (Sharma et al. 2018), and the atmospheric air by gas of MSW combustion or production of greenhouse gas (GHG). Currently, we see that the GHG production through the MSW sector more doubled from 30.3 Gg CO_2 (2002) to 76,623 Gg (2017) (Zhao et al. 2020). So, this continuous genesis of MSW requires an effective treatment to protect the environment.

There are several MSW treatment techniques (landfill, incineration, composting, and anaerobic digestion). Landfill is a classical way that requires a large land area and contributes to producing a large amount of GHG. Landfill participates ten times larger in climate change than others way treatment (Gao et al. 2017). Incineration reduces the volume of waste and produces energy, but it can produce atmospheric pollution. Linville et al. (2015) declare that anaerobic digestion (AD) is a better technology for MSW management than the current practice. The upward technologies adopted for the treatment, management, and valorization of MSW are AD and composting (Abdel-Shafy and Mansour 2018). Gao et al. (2017) have classified the MSW treatment ways in the suitable sequence from anaerobic digestion, composting, and incineration to landfill. So, AD and composting are the best-recommended treatment technologies of MSW.

Composting is an aerobic microbiological process, but AD is anaerobic microbial degradation. So, the composting and AD of MSW are microbial technologies for waste recovery based on the biodegradation of organic matter by diverse microbes, including bacteria and fungi. The conversion of MSW during the composting process indicates a succession microbial community (Peters et al. 2000). Meng et al. (2019) showed a large microbial diversity such as *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Ascomycota* in the composting technology. This treatment process produces a stable organic matter called compost. The stability and maturity of compost are linked strongly to microbial activities; when we have less microbial activity at the end of this biological process, we have better compost for agriculture activities (Kumar 2011).

The AD produces two products, biogas which contains methane and digestate. Methane gas is a source of thermal and electrical energy; conversely, digestate can be used as a soil fertilizer (Angelidaki et al. 2003; Mata-Alvarez et al. 2000). AD is a multi-stage microbial decomposition of organic matter in MSW; it comprises four phases (hydrolysis, acidogenesis, acetogenesis, and methanogenesis). The microbial populations are different among the stages of AD. More than 50 bacteria types provide the hydrolysis and acidogenesis phases; the principal phyla are *Clostridium*, *Butyrivibrio*, *Pseudomonas,* and *Bacillus* (Wang et al. 2018). The methanogenesis phase consists of 65 archaea species distributed to seven families, let us quote *Methanosarcina* and *Methanomicrobium* (Nielsen et al. 2007). The type of microbe community in the digester is an indicator of stable or failure AD treatment (Wang et al. 2018). So, the microbe's types play a significant role in the two treatment technologies of MSW.

This chapter is an excellent tool in which we will show the role of applications of microbes in MSW treatment technologies. For this, we have divided this chapter into three axes: a) We discussed in the first part the production of MSW in the world and their characteristics to determine the suitable remedy. b) The second axis consists of showing the role of microbial inoculation in the composting of MSW and the presentation of new microbial applications in this treatment field. c) We will highlight some new applications of microorganisms in anaerobic digestion and their importance in improving this process.

21.2 Municipal Solid Waste: Production, Composition, and Characteristics

21.2.1 Production of Municipal Solid Waste

Several researchers have described that the solid waste is not easy to define because it has great complexity and variety of chemicals substances (Albanna 2013). The definition of MSW is different among researchers from low developed countries to developed countries. Periathamby (2011) proposed a global definition: all wastes produced, collected, transported, and stored within the jurisdiction of a municipal authority (Periathamby 2011). The MSW has been made principally from

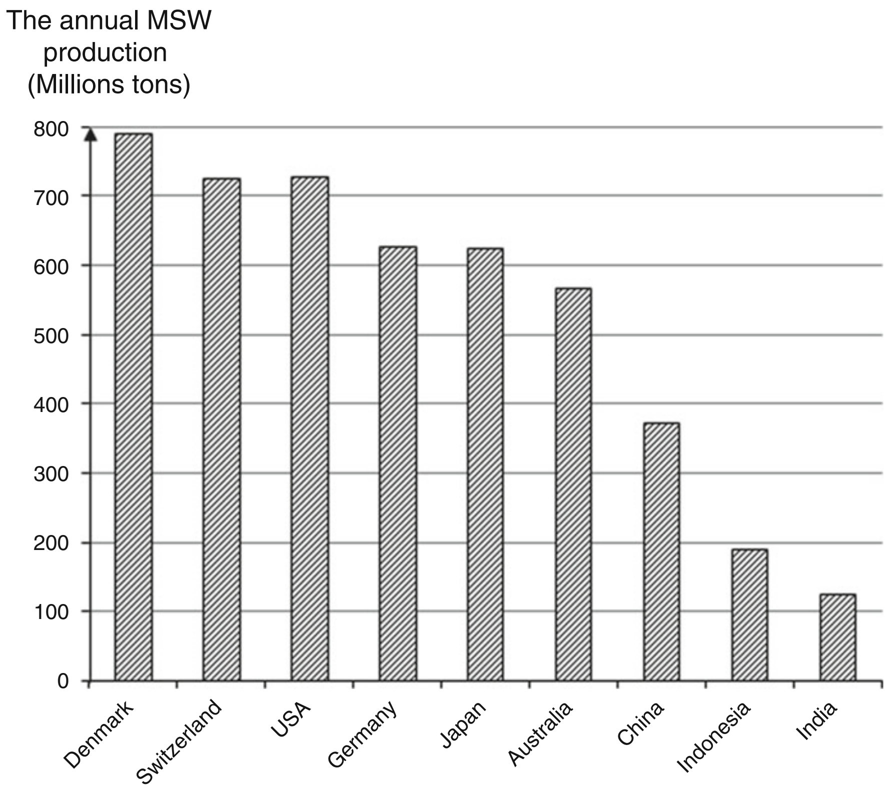

Fig. 21.1 The annual production of MSW by some countries

households (55–80%) and marketplaces (10–30%) (Abdel-Shafy and Mansour 2018). The current world situation has been described by Mushtaq et al. (2020); they have declared that all countries around the world produce 2 billion tons of MSW, but this amount will increase toward 3.4 billion tons in 2050. The annual municipal solid waste production differs in each country (Fig. 21.1). It is influenced by the economic situation, education, citizen custom, urbanization speed, and climatic parameters (Brindha and Schneider 2019). Currently, there is a tremendous scientific debate on the relationship between gross domestic product (GDP) and municipal solid waste production. Several researchers have confirmed the vital link between them (Ludwig et al. 2003). But other authors demonstrated no causality relationship (Lee et al. 2016). So, the production of MSW has a strong correlation between the citizen's income level and the size of the municipality.

21.2.2 Composition and Characteristics of Municipal Solid Waste

The annual production, composition, and characteristics of MSW are decisive for choosing the technique of treatment suitable. The comparison of waste composition shows that it differs remarkably from country to country. This difference depends mainly on the citizen custom, lifestyle, waste legislation, economic development, and industrial situation for each country (Abdel-Shafy and Mansour 2018). In a low-income country, the principal fraction of MSW is food waste, but it is mainly reduced in a high-income country such as Germany (Table 21.1). Ludwig et al. (2003) have described in their books that the composition of MSW is a mirror of the social structure of each country (Ludwig et al. 2003). Several studies confirmed that the large fraction of MSW is organic matter. The MSW of Morocco, Indonesia, Singapore, India, Italy, and Germany are about 65%, 61.35%, 44.4%, 42%, 31%, and 30% of organic fraction, respectively (Karouach et al. 2020; Khair et al. 2019; Mühle et al. 2010; Shekdar 2009). This organic fraction accounts for between 40% and 70% of biodegradable material in developing countries (Wei et al. 2017). So, the MSW is characterized by a large amount of biodegradable matter, which will serve as a substrate for microbes.

21.3 Applications of Microbes in Composting of MSW

Composting is a method of waste recovery based on the biological degradation of organic matter under aerobic conditions to produce compost (Jurado et al. 2014). This degradation is assured by the microbial community succession, which uses carbon and nitrogen of MSW as the energy sources (Wei et al. 2017). Partanen et al. (2010) have called composting an aerobic microbiological process, ensured by two types of microbes: mesophilic and thermophilic bacteria and fungi (Partanen et al. 2010). Anastasi et al. (2005) have shown 194 entities of fungi in the composting process. Nevertheless, Ryckeboer et al. (2003) have counted 175 dominant bacterial colonies in the compost of biowaste. So, the microbes play a vital role in this process. The microbe community is helpful as additives (or inoculum) in composting treatment. This inoculation can be specific microbial communities, i.e., mixtures of cultures, consortium, or a single strain of microbes. In this chapter part, we will discuss the different applications of microbial additives in composting of MSW.

21.3.1 Production of Different Enzymes for MSW Degradation

We have already shown that MSW contains a significant fraction of organic matter. The latter component requires biochemical degradation using microbial enzymes. Jurado et al. have successfully identified some fungi and bacteria such as *Alternaria tenuissima*, *Cladosporium lignicolous*, *Bacillus licheniformis*, *Gibellulopsis nigrescens,* and *Streptomyces albus* that have been characterized by a wide range of metabolic activity (pectinolytic, cellulolytic, hemicellulolytic, ligninolytic,

Table 21.1 Composition and characteristics of MSW in some countries

	Paper (%)	Wood (%)	Plastics (%)	Metals (%)	Textiles (%)	Glass (%)	Food (%)	References
USA	27	6	13	9	9	4	15	Abdel-Shafy and Mansour (2018)
Algeria	0.7–5.8	–	0.3–8.9	0.4–2	1.7–7.5	0.4–5.9	53.2–77.2	Naïma et al. (2012)
Australia	26	1	7.1	2.5	4	11	21	El Hanandeh and El-Zein (2009)
China	2–12	0.5–13	2–14	0.2–1.7	1–6	0.8–4	38–73	Wang and Nie (2001)
Japan	32.2	2.3	32.2	2	6.4	0.9	39.8	Yamada et al. (2017)
Morocco	7–10	7	4–7	1	3	1.5	60–80	Naimi et al. (2017)
Germany	4.6	–	6.1	3.9	1.5	11.5	27	Vehlow (1996)

amylolytic, proteolytic, lipolytic, ammonifying, and phosphate-solubilizing activities) (Table 21.2) (Jurado et al. 2014). These activities are effectuated through the enzymes produced by last microbes. These microbial enzymes are responsible for the hydrolysis of complicated macromolecules of MSW (Vargas-García et al. 2010). Also, these bacteria and fungi described important thermotolerance and the ability to deteriorate a large type of organic waste (Anastasi et al. 2005).

The significant organic fraction of MSW is plant-derived carbohydrates such as cellulose and lignin (Meor Hussin et al. 2013). The MSW is composed of cellulose (40–50%), hemicellulose (9–12%), and lignin (10–15%) on a dry weight waste (Barlaz 1998). Thus, we can add some fungi that secreted ligninolytic enzymes such as *Talaromyces emersonii* and *Thermoascus aurantiacus* thermostable product xylanases, *Aureobasidium pullulans* products b-xylosidase, *Ceriporiopsis subvermispora* product laccase, *Phanerochaete chrysosporium*, and *Chrysonilia sitophila* product lignin peroxidases that are capable of degrading a number of lignin model compounds (Jeffries 1994; Wan and Li 2012). So, these fungi can generate several ligninolytic enzymes that oxidize the lignin polysaccharide and produce aromatic radicals.

Also, we can add some bacteria such as *Bacillus stearothermophilus* and *Butyrivibrio fibrisolvens* that possess b-xylosidase and a-L-arabinofuranosidase, respectively (Jeffries 1994). Some researchers have screened many bacteria (*Novosphingobium* sp., *Cupriavidus basilensis*, and *Comamonas* sp.) for lignocellulose biorefinery (Zhuo et al. 2018). Lin et al. (2011) have created an artificial fungal consortium composed of 92% of *Trichoderma* sp. 6.7% of *Phanerochaete chrysosporium,* and 1.3% of *Aspergillus oryzae*. This fungal consortium produces many cellulolytic enzymes for strong saccharification of food waste (Lin et al. 2011).

The microbial degradation of lignocellulosic in MSW is separated into two phases: depolymerization of lignin by extracellular enzymes and intracellular degradation of residual aromatic compounds (Masai et al. 2007). So, these microbes that we described previously can secrete the extra- or intracellular enzymes to facilitate the decomposition of MSW. Thus, they are significant inoculants for the production of good compost (Table 21.3).

21.3.2 Improved the Environmental Parameters of MSW Composting

The environmental parameters like temperature, pH, C/N ratio, moisture content, ammonium, and nitrate within composting process are a significant key to producing an excellent compost of MSW. The diversity of microorganisms in composting process varied depending on the temperature of decomposition (Miyatake and Iwabuchi 2005). Under aerobic conditions, both bacteria such as *Escherichia coli* and *Lactobacillus* can decrease pH composting by oxidizing food waste (Song et al. 2018). Song et al. (2018) have described that the inoculation of pure cultured microbial strains is an alternative way to prevent the drop in pH (Song et al. 2018). Zhang et al. (2011) have confirmed the strong relationships between

Table 21.2 Comparison of biochemical activity of *Alternaria tenuissima*, *Cladosporium lignicola*, *Bacillus licheniformis*, *Gibellulopsis nigrescens*, and *Streptomyces albus* in composting of MSW (+: presence activity, −: lacking activity)

	Fungi			Bacteria		
	Ascomycota			Firmicutes	Proteobacteria	Actinobacteria
	Alternaria tenuissima	*Gibellulopsis nigrescens*	*Cladosporium lignicola*	*Bacillus licheniformis*	*Pseudomonas stutzeri*	*Streptomyces albus*
Cellulolytic	−	+	+	−	−	−
Hemicellulolytic	+	+	+	+	+	+
Pectinolytic	+	+	−	+	+	−
Amylolytic	+	+	+	+	−	+
Proteolytic	+	+	−	+	+	−
Ligninolytic	−	−	+	−	−	+
Lipolytic	+	+	−	+	+	+
Ammonifying	+	+	−	+	+	+

Table 21.3 Most bacteria used in different steps of anaerobic digestion

Phases of AD	Bacteria types
Hydrolysis	*Enterobacterium, Clostridium, Fusobacterium, Streptococcus, Selenomonas, Butyrivibrio*
Acidogenesis	*Pseudomonas, Bacillus, Clostridium, Lactobacillus, Eubacterium, Ruminococcus, Bacteroides, Micrococcus, Flavobacterium*
Acetogenesis	*Syntrophobacter, Syntrophomonas, Syntrophospora, Smithella*
Methanogenesis	*Methanococcus, Methanosarcina, Methanothrix, Methanospirillum, Methanobacterium, Methanobrevibacter*

microbial community and environmental parameters of the composting process (Zhang et al. 2011).

During the composting treatment of MSW, the ammonium can be oxidized to form nitrite by ammonia-oxidizing bacteria, which is then transformed into nitrate by nitrite-oxidizing bacteria (Yamamoto et al. 2010). These relationships have been found between ammonium and nitrate with the bacterial but not fungal species (Zhang et al. 2011). Several studies showed that inoculation in the compost of MSW could reduce ammonia emission and nitrogen loss by transforming ammonium (Ohtaki et al. 1998; Selvamani et al. 2019; Zhang et al. 2016). These microbial additives include *Nitrosomonas*, *Bacillus,* and *Nitrosospira* sp. (Kowalchuk et al. 1999). Wei et al. (2007) have recommended a mixture of microorganisms (Bacillus casei, *Lactobacillus buchneri,* and *Candida rugopelliculosa*) and lignocellulolytic microorganisms (*Trichoderma* and white-rot fungi) for MSW composting. Diverse researches have shown that microbial additives installed positive environmental parameters of composting treatment (Wei et al. 2007). So, it is clear that inoculation can establish good environmental conditions of MSW composting.

21.3.3 Reduced the MSW Composting Period

The composting time of MSW fluctuated from 10 days to 3 months (Elango et al. 2009). So, to reduce this parameter, the insertion of microbial inoculation is indispensable. Wei et al. indicated that microbial additives successfully decreased the processing time of MSW composting (Wei et al. 2007). Heidarzadeh et al. (2019) demonstrated that fungal inoculation by *Aspergillus niger* reduces the time of MSW composting to 18 days, thereby decreasing cost treatment (Heidarzadeh et al. 2019). Manu et al. (2019) recommended adding microbes at the start of MSW composting to activate the decomposition period within 30 days and reach maximum temperature in 3–6 days (Manu et al. 2019). Awasthi et al. (2014) have inoculated MSW with 5 liters of suspension microbial (*Trichoderma viride*, *Aspergillus niger,* and *Aspergillus flavus*), which leads to achieving faster compost maturity (Awasthi et al. 2014). Song et al. (2018) have demonstrated that the inoculation with the microbial consortium composed by *Dysgonomonas* sp., *Pseudomonas caeni, Aeribacillus pallidus, Pseudomonas* sp., *Lactobacillus salivarius, Bacillus thuringiensis,* and *Bacillus cereus* allows avoiding the lag phase in the pile temperature and enormously shorten the composting period (Song et al. 2018). So, the microbial additives in the composting MSW treatment can minimize the initial lag time and reduce the processing period.

21.3.4 Improved Humification During MSW Composting

Composting is also a humification process of MSW to produce both acids, humic and fulvic. They are humic substances that play a crucial role in soil fertility and plant growth (Allard et al. 1991; Canellas et al. 2015). Some studies measured these acids for characterizing the maturity and stability of compost products (Amir et al. 2005; Huang et al. 2006). Some researchers have used microbial inoculation to increase the production of humic acid and fulvic acid. Zeng et al. (2010) have used the fungi, *Phanerochaete chrysosporium*, to increase the production of humic acid (Zeng et al. 2010). Xi et al. (2012) recommended bacterial inoculation by *Nitrobacter* and *Thiobacillus* to increase the humic and fulvic acids to get a good humification degree of MSW (Xi et al. 2012). So, the microbial inoculation methods are efficiently applied to the improvement of the humification process of MSW.

21.3.5 Reduce the Odorous Emissions by Biofilter

One big problem for composting manufactory is odor emission within the neighboring environment (Sundberg et al. 2013). The major composting exhaust gases of MSW are volatile organic compounds that included sulfur, nitrogen, phenols, alcohols, ketones, esters, terpenes, and volatile fatty acids (Pagans et al. 2006). Only in the composting of livestock mortality can we measure more than 200 volatile organic compound types (Akdeniz et al. 2010). We can adapt microbial inoculation to reduce emissions from these processes, such as the biofiltration technique.

In the biofilter, odorous air is passed through a mixture of compost and woodchips that populated with microbes. These microorganisms convert the contaminants principally into carbon dioxide and water (McNevin and Barford 2000). Detchanamurthy (2010) has cited that fungi *Paecilomyces variotii* and *Scedosporium apiospermum* are effective biofilter for treating volatile organic compounds because they have essential elimination capacity (245 g/m^3h) (Detchanamurthy and Gostomski 2012). Several studies recommended using bacterial additives, including *Bacillus azotofixams*, *Bacillus megaterium*, and *Bacillus mucilaginosus* to reduce odorous gas emissions and stabilize composting products (Karnchanawong and Nissaikla 2014; Xi et al. 2005). Chung (2007) demonstrated that some microbes (Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes) are satisfactory to remove nitrogen, sulfur, and total hydrocarbons at 30 s in the retention time. So, the emission odors generated during the composting of different MSW can decrease using biofiltration technology based on fungi and bacteria.

21.4 Applications of Microbes in Anaerobic Digestion of MSW

In the last decades, the anaerobic digestion (AD) of MSW was a treatment before landfilling (Nguyen et al. 2007). Several researchers have described that AD will be a better treatment alternative for MSW (Albanna 2013). The AD is a treatment process of MSW that is microbiologically converted under anaerobic conditions into carbon dioxide, methane, and small amounts of nitrogen, hydrogen, ammonia, and hydrogen sulfide (Gujer and Zehnder 1983; Moletta et al. 1986). This mixture of gaseous products is called biogas, and the process of anaerobic degradation is often called anaerobic digestion (Schirmer et al. 2014). The term "anaerobic digestion" is widely used synonymously to biomethanization (Braun 2007). This process is characterized by four linked and successive phases (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) (Angelidaki et al. 2011; Moletta 2015). It generates two products, the biogas converted to green energy, and digestate is used as fertilizer for agriculture applications (Erraji et al. 2017; Laiche et al. 2017). Many studies have confirmed that the four steps of the anaerobic digestion process are assured principally by different microbes. The microbes are an essential key of this process; they are useful as inoculum (or additives) in AD steps. Several researchers have confirmed the importance of inoculum and microbes community for biogas production from MSW (Liu et al. 2017; Lopes 2004). This inoculation can be specific microbial communities such as pure culture or a complex mixture like rumen fluid, sewage sludge, and animal manure. In this chapter part, we will discuss the different applications of microbial inoculum in AD of MSW.

21.4.1 Increasing Methane Production During MSW Anaerobic Treatment

The biogas produced from MSW and its quality depend on the amount of methane that it contains because this gas has a high calorific value (9.94 kWh/m^3) (El et al. 2015). So, the concrete economies benefit from biogas produced by AD of MSW when it contains a high methane content. We have already shown in some works that the waste/inoculum ratio is crucial for methane production. The maximum output of methane (69.9%) by AD of chicken waste is achieved by the highest proportion (1/1) (Elasri et al. 2018). These results reflect the dependence of methane production in AD of MSW with several microbes present in the digester.

We have recommended using particular inoculation to increase methane production; we can cite that the methanogens genus such as *Methanosaeta* sp. improved the methane production under high acetic acid content conditions (Elasri et al. 2018). Liu and Whitman (2008) have indicated that *Methanosarcinaceae* sp. has the immense ability to adapt in digesters for assured production of methane because it is a fast-growing and substrate-versatile methanogen (Liu and Whitman 2008). In other work, the addition of both methanogenic bacteria (*Methanosarcina* sp. and *Methanosaeta* sp.) in a digester with municipal waste give a maximum yield of biogas and methane (Singh et al. 2010). Liaquat et al. (2017) recommended a complex consortium composed of *Bacillus*, *Clostridium*, *Enterobacter*, *Methanomicrobia*, and *Methanosarcina* to improve methane production (Liaquat et al. 2017). Mayumi et al. (2016) have discovered that pure cultures of *Methermicoccus shengliensis* produce methane by decomposition of coal residues (Mayumi et al. 2016). Some archaebacteria such as *Methanobrevibacter arboriphilus* and *Methanobacterium formicium* are the dominant methane-producing bacteria in AD of organic matter (Gerardi 2003). De Vrieze et al. (2015) declared that methane production was strongly correlated with *Methanosaetaceae* (De Vrieze et al. 2015). We can see that using the previous bacteria as a suitable inoculum for improved biogas quality increases methane content. Finally, the microbial inoculation of AD of MSW improves methane yield and affects the physicochemical characteristics of the process.

21.4.2 Improving the Hydrolysis Rate of MSW

The hydrolysis phase is the first and significant step of anaerobic treatment because it is the limiting step of this biotechnology, and it is directly affecting biogas production (El and Afilal 2017). We can, therefore, consider that the treatment efficiency by AD of MSW is estimated from efficient hydrolysis of their organic content within the digester. The fermentative and hydrolytic microbes evacuate different types of exoenzymes like cellulase, xylanase, amylase, lipase, and protease for converting the complex organic molecules of MSW into simple substrates (Mir et al. 2016). Ozbayram et al. have demonstrated that the anaerobic microorganisms (*Ruminococcus flavefaciens*, *Ruminococcus albus*, and *Fibrobacter succinogenes*) isolated of rumen fluid are the most effective inoculation for improving the hydrolysis phase because they have efficient cellulolytic activities (Ozbayram et al. 2018). Thus, without hydrolytic microorganisms, the AD processes cannot be naturally initiated, and every biogas unit of MSW should be started with an inoculum.

Also, we have already shown in the first part that MSW is rich in hardly biodegradable compounds. The MSW is composed of cellulose (40–50%), hemicellulose (9–12%), and lignin (10–15%) on a dry weight waste (Barlaz 1998). Therefore, it is necessary to add microbes that make these compounds biodegradable. Burrell et al. (2004) have demonstrated that the conversion of cellulosic content in MSW to biogas is mediated by *Firmicutes* phylum, principally the genus *Clostridium,* i.e., *Clostridium stercorarium*, *Clostridium thermocellum* (Burrell et al. 2004). Cotta et al. have recommended using two bacteria *Butyrivibrio fibrisolvens* and *Selenomonas ruminantium,* to degrade xylan in organic waste and utilization of their xylooligosaccharide (Cotta and Zeltwanger 1995). Pohlschroeder et al. (1994) use both cultures of strictly anaerobic bacteria *Spirochaeta caldaria* and *Clostridium thermocellum* to increase cellulose degradation rates (Pohlschroeder et al. 1994). Sun et al. (2016) showed a strong correlation between the degradation of lignocellulose and the presence of bacteria belonging to *Firmicutes* and *Bacteroidetes,* which are the leading producers of glycoside hydrolase (Sun et al. 2016). Other works have mentioned that diverse microbes of *Firmicutes* and *Bacteroidetes* can degrade the fiber of MSW into different organic acids, and they have positive resistance for the concentration of organic acids produced (Li et al. 2016). These studies demonstrated that the application of hydrolytic microbes could improve the hydrolysis yields of MSW within the anaerobic digester. For later use of hydrolytic microbes such as bacterial inoculum, we have selected all anaerobic hydrolytic microbes used as additives in biogas plants to increase the phenomena of hydrolysis in Fig. 21.2.

21.4.3 Shorten the Start-up Time

The inoculation in AD of MSW not only affects the quality of biogas production and physicochemical characteristics of this process and influences treatment kinetics. The start-up of AD of MSW is crucial because it can establish the stability and action of all steps of treatment. For obtaining a promising start-up for AD units with high matter inputs such as MSW, we must have a good balance of hydrolytic microbes, proton-reducing acetogenic bacteria, and methanogens (Griffin et al. 1998). So, microbial inoculation is one of the keys that could affect the start-up situation.

Microbial inoculation plays a major part in the digester startup because they control the populations balancing of syntrobacter and methanogens. Wu et al. (2016) have described the microbial cooperation of some bacteria such as Firmicutes, Bacteroidetes, and Thermotoga that assure the first three steps (hydrolysis, acidogenesis, acetogenesis) with thermophilic methanogens for giving the fast start-up of AD (Wu et al. 2016). Mir et al. (2016) describe that the balance makes syntrophic metabolism thermodynamically feasible in AD treatment (Mir et al. 2016). McMahon et al. (2004) declared that the units of MSW treatment by AD with an elevated amount of Archaea principally Methanosaeta concilii started up successfully. In contrast, digesters that present with a lower amount of *Archaea* or abundant *Methanosarcina* spp. have a hard start-up time (McMahon et al. 2004). Griffin et al. (1998) confirmed that the successful start-up of biogas units have the highest amount of *Methanosaeta concilii* and the lowest amount of *Methanobacteriaceae* (Griffin et al. 1998). Brummeler et al. (2007) have observed during the start-up of a dry anaerobic batch of MSW a rapid methane formation because they have found a microbial shift in the methanogenic biomass (Brummeler et al. 2007). Finally, we can see that a good inoculation ensures a good balance between all the microbes of the AD phases, allows the success of the start of the process, and reduces the time of appearance of methane.

21.5 Conclusion

The annual amount of MSW increases worldwide. We are faced with the need to treat, manage, and enhance this growing flow of MSW tons. Microbes are exciting tools because it improves and increases the performance of composting and anaerobic digestion treatment of MSW. In the compost treatment, the application of microbes such as inoculation or additives can play a crucial role: improvement of

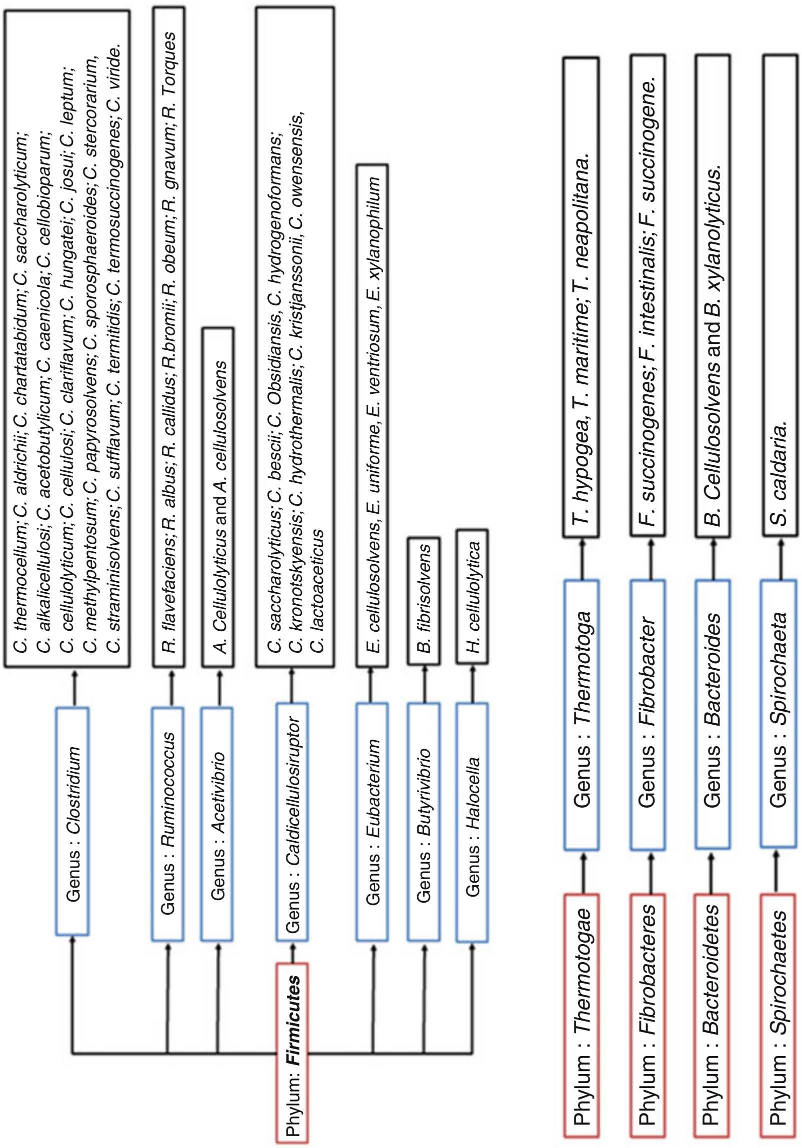

Fig. 21.2 Taxonomic classification of the most recognized hydrolytic microbes, they are an effective hydrolytic inoculum for biogas production by MSW

humification, secretion of catabolism enzymes, minimize the initial lag time, reduce the process treatment and odorous emissions. So, inoculation can provide good compost, which can be used to increase soil fertilization and improve agriculture. In anaerobic digestion, the addition of microbes in this process has become mandatory because it makes obtaining a large quantity of renewable energy in a short time. This goal is achieved thanks to an effective inoculum and chosen with great vigilance. The microbial inoculation for MSW treatment is an eco-friendly tool, less expensive, and efficient for a zero-waste economy.

References

Abdel-Shafy HI, Mansour MSM (2018) Solid waste issue: sources, composition, disposal, recycling, and valorization. Egypt J Pet 27:1275–1290. https://doi.org/10.1016/j.ejpe.2018.07.003

Akdeniz N, Koziel JA, Ahn H-K, Glanville TD, Crawford BP, Raman DR (2010) Laboratory scale evaluation of volatile organic compound emissions as indication of swine carcass degradation inside biosecure composting units. Bioresour Technol 101:71–78. https://doi.org/10.1016/j.biortech.2009.07.076

Albanna M (2013) Anaerobic digestion of the organic fraction of municipal solid waste. In: Malik A, Grohmann E, Alves M (eds) Management of microbial resources in the environment. Springer, Dordrecht, pp 313–340. https://doi.org/10.1007/978-94-007-5931-2_12

Allard B, Borén H, Grimvall A (1991) The different roles of humic substances in the environment. In: Allard B, Borén H, Grimvall A (eds) Humic substances in the aquatic and terrestrial environment. Springer, Berlin, pp 1–5. https://doi.org/10.1007/BFb0010454

Amir S, Hafidi M, Merlina G, Revel J-C (2005) Structural characterization of fulvic acids during composting of sewage sludge. Process Biochem 40:1693–1700. https://doi.org/10.1016/j.procbio.2004.06.037

Anastasi A, Varese GC, Filipello Marchisio V (2005) Isolation and identification of fungal communities in compost and vermicompost. Mycologia 97:33–44. https://doi.org/10.1080/15572536.2006.11832836

Angelidaki, I., Ellegaard, L., Ahring, B.K., 2003. Applications of the anaerobic digestion process, in: Ahring, Birgitte K., Ahring, B. K., Angelidaki, I., Dolfing, J., EUegaard, L., Gavala, H.N., Haagensen, F., Mogensen, A.S., Lyberatos, G., Pind, P.F., Schmidt, J.E., Skiadas, I.V., Stamatelatou, K. (Eds.), Biomethanation II Springer Berlin, pp. 1–33

Angelidaki I, Karakashev D, Batstone DJ, Plugge CM, Stams AJM (2011) Biomethanation and its potential. In: Rosenzweig A, Ragsdale S (eds) Methods in enzymology, Methods in methane metabolism. Elsevier, New York, pp 327–351

Awasthi MK, Pandey AK, Khan J, Bundela PS, Wong JWC, Selvam A (2014) Evaluation of thermophilic fungal consortium for organic municipal solid waste composting. Bioresour Technol 168:214–221. https://doi.org/10.1016/j.biortech.2014.01.048

Barlaz MA (1998) Carbon storage during biodegradation of municipal solid waste components in laboratory-scale landfills. Glob Biogeochem Cycles 12:373–380. https://doi.org/10.1029/98GB00350

Braun R (2007) Anaerobic digestion: a multi-faceted process for energy, environmental management and rural development. In: Ranalli P (ed) Improvement of crop plants for industrial end uses. Springer, Dordrecht, pp 335–416. https://doi.org/10.1007/978-1-4020-5486-0_13

Brindha K, Schneider M (2019) Impact of urbanization on groundwater quality. In: GIS and geostatistical techniques for groundwater science. Elsevier, pp. 179–196. doi:https://doi.org/10.1016/B978-0-12-815413-7.00013-4

Brummeler ET, Horbach HCJM, Koster IW (2007) Dry anaerobic batch digestion of the organic fraction of municipal solid waste. J Chem Technol Biotechnol 50:191–209. https://doi.org/10.1002/jctb.280500206

Burrell PC, O'Sullivan C, Song H, Clarke WP, Blackall LL (2004) Identification, detection, and spatial resolution of clostridium populations responsible for cellulose degradation in a methanogenic landfill leachate bioreactor. Appl Environ Microbiol 70:2414–2419. https://doi.org/10.1128/AEM.70.4.2414-2419.2004

Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P, Piccolo A (2015) Humic and fulvic acids as biostimulants in horticulture. Sci Hortic 196:15–27. https://doi.org/10.1016/j.scienta.2015.09.013

Chung Y-C (2007) Evaluation of gas removal and bacterial community diversity in a biofilter developed to treat composting exhaust gases. J Hazard Mater 144:377–385. https://doi.org/10.1016/j.jhazmat.2006.10.045

Cotta MA, Zeltwanger RL (1995) Degradation and utilization of xylan by the ruminal bacteria Butyrivibrio fibrisolvens and Selenomonas ruminantium. Appl Environ Microbiol 61:4396–4402. https://doi.org/10.1128/AEM.61.12.4396-4402.1995

De Vrieze J, Gildemyn S, Vilchez-Vargas R, Jáuregui R, Pieper DH, Verstraete W, Boon N (2015) Inoculum selection is crucial to ensure operational stability in anaerobic digestion. Appl Microbiol Biotechnol 99:189–199. https://doi.org/10.1007/s00253-014-6046-3

Detchanamurthy S, Gostomski PA (2012) Biofiltration for treating VOCs: an overview. Rev Environ Sci Biotechnol 11:231–241. https://doi.org/10.1007/s11157-012-9288-5

Dogaris I, Ammar E, Philippidis GP (2020) Prospects of integrating algae technologies into landfill leachate treatment. World J Microbiol Biotechnol 36. https://doi.org/10.1007/s11274-020-2810-y

El Asri O, Afilal ME (2017) Comparison of the experimental and theoretical production of biogas by monosaccharides, disaccharides, and amino acids. Int J Environ Sci Technol. https://doi.org/10.1007/s13762-017-1570-1

El Hanandeh A, El-Zein A (2009) Strategies for the municipal waste management system to take advantage of carbon trading under competing policies: the role of energy from waste in Sydney. Waste Manag 29:2188–2194. https://doi.org/10.1016/j.wasman.2009.03.002

El Asri O, Hafidi I, Afilal M e (2015) Comparison of biogas purification by different substrates and construction of a biogas purification system. Waste Biomass Valorization 6:459–464. https://doi.org/10.1007/s12649-015-9378-z

Elango D, Thinakaran N, Panneerselvam P, Sivanesan S (2009) Thermophilic composting of municipal solid waste. Appl Energy 86:663–668. https://doi.org/10.1016/j.apenergy.2008.06.009

Elasri O, Afilal M e (2014) Etude de risque de contamination des eaux marocaines par les fientes de poulet de chair/[Study a risk of contamination Moroccan waters by chickens droppings]. Int J Innov Appl Stud 7:593

Elasri O, Salem M, Ramdani M, Zaraali O, Lahbib L (2018) Effect of increasing inoculum ratio on energy recovery from chicken manure for better use in Egyptian agricultural farms. Chem Biol Technol Agric 5. https://doi.org/10.1186/s40538-018-0129-9

Erraji H, Afilal ME, Azim K, Laiche H, El Asri O (2017) Valorization of household anaerobic processed digestate: a case study of Morocco. J Mater Environ Sci 8:4024–4031

Gao A, Tian Z, Wang Z, Wennersten R, Sun Q (2017) Comparison between the technologies for food waste treatment. Energy Procedia 105:3915–3921. https://doi.org/10.1016/j.egypro.2017.03.811

Gerardi MH (2003) The microbiology of anaerobic digesters, Wastewater microbiology series. Wiley, Hoboken, NJ. https://doi.org/10.1002/0471468967

Griffin ME, McMahon KD, Mackie RI, Raskin L (1998) Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids. Biotechnol Bioeng 57:342–355

Gujer W, Zehnder AJB (1983) Conversion processes in anaerobic digestion. Water Sci Technol 15:127–167

Heidarzadeh MH, Amani H, Javadian B (2019) Improving municipal solid waste compost process by cycle time reduction through inoculation of Aspergillus niger. J Environ Health Sci Eng 17:295–303. https://doi.org/10.1007/s40201-019-00348-z

Huang GF, Wu QT, Wong JWC, Nagar BB (2006) Transformation of organic matter during co-composting of pig manure with sawdust. Bioresour Technol 97:1834–1842. https://doi.org/10.1016/j.biortech.2005.08.024

Jeffries TW (1994) Biodegradation of lignin and hemicelluloses. In: Biochemistry of microbial degradation. Springer, Dordrecht, pp 233–277

Jurado M, López MJ, Suárez-Estrella F, Vargas-García MC, López-González JA, Moreno J (2014) Exploiting composting biodiversity: study of the persistent and biotechnologically relevant microorganisms from lignocellulose-based composting. Bioresour Technol 162:283–293. https://doi.org/10.1016/j.biortech.2014.03.145

Karnchanawong S, Nissaikla S (2014) Effects of microbial inoculation on composting of household organic waste using passive aeration bin. Int J Recycl Org Waste Agric 3:113–119. https://doi.org/10.1007/s40093-014-0072-0

Karouach F, Bakraoui M, El Gnaoui Y, Lahboubi N, El Bari H (2020) Effect of combined mechanical–ultrasonic pretreatment on mesophilic anaerobic digestion of household organic waste fraction in Morocco. Energy Rep 6:310–314. https://doi.org/10.1016/j.egyr.2019.11.081

Khair H, Rachman I, Matsumoto T (2019) Analyzing household waste generation and its composition to expand the solid waste bank program in Indonesia: a case study of Medan City. J Mater Cycles Waste Manag 21:1027–1037. https://doi.org/10.1007/s10163-019-00840-6

Kowalchuk GA, Naoumenko ZS, Derikx PJ, Felske A, Stephen JR, Arkhipchenko IA (1999) Molecular analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in compost and composted materials. Appl Environ Microbiol 65:396–403

Kumar S (2011) Composting of municipal solid waste. Crit Rev Biotechnol 31:112–136. https://doi.org/10.3109/07388551.2010.492207

Laiche H, El Asri O, Erraji H, Afilal ME (2017) Quality comparison of two methacomposts comes from animalrearing of laboratory and University Restaurant of Oujda University in Morocco. J. Mater. Environ. Sci. 8:2592–2598

Lee S, Kim J, Chong WKO (2016) The causes of the municipal solid waste and the greenhouse gas emissions from the waste sector in the United States. Waste Manag 56:593–599. https://doi.org/10.1016/j.wasman.2016.07.022

Li Y-F, Shi J, Nelson MC, Chen P-H, Graf J, Li Y, Yu Z (2016) Impact of different ratios of feedstock to liquid anaerobic digestion effluent on the performance and microbiome of solid-state anaerobic digesters digesting corn stover. Bioresour Technol 200:744–752. https://doi.org/10.1016/j.biortech.2015.10.078

Liaquat R, Jamal A, Tauseef I, Qureshi Z, Farooq U, Imran M, Ali M (2017) Characterizing bacterial consortia from an anaerobic digester treating organic waste for biogas production. Pol J Environ Stud 26:709–716. https://doi.org/10.15244/pjoes/59332

Lin H, Wang B, Zhuang R, Zhou Q, Zhao Y (2011) Artificial construction and characterization of a fungal consortium that produces cellulolytic enzyme system with strong wheat straw saccharification. Bioresour Technol 102:10569–10576. https://doi.org/10.1016/j.biortech.2011.08.095

Linville JL, Shen Y, Wu MM, Urgun-Demirtas M (2015) Current State of Anaerobic Digestion of Organic Wastes in North America. Curr Sustain Energy Rep 2:136–144. https://doi.org/10.1007/s40518-015-0039-4

Liu Y, Whitman WB (2008) Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann N Y Acad Sci 1125:171–189. https://doi.org/10.1196/annals.1419.019

Liu T, Sun L, Müller B, Schnürer A (2017) Importance of inoculum source and initial community structure for biogas production from agricultural substrates. Bioresour Technol 245:768–777. https://doi.org/10.1016/j.biortech.2017.08.213

Lopes W (2004) Influence of inoculum on performance of anaerobic reactors for treating municipal solid waste. Bioresour Technol 94:261–266. https://doi.org/10.1016/j.biortech.2004.01.006
Ludwig C, Hellweg S, Stucki S (eds) (2003) Municipal solid waste management. Springer, Berlin. https://doi.org/10.1007/978-3-642-55636-4
Manu MK, Kumar R, Garg A (2019) Decentralized composting of household wet biodegradable waste in plastic drums: effect of waste turning, microbial inoculum and bulking agent on product quality. J Clean Prod 226:233–241. https://doi.org/10.1016/j.jclepro.2019.03.350
Masai E, Katayama Y, Fukuda M (2007) Genetic and biochemical investigations on bacterial catabolic pathways for lignin-derived aromatic compounds. Biosci Biotechnol Biochem 71:1–15. https://doi.org/10.1271/bbb.60437
Mata-Alvarez J, Macé S, Llabrés P (2000) Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresour Technol 74:3–16. https://doi.org/10.1016/S0960-8524(00)00023-7
Mayumi D, Mochimaru H, Tamaki H, Yamamoto K, Yoshioka H, Suzuki Y, Kamagata Y, Sakata S (2016) Methane production from coal by a single methanogen. Science 354:222–225
McMahon KD, Zheng D, Stams AJM, Mackie RI, Raskin L (2004) Microbial population dynamics during start-up and overload conditions of anaerobic digesters treating municipal solid waste and sewage sludge. Biotechnol Bioeng 87:823–834. https://doi.org/10.1002/bit.20192
McNevin D, Barford J (2000) Biofiltration as an odour abatement strategy. Biochem Eng J 5:231–242. https://doi.org/10.1016/S1369-703X(00)00064-4
Meng Q, Yang W, Men M, Bello A, Xu X, Xu B, Deng L, Jiang X, Sheng S, Wu X, Han Y, Zhu H (2019) Microbial community succession and response to environmental variables during cow manure and corn straw composting. Front Microbiol 10:529. https://doi.org/10.3389/fmicb.2019.00529
Meor Hussin AS, Collins SRA, Merali Z, Parker ML, Elliston A, Wellner N, Waldron KW (2013) Characterisation of lignocellulosic sugars from municipal solid waste residue. Biomass Bioenergy 51:17–25. https://doi.org/10.1016/j.biombioe.2012.12.015
Mian MM, Zeng X, al Naim Bin Nasry A, Al-Hamadani SMZF (2017) Municipal solid waste management in China: a comparative analysis. J Mater Cycles Waste Manag 19:1127–1135. https://doi.org/10.1007/s10163-016-0509-9
Minghua Z, Xiumin F, Rovetta A, Qichang H, Vicentini F, Bingkai L, Giusti A, Yi L (2009) Municipal solid waste management in Pudong new area. China Waste Manag 29:1227–1233. https://doi.org/10.1016/j.wasman.2008.07.016
Mir MA, Hussain A, Verma C (2016) Design considerations and operational performance of anaerobic digester: A review. Cogent Eng 3. https://doi.org/10.1080/23311916.2016.1181696
Miyatake F, Iwabuchi K (2005) Effect of high compost temperature on enzymatic activity and species diversity of culturable bacteria in cattle manure compost. Bioresour Technol 96:1821–1825. https://doi.org/10.1016/j.biortech.2005.01.005
Moletta R (2015) La méthanisation. Editions Tec & Doc, Paris
Moletta R, Verrier D, Albagnac G (1986) Dynamic modelling of anaerobic digestion. Water Res 20:427–434. https://doi.org/10.1016/0043-1354(86)90189-2
Mühle S, Balsam I, Cheeseman CR (2010) Comparison of carbon emissions associated with municipal solid waste management in Germany and the UK. Resour Conserv Recycl 54:793–801. https://doi.org/10.1016/j.resconrec.2009.12.009
Munawar E, Yunardi Y, Lederer J, Fellner J (2018) The development of landfill operation and management in Indonesia. J Mater Cycles Waste Manag 20:1128–1142. https://doi.org/10.1007/s10163-017-0676-3
Mushtaq J, Dar AQ, Ahsan N (2020) Spatial–temporal variations and forecasting analysis of municipal solid waste in the mountainous city of north-western Himalayas. SN Appl Sci 2:1161. https://doi.org/10.1007/s42452-020-2975-x
Naïma TD, Guy M, Serge C, Djamel T (2012) Composition of municipal solid waste (MSW) generated by the City of Chlef (Algeria). Energy Procedia 18:762–771. https://doi.org/10.1016/j.egypro.2012.05.092

Naimi Y, Saghir M, Cherqaoui A, Chatre B (2017) Energetic recovery of biomass in the region of Rabat. Morocco Int J Hydrog Energy 42:1396–1402. https://doi.org/10.1016/j.ijhydene.2016.07.055

Nguyen PHL, Kuruparan P, Visvanathan C (2007) Anaerobic digestion of municipal solid waste as a treatment prior to landfill. Bioresour Technol 98:380–387. https://doi.org/10.1016/j.biortech.2005.12.018

Nielsen H, Uellendahl H, Ahring B (2007) Regulation and optimization of the biogas process: Propionate as a key parameter. Biomass Bioenergy 31:820–830. https://doi.org/10.1016/j.biombioe.2007.04.004

Ohtaki A, Akakura N, Nakasaki K (1998) Effects of temperature and inoculum on the degradability of poly-ε-caprolactone during composting. Polym Degrad Stab 62:279–284. https://doi.org/10.1016/S0141-3910(98)00008-1

Ouigmane A, Boudouch O, Hasib A, Berkani M (2018) Management of municipal solid waste in Morocco: the size effect in the distribution of combustible components and evaluation of the fuel fractions. In: Hussain CM (ed) Handbook of environmental materials management. Springer, Cham, pp 1–13. https://doi.org/10.1007/978-3-319-58538-3_82-1

Ozbayram EG, Akyol Ç, Ince B, Karakoç C, Ince O (2018) Rumen bacteria at work: bioaugmentation strategies to enhance biogas production from cow manure. J Appl Microbiol 124:491–502. https://doi.org/10.1111/jam.13668

Pagans E, Font X, Sanchez A (2006) Emission of volatile organic compounds from composting of different solid wastes: abatement by biofiltration. J Hazard Mater 131:179–186. https://doi.org/10.1016/j.jhazmat.2005.09.017

Partanen P, Hultman J, Paulin L, Auvinen P, Romantschuk M (2010) Bacterial diversity at different stages of the composting process. BMC Microbiol 10:94. https://doi.org/10.1186/1471-2180-10-94

Periathamby A (2011) Municipal waste management. In: Waste. Elsevier, pp. 109–125. doi:https://doi.org/10.1016/B978-0-12-381475-3.10008-7

Peters S, Koschinsky S, Schwieger F, Tebbe CC (2000) Succession of microbial communities during hot composting as detected by PCR–single-strand-conformation polymorphism-based genetic profiles of small-subunit rRNA genes. Appl Environ Microbiol 66:930–936. https://doi.org/10.1128/AEM.66.3.930-936.2000

Pohlschroeder M, Leschine SB, Canale-Parola E (1994) Spirochaeta caldaria sp. nov., a thermophilic bacterium that enhances cellulose degradation by Clostridium thermocellum. Arch Microbiol 161:17–24. https://doi.org/10.1007/BF00248889

Raghab SM, Abd El Meguid AM, Hegazi HA (2013) Treatment of leachate from municipal solid waste landfill. HBRC J 9:187–192. https://doi.org/10.1016/j.hbrcj.2013.05.007

Ryckeboer J, Mergaert J, Coosemans J, Deprins K, Swings J (2003) Microbiological aspects of biowaste during composting in a monitored compost bin. J Appl Microbiol 94:127–137. https://doi.org/10.1046/j.1365-2672.2003.01800.x

Scarlat N, Fahl F, Dallemand J-F (2019) Status and opportunities for energy recovery from municipal solid waste in Europe. Waste Biomass Valorization 10:2425–2444. https://doi.org/10.1007/s12649-018-0297-7

Schirmer WN, Jucá JFT, Schuler ARP, Holanda S, Jesus LL (2014) Methane production in anaerobic digestion of organic waste from Recife (Brazil) landfill: evaluation in refuse of diferent ages. Braz J Chem Eng 31:373–384. https://doi.org/10.1590/0104-6632.20140312s00002468

Selvamani K, Annadurai V, Soundarapandian S (2019) Improved co-composting of poultry manure with complementary consortium of indigenous Bacillus spp. Biotech 3:9. https://doi.org/10.1007/s13205-019-1745-1

Sharma A, Gupta AK, Ganguly R (2018) Impact of open dumping of municipal solid waste on soil properties in mountainous region. J Rock Mech Geotech Eng 10:725–739. https://doi.org/10.1016/j.jrmge.2017.12.009

Shekdar AV (2009) Sustainable solid waste management: An integrated approach for Asian countries. Waste Manag 29:1438–1448. https://doi.org/10.1016/j.wasman.2008.08.025

Singh R, Mandal SK, Jain VK (2010) Development of mixed inoculum for methane enriched biogas production. Indian J Microbiol 50:26–33. https://doi.org/10.1007/s12088-010-0060-7

Song C, Zhang Y, Xia X, Qi H, Li M, Pan H, Xi B (2018) Effect of inoculation with a microbial consortium that degrades organic acids on the composting efficiency of food waste. Microb Biotechnol 11:1124–1136. https://doi.org/10.1111/1751-7915.13294

Sun L, Liu T, Müller B, Schnürer A (2016) The microbial community structure in industrial biogas plants influences the degradation rate of straw and cellulose in batch tests. Biotechnol Biofuels 9:128

Sundberg C, Yu D, Franke-Whittle I, Kauppi S, Smårs S, Insam H, Romantschuk M, Jönsson H (2013) Effects of pH and microbial composition on odour in food waste composting. Waste Manag 33:204–211. https://doi.org/10.1016/j.wasman.2012.09.017

Vargas-García MC, Suárez-Estrella F, López MJ, Moreno J (2010) Microbial population dynamics and enzyme activities in composting processes with different starting materials. Waste Manag 30:771–778. https://doi.org/10.1016/j.wasman.2009.12.019

Vehlow J (1996) Municipal solid waste management in Germany. Waste Manag 16:367–374. https://doi.org/10.1016/S0956-053X(96)00081-5

Wan C, Li Y (2012) Fungal pretreatment of lignocellulosic biomass. Biotechnol Adv 30:1447–1457. https://doi.org/10.1016/j.biotechadv.2012.03.003

Wang H, Nie Y (2001) Municipal solid waste characteristics and management in China. J Air Waste Manag Assoc 51:250–263. https://doi.org/10.1080/10473289.2001.10464266

Wang P, Wang H, Qiu Y, Ren L, Jiang B (2018) Microbial characteristics in anaerobic digestion process of food waste for methane production–A review. Bioresour Technol 248:29–36. https://doi.org/10.1016/j.biortech.2017.06.152

Wei Z, Xi B, Zhao Y, Wang S, Liu H, Jiang Y (2007) Effect of inoculating microbes in municipal solid waste composting on characteristics of humic acid. Chemosphere 68:368–374. https://doi.org/10.1016/j.chemosphere.2006.12.067

Wei Y, Li J, Shi D, Liu G, Zhao Y, Shimaoka T (2017) Environmental challenges impeding the composting of biodegradable municipal solid waste: A critical review. Resour Conserv Recycl 122:51–65. https://doi.org/10.1016/j.resconrec.2017.01.024

Wu B, Wang X, Deng Y-Y, He X-L, Li Z-W, Li Q, Qin H, Chen J-T, He M-X, Zhang M, Hu G-Q, Yin X-B (2016) Adaption of microbial community during the start-up stage of a thermophilic anaerobic digester treating food waste. Biosci Biotechnol Biochem 80:2025–2032. https://doi.org/10.1080/09168451.2016.1191326

Xi B, Zhang G, Liu H (2005) Process kinetics of inoculation composting of municipal solid waste. J Hazard Mater 124:165–172. https://doi.org/10.1016/j.jhazmat.2005.04.026

Xi B-D, He X-S, Wei Z-M, Jiang Y-H, Li M-X, Li D, Li Y, Dang Q-L (2012) Effect of inoculation methods on the composting efficiency of municipal solid wastes. Chemosphere 88:744–750. https://doi.org/10.1016/j.chemosphere.2012.04.032

Yamada T, Asari M, Miura T, Niijima T, Yano J, Sakai S (2017) Municipal solid waste composition and food loss reduction in Kyoto City. J. Mater. Cycles Waste Manag. 19:1351–1360. https://doi.org/10.1007/s10163-017-0643-z

Yamamoto N, Otawa K, Nakai Y (2010) Diversity and abundance of ammonia-oxidizing bacteria and ammonia-oxidizing archaea during cattle manure composting. Microb Ecol 60:807–815. https://doi.org/10.1007/s00248-010-9714-6

Zeng G, Yu M, Chen Y, Huang D, Zhang J, Huang H, Jiang R, Yu Z (2010) Effects of inoculation with Phanerochaete chrysosporium at various time points on enzyme activities during agricultural waste composting. Bioresour Technol 101:222–227. https://doi.org/10.1016/j.biortech.2009.08.013

Zhang J, Zeng G, Chen Y, Yu M, Yu Z, Li H, Yu Y, Huang H (2011) Effects of physico-chemical parameters on the bacterial and fungal communities during agricultural waste composting. Bioresour Technol 102:2950–2956. https://doi.org/10.1016/j.biortech.2010.11.089

Zhang Y, Zhao Y, Chen Y, Lu Q, Li M, Wang X, Wei Y, Xie X, Wei Z (2016) A regulating method for reducing nitrogen loss based on enriched ammonia-oxidizing bacteria during composting. Bioresour Technol 221:276–283. https://doi.org/10.1016/j.biortech.2016.09.057

Zhao Z, Bian R, Zhao F, Chai X (2020) Implications of municipal solid waste disposal methods in China on greenhouse gas emissions. Environ Prog Sustain Energy 39. https://doi.org/10.1002/ep.13372

Zhuo S, Yan X, Liu D, Si M, Zhang K, Liu M, Peng B, Shi Y (2018) Use of bacteria for improving the lignocellulose biorefinery process: importance of pre-erosion. Biotechnol Biofuels 11. https://doi.org/10.1186/s13068-018-1146-4

22 Applications of Waste Decomposer in Plant Health Protection, Crop Productivity and Soil Health Management

Aruna Jyothi Kora

Abstract

As an alternative to currently available, commercial biocontrol agents and biofertilizers, waste decomposer was released for the farmers by National Centre of Organic Farming for enhancing the crop productivity and plant disease management. The waste decomposer is a consortium of few beneficial bacteria, isolated from *desi* cow dung and can be easily multiplied with jaggery at farmer level. The waste decomposer exhibits multifaceted uses in agriculture including in situ composting of crop residues, quick composting of organic wastes, seed dressing, soil irrigant, biocontrol agent, biofertilizer, soil health reviver, etc. It is bestowed with virtues such as low cost, easier multiplication, fast growth rate, superior shelf life and broad spectrum activity on phytopathogens. The jaggery propagated waste decomposer indicated the presence of cellulolytic, phosphate and potassium solubilizing; siderophore producing bacteria on selective culture media. The consortium is also abundant in nitrogen fixing bacteria (*Azotobacter, Azospirillum, Rhizobium, Acetobacter*) and *Pseudomonas fluorescence*. The lignocellulolytic action of waste decomposer on the crop residues aids in greenhouse gas mitigation. It can control different types of soilborne, seedborne, rootborne, shootborne and foliar diseases; insects and pests as a plant protection agent. The biocontrol action of waste decomposer is possibly via nutrient and space competition; antagonistic action, extracellular lytic enzyme, antibiotic, siderophore, secondary metabolite production; and systemic resistance induction

A. J. Kora (✉)
National Centre for Compositional Characterisation of Materials (NCCCM), Bhabha Atomic Research Centre (BARC), Hyderabad, India

Homi Bhabha National Institute (HBNI), Mumbai, India
e-mail: koraaj@barc.gov.in

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in plants. The application of waste decomposer improves the crop productivity due to its biofertilizer, biocontrol and mineral solubilizing action. The keratinolytic action of waste decomposer also finds its application in degradation of poultry feathers and human hair. Further, other potential applications of waste decomposer need to be exploited.

Keyword

Waste decomposer · composting · biofertilizer · biocontrol agent · bacteria · organic acids · siderophore · keratin degrading · disease control · application

22.1 Introduction

In market, an array of organic, natural products/formulations are available for farmers for enhancing the crop productivity and plant disease management. These concoctions are marketed for applying as foliar spray, soil irrigant, seed dressing etc. for improving soil fertility, pest repellence and disease resistance. A small scale evaluation study on different commercial organic natural products/concoctions was carried out for the presence of beneficial microbes which exhibit pesticidal activity. It is noted that some of the commercially available, natural products marketed under the name of biofertilizers, biocontrol agents and biopesticides are adulterated, low in quality and poor in performance. Thus, farmers are losing confidence on these organic inputs (Sridevi et al. 2017). Hence, there is an imperative need for the development of beneficial microbe enriched formulations which can be easily prepared at farmer level, with locally available renewable sources.

In this scenario, waste decomposer culture was released by National Centre of Organic Farming (NCOF), Ghaziabad, India under the aegis of Ministry of Agriculture and Farmers Welfare, Government of India. It was developed by Dr. Krishan Chandra and his team after a continuous research for more than 10 years. It is a consortium of beneficial bacteria isolated from *desi* (native) cow dung. It is utilized for rapid composting of various organic wastes, soil heath management and as a plant protection agent (Verma 2020; Chandra et al. 2019). The waste decomposer is sold in a bottle of 30 g costing INR 20/bottle directly through NCOF and Regional Organic Farming Centres (RCOF) to farmers and also validated by Indian Council of Agriculture Research (ICAR) [Fig. 22.1(a)] (Kosaraju 2018a).

22.2 Mass Multiplication and Composition of Waste Decomposer Solution

For mass multiplication of waste decomposer at farmer lever, 2 kg of jaggery should be dissolved in 200 L of water stored in a plastic drum. To this, the contents of 1 bottle of waste decomposer should be added and mixed thoroughly with a wooden stick for even distribution of the inoculum. Then, the contents of covered drum

Fig. 22.1 (**a**) A bottle of waste decomposer and (**b**) mass multiplied waste decomposer solution in jaggery

should be stirred twice a day and after 5 days of incubation cream coloured solution of mass multiplied waste decomposer is ready for application [Fig. 22.1(b)]. For further and continuous propagation by farmers, the process can be repeated with 20 L of mass multiplied waste decomposer solution (Singh 2017; Mondal 2017; Chandra et al. 2019). The sweetener jaggery is a rich source of sucrose (65–85%), glucose and fructose (10–15%); protein (0.35–0.4%), fat (0.1–0.6%), chloride (0.2–0.34%), Ca (0.2–0.4%), P (0.04–0.22%), K (0.10–0.16%), Na (6–25 mg/100 g), Fe (5.8–20 mg/100 g), Mg (8–125 mg/100 g), Cu (7–10 mg/100 g), thiamine (18–30 mg/100 g), riboflavin (42–46 mg/100 g), nicotinic acid (4–4.5 mg/100 g), vitamin C (5.2–30 mg/100 g) and functions as a rich source of carbon, nitrogen, minerals and vitamins (383 Kcal/100 g) for microbial growth (Chikkappaiah et al. 2017; Sahu and Saxena 1994).

The waste decomposer multiplied in jaggery contains various cellulose degrading bacteria which is evident from the clear zone formation around the inoculum in carboxymethylcellulose (CMC) agar plates, detected by iodine staining [Fig. 22.2(a)]. The CMC agar is used for the detection of cellulolytic, hydrolytic enzymes such as glucanase, xylanse and hemicellulase production by microbes (Adlakha et al. 2011; Kasana et al. 2008). Besides, the consortium is also augmented with organic acid producing, phosphate, potassium and zinc solubilizing bacteria. The presence of phosphate solubilizers is demonstrated from yellow coloured halo zonation around the inoculum in Pikovskayas agar (PA) plates amended with acid-base indicator dye, bromothymol blue [Fig. 22.2(b)]. The PA contains insoluble phosphate source, calcium phosphate and it is a selective medium used for the isolation and detection of phosphate solubilizing microorganisms (Nagaraju et al. 2017). Further, the occurrence potassium solubilizing bacteria is indicated as yellow coloured halo

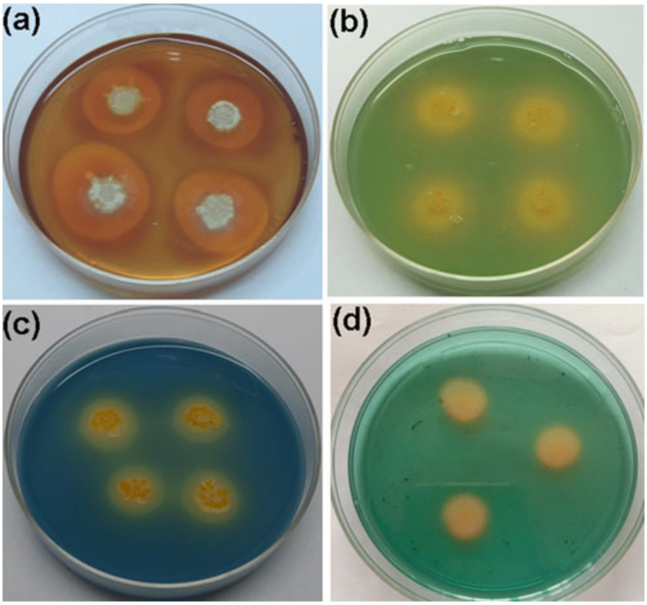

Fig. 22.2 The growth of waste decomposer bacteria on differential media, (**a**) carboxymethylcellulose agar, (**b**) Pikovskayas agar, (**c**) Aleksandrow agar and (**d**) chrome azurol S agar, indicating cellulose degradation, phosphate solubilization, potassium solubilization and siderophore production, respectively

zone formation around the inoculum in Aleksandrow agar (AA), amended with bromothymol blue [Fig. 22.2(c)]. The AA contains insoluble potassium source, potassium alumino silicate (mica) and it is a selective medium used for the isolation and detection of potassium solubilizing microorganisms (Singh et al. 2018). The bromothymol blue used in both the media detects the decrease in pH/acidic pH via a visual medium colour change from blue to yellow due to organic acid secretion (Rajawat et al. 2016). The organic acid production by the consortium is further confirmed from the solution pH of 4.5–6 (Chandra and Kanojia 2018). Also, the consortium produces siderophores for chelating the non-bioavailable iron in the soil and siderophore production is confirmed from the yellow orange coloured halo zone formation around the inoculum in chrome azurol S (CAS) agar [Fig. 22.2(d)]. The CAS agar is a selective medium used for the detection of siderophore production by various microbes (Mumtaz et al. 2017).

Notably, the propagated waste decomposer solution is abundant in various nitrogen fixing bacteria such as *Azotobacter, Azospirillum, Rhizobium* and *Acetobacter*. *Azotobacter* is one of the important Gram negative, rod shaped, aerobic, heterotrophic, free living, diazototrophic, nitrogen fixing, rhizospheric, beneficial bacteria present in the 7-8 day old mass multiplied waste decomposer solution. A selective agar medium that contains soil extract and mannitol is used to for the isolation, identification and cultivation of *Azotobacter* [Fig. 22.3(a)]. It

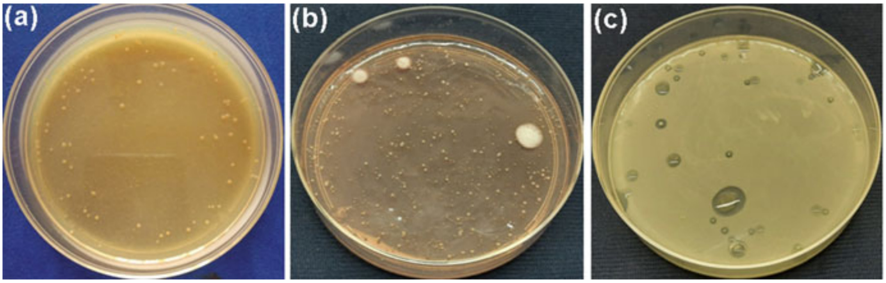

Fig. 22.3 The presence of nitrogen fixing bacteria, (**a**) *Azotobacter,* (**b**) *Rhizobium* and (**c**) *Acetobacter* in jaggery grown waste decomposer solution

produces pigments and also able to survive at an acidic solution pH of 4.5. It fixes the atmospheric, nonavailable nitrogen into available form for the plants and increases the soil fertility. In addition, it also synthesizes phytohormones; auxins and indole acetic acid; vitamins and stimulates plant growth (Dhevendaran et al. 2013). Another, free living, nitrogen fixing (40 kg/ha) bacteria, *Azospirillum* belonging to the same family of Azotobacteriaceae is also known be abundant in the prepared waste decomposer solution. Interestingly, another fast growing, organic acid producing, nitrogen fixing, symbiotic, nodulating bacteria *Rhizobium* is also present in 7-8 day old jaggery propagated waste decomposer solution. It shows creamy white, translucent, glistening colonies on selective yeast extract mannitol Congo red agar medium [Fig. 22.3(b)]. It is known to fix nitrogen in root nodules of leguminous plants such as green gram (20 kg/ha) and barseem clover (300 kg/ha) and reduces the urea based nitrogen requirement of the crops. The waste decomposer solution is also enriched with *Acetobacter*, a nitrogen fixing and organic acid producing bacteria. The presence is detected from the clear halo zone formation around the colonies due to the production of acid in medium [Fig. 22.3(c)]. The waste decomposer solution is also fortified with other important bacteria, *Pseudomonas fluorescence* and is selectively identified on King's B medium, which is used for the isolation and identification of *P. fluorescence* from various sources [Fig. 22.4(a)]. The release of UV florescent pigment, fluorescein into the medium by *P. fluorescence* is evident from characteristic fluorescence under 254 nm of UV light [Fig. 22.4(b)] (Scales et al. 2014).

22.3 Unique and Important Characteristics of Waste Decomposer (Chandra and Kanojia 2018)

1. Simple and reliable preparation at the user level.
2. Facile mass multiplication with cheap, locally available nutrient source, jaggery.
3. Lesser preparation time of 5 days.
4. Superior shelf life of 3 years.
5. Low cost (INR 20/30 g bottle) and affordable for users.
6. Reuse/replication of inoculum for many generations.

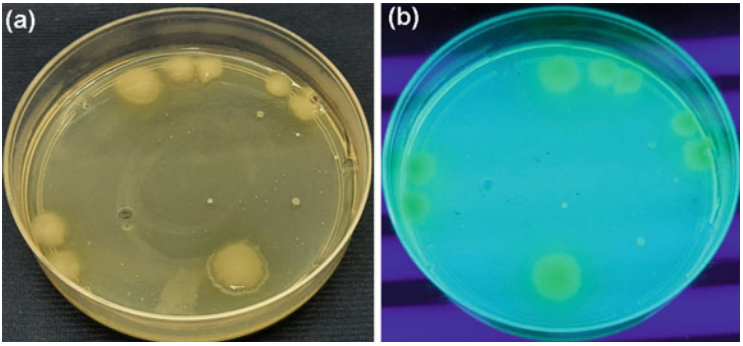

Fig. 22.4 The occurrence of *Pseudomonas fluorescence* in propagated waste decomposer solution evident from (**a**) growth on King's medium and (**b**) fluorescence under UV light

7. Recommended for all varieties of crops.
8. Superior crop response.
9. Fulfills the mandates of *Swachh Bharat* (Clean India) mission initiated by Ministry of Drinking Water and Sanitation, Government of India.
10. Capable of producing organic manure of over 0.1 million tonne/bottle/year by the farmer.
11. Safe to use and no reported toxicity towards humans and other mammals.

22.4 Applications of Waste Decomposer in Agriculture

The waste decomposer exhibits multifaceted uses in agriculture including in situ composting of crop residues, quick composting of organic wastes, seed dressing, soil irrigant, biocontrol agent, biofertilizer, soil health reviver, etc.

22.4.1 In Situ Composting of Crop Residues

Generally, the stubbles of different crops are burnt in the fields by the farmers as a preparatory step for the next crop, improvement of tillage efficiency and seeding operations; weed control and reduction of pesticide usage (Singh and Irungbam 2018). Due to an increase in mechanized farming (use of combine harvester), shortage of labour, high transportation costs, slow stubble decomposition, nonavailability of suitable technologies and lack of awareness, stubble burning became more common in the states of Punjab, Haryana, Uttar Pradesh and Manipur, as a quick, easy and cheap way of stubble management. The practice of stubble burning not only decreases local microbial population and organic carbon, nitrogen, potassium, phosphorous and sulphur content in soil; but also enhances air pollution. It releases suspended particulate matter, dioxins, furans, volatile organic compounds, carcinogenic polycyclic aromatic hydrocarbons, greenhouse gases such as carbon monoxide, carbon dioxide, sulphur dioxide, nitrogen oxides and methane into the atmosphere, which in turn affects the air quality. The reduced air quality leads to decreased visibility and impacts human health. For example, smog formation and air pollution during the winter season in Indian city of New Delhi is due to stubble burning (Devi et al. 2018; Shashidhar et al. 2018; Ansari et al. 2018; Moirangthem et al. 2018; Jha et al. 2018).

The stubbles of various cereal crops including wheat, paddy; and different other postharvest crop residues (sugarcane, cotton stalks) are composted in situ within 30 days by spraying and flooding of prepared waste decomposer solution mixed with irrigation water. A prepared solution of 200 L is sufficient for in situ composting of 0.4 ha of crop residues and the process can be hastened by repetitive sprays and shredding of crop residues. Thus, in situ composting of crop residues by waste decomposer treatment for many years produces a layer of organic matter on soil surface, reduces the burden of commercial chemical fertilizer application, increases fertilizer retention and enhances the soil porosity, water holding capacity, aeration and soil fertility (Chandra and Kanojia 2018; Devi et al. 2018).

22.5 Quick Composting of Organic Wastes

Every year, India produces approximately 620–700 million tonnes of biowastes, with varying characteristics and composition. Of which, ten million tonnes is cattle and cow dung, 11 million tonnes is sugarcane press mud and the remaining is postharvest agriculture residues and municipal solid wastes. However, on average each Indian generates waste of 200–600 g/day in cities. While in village with an average households of 300–400 produces 2 tonnes/day of agriwaste that includes animal dung, animal shed waste, husk, trash, biomass, stems, sticks, etc. (Thacker 2019; Mondal 2017; Pan et al. 2011). Especially, the release of undecomposed animal dung into agricultural fields causes ground water contamination and poses a threat to public health. While, the release of nitrogen rich manures into water bodies leads to eutrophication (Kora et al. 2017; Kora 2019).

The process of composting is an exothermic, aerobic biodegradation process in which organic matter is transformed into humic substances by various bacteria, fungi and actinomycetes, thus augments the physicochemical and biological characteristics of soils. The microbial growth during the composting is effected by different factors such as nutrient, moisture and oxygen content; carbon nitrogen ratio, pH, temperature etc. In India, an array of composting methods are practiced which are limited by adaptability, high labour cost, high construction cost, low replicability, low popularity, technical difficulties and low quality compost (Singh et al. 2012; Pan et al. 2011). Generally, for composting a tonne of biowastes, minimum of 1–2 kg of microbial culture is needed which is usually expensive and ineffective towards broad variety of organic wastes (Kosaraju 2018b). Most of these difficulties are easily surpassed by the use of versatile waste decomposer solution. The mass propagated waster decomposer solution is used for quick composting of various organic wastes such as crop residues, agriculture wastes, market yard wastes,

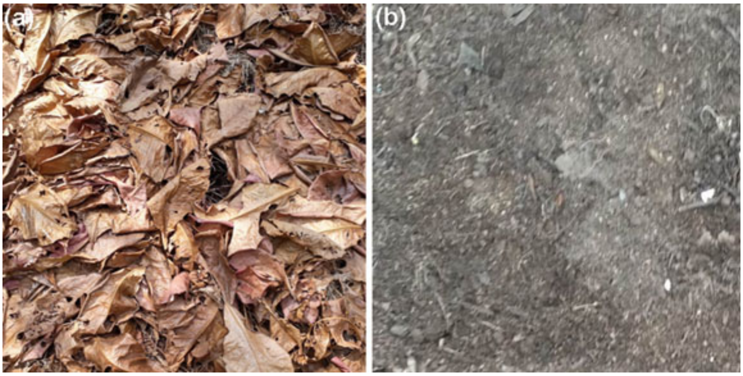

Fig. 22.5 Pile of garden leaf litter (**a**) before and (**b**) after 45 days of mass multiplied waste decomposer application

domestic kitchen wastes, animal dung, aquatic floating weeds etc. within 30–45 days (India To 2019). The composting process depends upon the nature of crop residue, temperature, moisture content and aeration. Under shade, a tonne of crop residues should be layered at a thickness of 18–20 cm either on ground or plastic sheet and sprinkled with 10–20 L of the mass multiplied waste decomposer solution. Again, layer another tonne of crop residue over the existing one and spray with 10–20 L of solution and continue for a total of thickness of 30–45 cm. A moisture content of 60% should be maintained throughout the composting process. At a weekly interval, turning over the pile of residues is needed for uniform composting and additional spraying is needed for faster composting (Kumar and Kumar 2019; Vootla and Chandra 2018). A typical picture of composting of dried garden leaves within 45 days of application of mass multiplied waste decomposer is shown in Fig. 22.5. Most importantly, the construction of standard structure such as brick lining, bottom concrete lining, trench, bins or pit is not required for composting. Also, high quality compost can be produced without depending on parameters such as heap size (height, width, thickness), covering with plastic/jute materials and ventilating stacks (Thacker 2019; Kosaraju 2018b).

A bottle of waste decomposer is capable of producing organic manure of over 0.1 million tonne/year. The compost produced after 30–45 days is dark brown in colour, dry and contains high organic carbon and other nutrients. The cow dung composted for 35 days with waste decomposer shows a pH of 7.5, electrical conductivity of 3.8 dS/cm, organic carbon of 18%, nitrogen of 1.2%, C/N ratio of 18:1, potassium of 0.8%, phosphorus of 0.6% and total bacterial count of 10^{12} CFU/gm. It neither emits foul smell (rotten eggs, rancid butter, vinegar, ammonia) nor attracts flies, millipedes, slugs, fire ants, other insects and rodents, which are common problems faced during the composting by farmers and surrounding people. Hence, it is very much attractive for kitchen and terrace gardening; municipal corporations, housing societies and communities also (Thacker 2019). It completely decomposes even the tough matrices like matted leaves and grass clippings. The waste decomposer multiplied in jaggery contains various cellulose degrading bacteria and they show vigorous growth, enhanced pH tolerance and quicker production of lignocellulytic enzymes including glucanase and β-1,3 glucanase, thus making it a superb lignocellulose decomposer (Thacker 2019). Also, the propagated waste decomposer solution is abundant with phosphate, potassium and zinc solubilizing and siderophore producing bacteria (Chandra and Kanojia 2018). The existence of cellulose (1×10^7 CFU/mL) and xylan (2.4×10^6 CFU/mL) degrading, phosphorus (2×10^7 CFU/mL) and potassium (8×10^4 CFU/mL) solubilizing bacteria in waste decomposer consortium is established from a study carried out on waste decomposer efficiency in crop residue (wheat, rice and sugar cane residues) management. The crop residues of rice, wheat and sugarcane treated with waste decomposer under laboratory and pot culture techniques for a period of 54 days produced soil available nitrogen of 176 kg/ha, 184 kg/ha and 184 kg/ha, respectively due to waste decomposer induced decomposition. The pH, organic carbon and microbial parameters were analyzed at different days. It was found that pH was in the range of 4 to 6 while the organic carbon in the range of 0.4–0.6% (Chandra and Kanojia 2018). It is known that the soil microorganisms play a significant role in improving the bioavailability of potassium, zinc, iron and phosphate through the biosynthesis of organic acids, siderophores, organic ligands and polysaccharides (Rajawat et al. 2016).

22.6 Plant Health Protection

Various chemical pesticides are available in the market under different brand names for controlling an array of plant diseases, insects and pests. Most of them are expensive, show indiscriminate action against beneficial and pathogenic microbes and hazardous to environment and humans. While, the biological plant protection agents are cheap, renewable and nontoxic to human and animals (Massart and Jijakli 2007). The important qualities of an ideal biocontrol agent includes, rapid growth rate, high survival rate, high shelf life, high pathogenicity, broad spectrum activity, compatibility with chemical fertilizers and pesticides; and nonpathogenic towards plants, animals and humans. In addition to disease control, they play a substantial function in integrated disease management (IPM) (Spadaro and Gullino 2005). The biocontrol agents function based on complex mechanisms such as nutrient competition, antibiosis, parasitism, pathogen enzyme inactivation, systemic resistance induction and stress tolerance in plants (Raymaekers et al. 2020).

The waste decomposer falls under the category of effective, reliable, economical, high quality, easily propagated, broad spectrum biocontrol agent (Harman 1991). It can be utilized as a foliar spray, seed dressing material and drip irrigant. It is known to control a wide range of bacterial, fungal and viral diseases of various crops which are soilborne, seedborne, rootborne, shootborne and foliar in origin. It is known to control damping off disease in solanaceous (chilli, tomato, potato, brinjal), fabaceous (peanut, soybean) plants; cabbage and maize. The rhizome root diseases in turmeric, ginger and onion; root rot diseases in pineapple, fenugreek, citrus and barseem clover; and wilt diseases in chilli, tomato, potato, brinjal, banana, cotton, peanut, coffee, betel and black pepper are effectively managed by its application. Thus, its application eliminates the necessity of commercial, chemical based bactericides, fungicides, pesticides and insecticides (Kosaraju 2018a, b).

22.6.1 Foliar Spray

A 2.5–10% solution of waste decomposer in water can be sprayed on the leaves of standing crops for about 4 times at an interval of 10 days. The foliar spray helps in controlling the various foliar plant diseases and pests. Further, the foliar spray prevents the grazing and foraging of crops by animals such as Blue bull commonly found in Faridabad and Kutch regions (Thacker 2019; Mondal 2017).

22.6.2 Seed Dressing

The seed dressing or treatment is a technique that involves the application of physical methods, chemicals, biological extracts or biocontrol agents to seeds, seedlings or plants before sowing for suppressing, controlling, repelling the phytopathogens, insects or other pests by forming a protective coating around the surface of the seeds or propagules (Naguri et al. 2020; Sharma et al. 2015). The seed treatment protects the seed during storage and after soil planting; reduces the initial inoculum of pathogens, reduces the environmental hazards caused by pesticide spray, increases seed vigour, breaks seed dormancy and improves seedling emergence. Most importantly, the seed dressing is the only technique which prevents the plant from viral diseases. While the biocontrol agent based seed dressing is an alternative to chemicals due to its low cost, renewability, biodegradability, food safety and sustainability (Sharma et al. 2015; Nandini and Naidu 2018).

The mass multiplied waste decomposer solution, slurry or the powder formulation can be used for the seed treatment. The seeds of all the crop varieties can be sprayed uniformly with the solution of waste decomposer. A lot of 20 kg of seeds can be treated with one bottle waste decomposer mixed with 30 g of jaggery. After 30 min, the waste decomposer treated and shade dried seeds can be used for sowing. The seed dressing with waste decomposer helps in controlling the seed and soilborne diseases, enhances seed germination (up to 98%) and reduces the time of seedling emergence (by 4 days). Further, the treatment alleviates the biotic (seed and seedling diseases), abiotic (salinity, cold, heat and osmotic shock) and physiological (aging induced poor seed quality) stresses, thereby enhances the plant growth and yield (Thacker 2019; Kosaraju 2018a; Chandra et al. 2019).

22.6.3 Drip Irrigation/Fertigation/Microbigation

The prepared solution can be mixed with irrigation water and used for drip irrigation. The produced solution of 200 L is sufficient for drip irrigation of 0.4 hectare of crop land. The microbigation with waste decomposer revives soil health and acts as a biofertilizer and biocontrol agent for various soilborne and foliar diseases (Chandra and Kanojia 2018; Thacker 2019). The delivery of biocontrol agents via systematic drip irrigation is known as microbigation (Boari et al. 2008).

22.7 Crop Productivity

After green revolution, in India the increased consumption of nitrogenous chemical fertilizers resulted in high prevalence of pests and diseases in plants, which in turn resulted in decreased yield and quality of the produce. Hence, utilization of natural, alternate sources gained momentum in terms of integrated nutrient management. The usage of waste decomposer for various crops enhances both the quality and yield of the crops. Its application acts an alternative to chemical fertilizers such as urea, diammonium phosphate, muriate of potash, etc. and serves as a key component in organic farming. Its application can drop the chemical fertilizer usage by 60% due to an increase in organic carbon (Thacker 2019; Kosaraju 2018b). In sugarcane variety Co238, the waste decomposer treatment improved tillering, cane height, cane weight, yield and no of millable canes over control and other treatments (Punia et al. 2019). The regular utilization of waste decomposer can reduce the input cost and the capable of doubling the crop income (Kosaraju 2018a). Since launching, more than one million farmers have adapted this technology and rejuvenated their soils, protected their crops from various insects and diseases; and enhanced their crop productivity (Kosaraju 2018b).

The major and micronutrient enriched nutrient mixture can be made by fermenting 50 L jaggery propagated waste decomposer solution supplemented with 2 kg each of powders of different pulses (red gram, black gram, green gram, chickpea, soybean) and oil seeds (mustard, sunflower, sesame); 100 g of iron nails, 250 g of copper wire/foil and 100 g of zinc powder for 15 days. The sieved solution can be sprayed for all crops during different stages for flowering and fruiting enhancement. The major and micro nutrients in the mixture increase the size, quality and yield of various crops. It can applied once/month for horticultural crops and thrice/month for vegetable crops (Kosaraju 2018a).

22.8 Soil Health Management

The presence of high levels of soluble salts in the soil is responsible for an increase in its salinity. The excessive usage of chemical fertilizers in many regions has increased the salinity of soils in India. The high osmotic pressure induced by salinity hinders the soil water absorption, oxygen transfer, water uptake and essential nutrient

Table 22.1 The effect of waste decomposer solution on soil mineral composition before and after treatment for 6 months

Parameter (mg/Kg)	Control soil	Waste decomposer treated soil
Total nitrogen	18	760
Phosphate	23	54.6
Potash	60.1	641
Iron	1	24
Manganese	0.2	3.1
Zinc	0.09	1.3
Copper	0.05	1.1

adsorption by roots (Mohan et al. 2019). It intern effects the plant growth and crop production and reflects in terms of various unfavourable symptoms such as poor seed germination, small and blue green coloured leaves; dwarf stems and branches; stunted growth, wilting and desiccation of plants; physiological drought, susceptibility to root and soilborne diseases; retarded flowering, sterility, smaller seeds, low yields and growth of halophilous weeds (Kosaraju 2018b).

Generally, gypsum, a rich source of calcium and sulphur is added at a dosage of 1–2 tonne/0.4 ha for alleviating the soil salinity, increasing the soil porosity and retention of organic carbon (Mohan et al. 2019). Most of the Indian soils, the organic carbon ranged from 0.1–0.5% and they are deficient in secondary and micronutrients (phosphorous, potassium; zinc, manganese, iron, boron and copper) (Amirneni 2020). The surface/top soil should contain a minimum of 0.2% organic carbon. While in some places of Indian states such as Punjab, Haryana, Uttar Pradesh, Tamil Nadu, Andhra Pradesh, it is limited to 0.05% only (Sathguru 2019). As an alternative, waste decomposer can be used for the reduction of soil salinity and enhancement of organic carbon in soil (Thacker 2019). The regular and continuous (6–12 months) application of waste decomposer in soil alters the physicochemical and biological qualities of the soil (Mondal 2017). At an application rate of 400 L/0.4 ha for 5 times is needed for the induction of plant growth in saline soils (Kosaraju 2018a). It is reported that a change in soil texture and structure; and enhancement in porosity made the potato harvesting very easy with bare hand, without applying any farming tools. Also, the application of waste decomposer increased the quality and shiny appearance of pomegranates. It also affects the weed pattern and aids in their control in crops such as pea and chickpea (Kosaraju 2018b, a). It enhances the soil porosity and various beneficial macro and micro biota of soil; and quantity of earthworms in soil. It favours the plant growth by decomposing crop residues in fields, enhances organic carbon and provides amiable rhizosphere for nutrient release due to the activities of cellulose degradation and mineral solubilization. The effect of waste decomposer solution on soil mineral composition before and after treatment for 6 months is shown in Table 22.1 (Kosaraju 2018a).

The available bacterial consortium in waste decomposer solution includes nitrogen fixing bacteria (*Azotobacter, Azospirillum, Rhizobium, Acetobacter*), *P. fluorescence* and organic acid producers and thus functions as a biofertilizer for all the crops. The nodulating, host specific *Rhizobium* bacteria fixes nitrogen (20–80 kg/0.4 ha) in various leguminous crops such as peanut, pea, barseem clover, soybean, beans, horse gram, cow pea, etc. The *Azotobacter* bacteria fixes nitrogen around 8–16 kg/0.4 ha and is used as a biofertilizer in chilli, cotton, rice, sugarcane, sorghum, pearl millet, tobacco, tea, coffee, vegetable, ornamental and horticultural crops. While, the biofertilizer *Azospirillum* strains are utilized in rice, maize, wheat, sorghum, finger millet, sugarcane, cotton, forage and horticultural crops for nitrogen fixation (8–16 kg/0.4 ha). And, *Acetobacter* is utilized in sugarcane. The consortium is enriched with *P. fluorescence*, which is known to stimulate plant growth via production of phytohormones, antibiotics, volatile compounds (ammonia, hydrogen cyanide), siderophores and organic acids. Hence, it acts as a dual functional biofertilizer and biocontrol agent. The siderophores are small molecular mass, high affinity iron chelating molecules biosynthesized by bacteria and fungi. They complex iron that is poorly soluble (iron oxides and hydroxides) and facilitate the iron uptake by plants. Its application increases leaf size, photosynthesis rate; fixes phosphorous (10–12 kg/0.4 ha) and controls blight, root rot and other fungal diseases (Madhavi and Kumar 2018). In soils, the minerals such as phosphorous, potassium and zinc are either deficient or tightly bound and unavailable for plant uptake. Due to the production various enzymes and organic acids by the consortium of waste decomposer, the minerals such as phosphorous, potassium, iron and zinc are bioavailable for plants (Mumtaz et al. 2017; Nagaraju et al. 2017; Singh et al. 2018).

Notably, the waste decomposer functions as an eco-friendly biocontrol agent and inhibits the soilborne pathogen growth via nutrient and space competition; antagonistic action against phytopathogens, and production of extracellular lytic enzymes and volatile and nonvolatile antibiotics, secondary metabolites such as polyketides and alkanes in the rhizosphere and stimulation of systemic resistance in plants via production of glucanase and β-1,3 glucanases (Thacker 2019; Kosaraju 2018b). In addition, its application stimulates the plant growth and development due to the production of plant growth promoting substances like auxins, indole acetic acid, siderophores, etc. (Dhevendaran et al. 2013).

22.8.1 Other Applications

It is also used for toilet cleaning and reduction of foul odour generation from septic tanks in villages, thus meeting the directives of *Swachh Bharat* mission (Thacker 2019; Kosaraju 2018b). Especially, the waste decomposer bacteria were found to degrade keratin present in poultry feathers and human hair (Fig. 22.6). Keratin is the main, structural fibrous protein present in feathers, wool, hair and nails. The keratin wastes are mostly produced from poultry farms, slaughter houses and leather industries and it is considered as an environmental pollutant. Hence, the keratinolytic activity of waste decomposer could be exploited for poultry waste recycling and nitrogenous fertilizer and animal feed production.

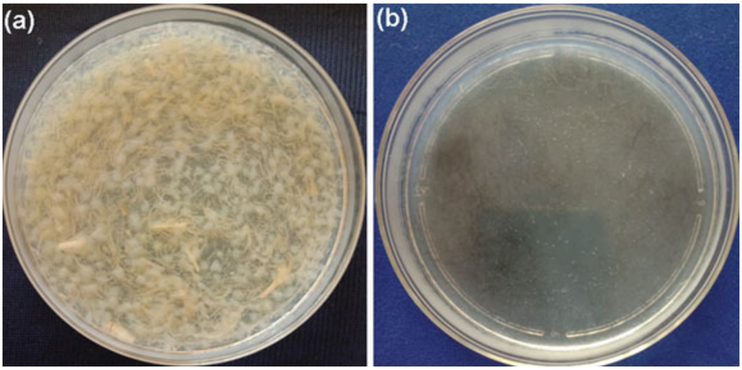

Fig. 22.6 The keratinolytic activity of waste decomposer bacteria towards (***a***) poultry feathers and (**b**) human hair

22.9 Conclusions

The mass multiplied waste decomposer solution is enriched with cellulolytic, organic acid, siderophore producing; bacteria in addition to nitrogen fixing bacteria and *P. fluorescence*. Thus, the consortium exhibits multifaceted activities such as composting, biofertilizer, biocontrol agent and soil heath reviver, thus leading to enhanced crop productivity, plant disease resistance and soil characteristics. Based on these virtues, it became more popular among farming communities and many more promising applications of waste decomposing are arising. Further studies on applicability of waste decomposer under varying agro climatic conditions, resistance development by phytopathogens and effects on beneficial and nontarget microbes are envisaged.

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References

Adlakha N, Rajagopal R, Kumar S, Reddy VS, Yazdani SS (2011) Synthesis and characterization of chimeric proteins based on cellulase and xylanase from an insect gut bacterium. Appl Environ Microbiol 77(14):4859–4866. https://doi.org/10.1128/AEM.02808-10

Amirneni NR (2020) Better results with organic carbon enhancement. Annadata 52(1):58–59

Ansari MA, Roy SS, Sharma SK, Shamurailatpam D, Park P, Meitei CB, Chanu NG, Lamnganbi M, S Mari, Ansari MH, Prakash N, Singh IM (2018) Burning of rice residue: hazards and solutions. Souvenier of brainstorming workshop on rice residue burning in Manipur-Issues and strategies for sustainable management: 44–50

Boari A, Zuccari D, Vurro M (2008) 'Microbigation': delivery of biological control agents through drip irrigation systems. Irrig Sci 26(2):101–107. https://doi.org/10.1007/s00271-007-0076-x

Chandra K, Kanojia P (2018) Microbes for rice residue management-options and strategies. Souvenier of brainstorming workshop on rice residue burning in Manipur-Issues and strategies for sustainable management: 6–13

Chandra K, Vootla PK, Singh R, Kannojia P (2019) Waste decomposer (a way of farmers doubling income). Ministry of Agriculture & Farmers Welfare, Government of India, Ghaziabad

Lava Chikkappaiah, Harish Nayaka MA, Manohar MP, Vinutha C, Prashanth Kumar GM (2017) Effect of plant mucilage clarificants on physical and chemical propperties of jaggery. Int J Recent Sci Res 8(10):20663–20669. doi:https://doi.org/10.24327/ijrsr

Devi MT, Layek J, Kumar B, Kannan R, Babu S, Das A (2018) Management of rice residue for sustainable soil health in conservation agriculture Souvenier of brainstorming workshop on rice residue burning in Manipur-Issues and strategies for sustainable management:20–29

Dhevendaran K, Preetha G, Hari BNV (2013) Studies on nitrogen fixing bacteria and their application on the growth of seedling of *Ocimum sanctum*. Pharm J 5(2):60–65

Harman GE (1991) Seed treatments for biological control of plant disease. Crop Prot 10 (3):166–171. https://doi.org/10.1016/0261-2194(91)90038-S

India To (2019) Compost technology to convert weeds into organic manure. Times of India, 19th December 2019,

Jha, A.K., Rymbai H, Verma VK, Deshmukh NA, Assumi SR, Talang HD, Devi MB (2018) Utility of rice residue in horticultural sector. Souvenier of brainstorming workshop on rice residue burning in Manipur-Issues and strategies for sustainable management:56–61

Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A (2008) A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Curr Microbiol 57(5):503–507. https://doi.org/10.1007/s00284-008-9276-8

Kora AJ (2019) Leaves as dining plates, food wraps and food packing material: importance of renewable resources in Indian culture. Bulletin of the National Research Centre 43(1):205. https://doi.org/10.1186/s42269-019-0231-6

Kora AJ, Rastogi L, Kumar SJ, Jagatap BN (2017) Physico-chemical and bacteriological screening of Hussain Sagar lake: an urban wetland. Water Science 31(1):24–33. https://doi.org/10.1016/j.wsj.2017.03.003

Kosaraju CR (2018a) Waste decomposer that offers many benefits. Raitu Nestam 14(1):43–46

Kosaraju CR (2018b) Zero productive cost, double income in crop cultivation with waste decomposer. Raitu Nestam 13(11):61–63

Kumar A, Kumar B (2019) Innovative method of preparing compost from farmyard manure with waste decomposer. In: Rana RK, Singh R, Thakur AK, Chahal VP, Singh AK (eds) Contemplating agricultural growth through farmers' frugal innovations. ICAR-Agricultural Technology Application Research Institute, Punjab, pp 95–96

Madhavi GB, Kumar PN (2018) Impooratnce of biofertilzers in cultivation of crops. Prakruti Nestam 5(10):10–12

Massart S, Jijakli HM (2007) Use of molecular techniques to elucidate the mechanisms of action of fungal biocontrol agents: a review. J Microbiol Methods 69(2):229–241. https://doi.org/10.1016/j.mimet.2006.09.010

Mohan MM, Madhuri KVN, Reddy BR, Prasad PR (2019) Why gysum should be applied to soil? Annadata 51(10):12

Moirangthem P, Hazarika S, Das A (2018) Rice residue burning-impacts on air quality nad greeenhouse gas emission. Souvenier of brainstorming workshop on rice residue burning in Manipur-Issues and strategies for sustainable management:51–55

Mondal M (2017) One oragnic solution for just Rs 20. Krishi Jagran. Accessed 12 February 2018

Mumtaz MZ, Ahmad M, Jamil M, Hussain T (2017) Zinc solubilizing *Bacillus* spp. potential candidates for biofortification in maize. Microbiol Res 202:51–60. https://doi.org/10.1016/j.micres.2017.06.001

Nagaraju Y, Triveni S, Gopal AV, Thirumal G, Kumar BP, Jhansi P (2017) *In vitro* screening of Zn solubilizing and potassium releasing isolates for plant growth promoting (PGP) characters. Bull Environ Pharmacol Life Sci 6(SI 3):590–597

Naguri S, Firdouz S, Nayak J, Swathi S, Sreedhar (2020) Benefits of seed dressing. Annadata 52 (6):14–15
Nandini MLN, Naidu MSM (2018) Farmers should know the importance of seed treatment in plant protection. Raitu Nestam 14(4):49–51
Pan I, Dam B, Sen SK (2011) Composting of common organic wastes using microbial inoculants. 3. Biotech 2(2):127–134. https://doi.org/10.1007/s13205-011-0033-5
Punia P, Prusty AK, Kashyap P, Kumar S, Chandrabhanu S, Meena LR (2019) physiological approaches for improving productivity of promising cropping systems. Annual report 2018-19. ICAR-Indian Institute of farming systems research, Modipuram, Meerut, India
Rajawat MVS, Singh S, Tyagi SP, Saxena AK (2016) A modied plate assay for rapid screening of potassium-solubilizing bacteria. Pedosphere 26(5):768–773
Raymaekers K, Ponet L, Holtappels D, Berckmans B, Cammue BPA (2020) Screening for novel biocontrol agents applicable in plant disease management – a review. Biol Control 144:104240. https://doi.org/10.1016/j.biocontrol.2020.104240
Sahu AR, Saxena AK (1994) Enhanced translocation of particles from lungs by jaggery. Environ Health Perspect 102(supplement 5):211-214
Sathguru (2019) The survival of the farmer depends on protecting the soil. Annadata 51(9):54–55
Scales BS, Dickson RP, LiPuma JJ, Huffnagle GB (2014) Microbiology, genomics, and clinical significance of the *Pseudomonas fluorescens* species complex, an unappreciated colonizer of humans. Clin Microbiol Rev 27(4):927–948. https://doi.org/10.1128/CMR.00044-14
Sharma KK, Singh US, Sharma P, Kumar A, Sharma L (2015) Seed treatments for sustainable agriculture-a review. J Appl Nat Sci 7(1):521-539. doi:10.31018/jans.v7i1.641
Shashidhar KS, Babu S, Premaradhya N, Kondareddy AN, Gopal D (2018) Status of rice cultivation and viable options for rice residue management in Manipur. Souvenier of brainstorming workshop on rice residue burning in Manipur-Issues and strategies for sustainable management:30–43
Singh J (2017) Farmer friendly techniques-waste decomposer. SSIAST art of living. Accessed 18 Aug 2020
Singh LN, Irungbam P (2018) Practice of burning rice stubble in zero tillage rabi crops-merits & demerits. Souvenier of brainstorming workshop on rice residue burning in Manipur-Issues and strategies for sustainable management:14–17
Singh J, Beniwal V, Yadav AK (2012) 45 days safal compost from dung heap. In: Devi R, Kidwai MK, Rose PK, Saran AK (eds) International conference on energy-water-waste Nexus for environmental management (ICEWWNEM - 2012). Narosa Publishing House, New Delhi, India, p 122
Singh S, Maurya BR, Bahadur I (2018) Solubilization of potassium containing various K-mineral sources by K-solubilizing bacterial isolates on Aleksandrov medium. Int J Curr Microbiol Appl Sci 7 (03):1142-1151. doi:10.20546/ijcmas.2018.703.136
Spadaro D, Gullino ML (2005) Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Protect 24(7):601-613
Sridevi T, Saikia N, Rao CS (2017) Natural products-a study to understand the presence of useful microbes with pesticidal activity. NIPHM Plant Health Newsletter 7(1)
Thacker H (2019) CSR: innovation to manage waste and facilitate organic farming. The CSR J
Verma B (2020) An revolutionary product of NCOF, India for "100% organic farming" throughout the world. Accessed 18th Aug 2020
Vootla P, Chandra K (2018) Challenges and opportunities for small and marginal farmers in organic farming. In: Dulloo A (ed) Proceedings of the consultation on “from sustainable agriculture to organic farming”. Ministry of Rural Development, Government of India, New Delhi, pp 51–76

Environmental Sulfate-Reducing Microorganisms

23

Mostafa Mostafa Abo Elsoud and Mohamed I. Abo-Alkasem

Abstract

Sulfate-reducing bacteria (SRB) are a group of obligate anaerobic microorganisms that use sulfate group as a final electron acceptor. They play a crucial role in the sulfur cycle in the environment. This role may be direct due sulfate reduction or indirect due to the effect of microbial metabolic activity and physical localization (biofilm formation). SRB activity deems to be desired or not based on the human requirements. On the other hand, the presence of SRB, in nature, aims to reach the environmental and ecological balance, which is a state of dynamic equilibrium within material and microbial community. In nature, this process is continuous (in circulation), and steady state cannot be reached which guarantees dynamic environment, only, in the presence of water.

Keywords

Sulfate-reducing bacteria (SRB) · Acid mine drainage (AMD) · Bio-precipitation · Bioremediation · Metal · Microbial corrosion

Abbreviations

AMD	Acid mine drainage
AMP	Adenosine monophosphate

M. M. Abo Elsoud (✉)
Genetic Engineering and Biotechnology Research Division, National Research Centre, Dokki, Egypt

M. I. Abo-Alkasem (✉)
Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Egypt

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APB	Acid-producing bacteria
APS	Adenosine phosphosulfate
ATP	Adenosine triphosphate
COD	Chemical oxygen demand
DMS	Dimethyl sulfide
EPS	Exopolysaccharides
GNP	Gross national product
HMW	Higher molecular weight
IBD	Inflammatory bowel disease
IOB	Iron-oxidizing bacteria
IRB	Iron-reducing bacteria
LMW	Low molecular weight
MIC	Microbiologically induced corrosion
MIW	Mining influenced water
MOB	Manganese-oxidizing bacteria
PAHs	Polycyclic aromatic hydrocarbons
SOB	Sulfur-oxidizing bacteria
SRA	Sulfate-reducing archaea
SRB	Sulfate-reducing bacteria
SR-PRZs	Sulfate-reducing permeable reactive zones
TNT	2,4,6-Trinitrotoluene
UC	Ulcerative colitis
WHO	World Health Organization

23.1 Introduction

Sulfur presents in air, water, and soil in various organic and inorganic structures, whether in gaseous, dissolved, or insoluble form. In drinking water derived from private wells, sulfate represents up to 20 mM (National Research Council 1977; Gomex et al. 1995). Sulfates (Fig. 23.1) contribute in the dissolution of numerous minerals (Greenwood and Earnshaw 1984), including sodium (Na_2SO_4), magnesium ($MgSO_4 \cdot 7H_2O$), potassium (K_2SO_4), and many other minerals which represents a huge problem during water treatment for its reuse.

It has been reported that sulfate in wastewater can reach as high concentrations of (4000 g/m^3) which is considered above the acceptable levels (500 g/m^3) approved by

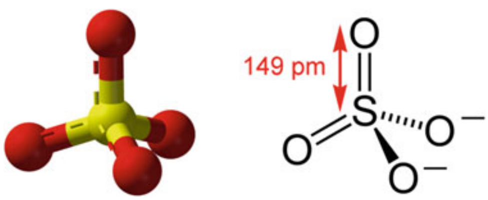

Fig. 23.1 3D form of sulfate ion

environmental legislations of many countries according to Al-Zuhair et al. (2008), while the concentration of (250 g/m^3) was recommended by the World Health Organization (WHO) (Visser et al. 2001). In industry, products of sulfuric acid and sulfate are used in chemical, soap, textile, dye, paper, glass, and fungi- and insecticide manufacture and used for leather processing, metal and plating industries, wood pulp, mining (Greenwood and Earnshaw 1984), and petroleum refineries (Al-Zuhair et al. 2008) which explains the presence of sulfur in all types of the environments. Furthermore, aluminum sulfate (alum) was used for drinking water treatment (as a sedimentation agent), and copper sulfate ($CuSO_4$) was used to control algal growth in public water supplies and swimming pools (McGuire et al. 1984). The atmospheric sulfur compounds such as sulfur dioxide and sulfur trioxide are produced from the metallurgical industries and combustion of fossil fuels which may combine with rainwater or water vapor to form acid water "acid rain" (Delisle and Schmidt 1977) and, subsequently, adsorbed by soil to reduce its pH to acidic levels resulting in soil destruction.

The effect of sulfur compounds on human ranges from simple side effects, e.g., catharsis and dehydration from diarrhea, to complicated side effects, e.g., carcinogenesis and toxicity and death (Cocchetto and Levy 1981; Morris and Levy 1983; US EPA 1999a, b). In the human large intestine, it has been reported that sulfomucins are responsible for the daily production of about 1.5–2.6 mM sulfate. This concentration of sulfate can support the growth and metabolism of SRB (Willis et al. 1996). The sulfation degree of mucin varies not only from one animal species to another but also among individual species or human (Sheahan and Jervis 1976; Nieuw Amerongen et al. 1998). It has been observed that SRB concentration is directly proportional to the density of sulfomucins in intestinal segments (Deplancke et al. 2000). It has been reported that the hydrogen sulfide molecule produced by the human intestinal sulfate-reducing bacteria (SRB) is an etiopathogenic agent responsible for ulcerative colitis (UC) and colorectal cancer chronic inflammatory bowel diseases (Kanazawa et al. 1996; Roediger et al. 1997). However, sulfur is an essential element for all kinds of life (Domagal-Goldman et al. 2011) as it is present in some coenzymes, iron sulfur clusters, and two amino acids (cysteine and methionine) representing about 1% of the living organism dry mass.

The presence of hydrogen sulfide may result in three major events: (1) odor of rotten eggs, (2) reaction with iron to form black iron sulfides, and (3) stimulation of electrolytic corrosive processes (Cullimore 2000). In addition, it has the ability to inhibit the growth of both aerobic and anaerobic microorganisms due to its strong reducing properties (Gibson 1990).

23.2 Sulfate-Reducing Bacteria (SRB)

23.2.1 Sites of SRB Isolation

The occurrence of sulfate-reducing bacteria (SRB) is highly associated with the presence of sulfates and anoxic conditions. Therefore, SRB can be found in many environmental locations including, but not confined to, wastewater, seawater and

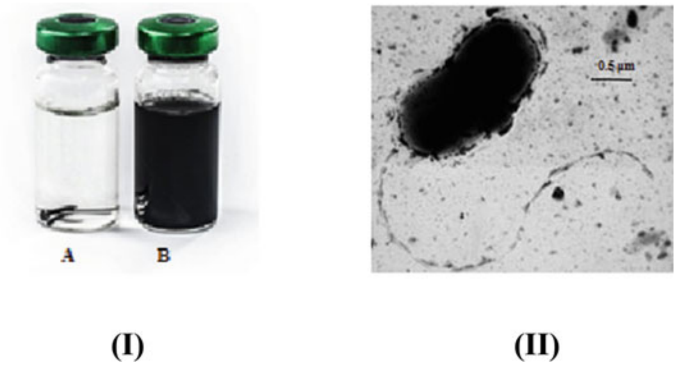

Fig. 23.2 (1) Reduction of sulfate and production of iron sulfide by SRB (B) compared with control (A). (**II**) Microscopic figure of *Desulfovibrio vulgaris*, the best studied SRB

sediments, water rich in organic matter (Barton 1995), and soils (Ouattara and Jacq 1992) and were reported by many researchers in the human large intestine (Gibson and Gibson 1988) and human-impacted environments such as paper mills and rice paddies and found in cattle rumens, wetland and lacustrine sediments, and geothermal vents (Postgate 1966). SRB can, also, be isolated at extreme environmental conditions such as acid mine drainage sites, deep subsurface, oil fields, and even hydrothermal vents (Muyzer and Stams 2008). Mostly, SRB utilize hydrogen (H_2) or short-chain organic compounds as electron donors; therefore, SRB depend on other aerobic and anaerobic microorganisms capable of degrading higher molecular weight (HMW) organic compounds such as cellulose, starches, and lignin to simpler forms (Logan et al. 2003) (Fig. 23.2).

23.3 Classification of Sulfate-Reducing Microorganisms

Sulfate-reducing bacteria represent an integral group of "sulfur bacteria" and are known to be noising bacteria in many fields and industries (American Water Works Association [AWWA] 1995). Sulfate-reducing microorganisms (SRM) or prokaryotes (SRP) were first described by Beijerinck (1895) and considered to be among the oldest microbial forms that can be 3.5 billion years back traced and comprising two types of microorganisms: sulfate-reducing archaea (SRA) and bacteria (SRB) (Barton and Fauque 2009). Although most of SRB cells stain Gram-negative, the Gram staining behavior of SRB was reported to be diagnostically unreliable (Zehnder 1988; Boopathy et al. 1998).

Sulfate-reducing bacteria (SRB) can be divided based on:

1. Type of the utilized organic substrates (Rzeczycka and Blaszczyk 2005).

SRB are considered morphologically diverse but physiologically unified microorganisms. Physiologically, two broad SRB subgroups have been recognized: the first group uses lactate, ethanol, pyruvate, or some specific fatty acids as sources of energy and carbon. The first SRB group includes *Desulfotomaculum*, *Desulfomonas*, *Desulfovibrio*, and *Desulfobulbus*. The second group oxidizes fatty acids, especially acetate. This group includes *Desulfococcus*, *Desulfosarcina*, *Desulfobacter*, and *Desulfonema* (Madigan et al. 1997). Using biomarkers such as fatty acids has been verified as a promising method for the detection and differentiation of SRB (Parkes et al. 1993; Lillebæk 1995).

2. Their degradation mode (organic complete or incomplete heterotrophic oxidation).

The genera of *Desulfotomaculum*, *Desulfosarcina*, *Desulfococcus*, *Desulfomonas*, and *Desulfobacter* are representatives of SRB that completely degrade organic carbon sources (23.1) producing CO_2 and H_2O (e.g., acetate), whereas the genera of *Desulfobulbus* and *Desulfovibrio* incompletely degrade organic compounds (e.g., 23.2) (Fauque et al. 1991; Castro et al. 2000).

$$4\ CH_3COCOONa + 5\ MgSO_4 \rightarrow 5MgCO3 + 2Na_2CO_3 + 5H_2S + 5CO_2 + H_2O \tag{23.1}$$

$$2\ CH_3CHOHCOO^{-} + SO_4^{2-} \rightarrow 2\ CH_3COO^{-} + 2\ HCO^{3-} + H_2S \tag{23.2}$$

The previous equations showed that the gaseous hydrogen sulfide (H_2S) is a main product of sulfate anaerobic respiration by SRB which reacts with heavy metals (Me^{2+} – metal cation) to form insoluble metal sulfide (23.3).

$$Me^{2+} + H_2S \rightarrow MeS + 2H^{+} \tag{23.3}$$

3. SRB energy source (Odom and Rivers Singleton 1993).

Two types of sulfate anaerobic respiration:

1. Autotrophic reduction: in which the gaseous hydrogen is the energy source for SRB and represented by Eq. 23.4:

$$4\ H_2 + SO_4^{2-} \rightarrow S^{2-} + 4\ H_2O \tag{23.4}$$

2. Heterotrophic reduction: in which the energy source is simple organic substances (some alcohols, pyruvate, fumarate, lactate, and the like).

Although physiological and morphological methods have been applied for the detection and identification of SRB, it was laborious and time-consuming. This fact raises the need for direct and rapid detection method.

For the characterization of SRB, much attention is being paid to the application of phylogenetic studies using 16S rRNA sequence information (Deveraux and Stahl 1992). It has been reported by Woese (1987) that the genetic materials, particularly the 16S rRNA, are an ideal tool for phylogenetic analyses of bacterial species. This is because ribosomes are structurally and functionally conserved and occur in all cellular microorganisms. The most initial 16S rRNA sequence comparisons have been provided by the data obtained by oligonucleotide cataloguing procedures. These comparisons among sequences and data analysis resulted in the recognition of a primary evolutionary group "archaea" which is distinct from both the "typical" bacteria and eukaryotes and the development of different bacterial phylogenetic scheme (Woese and Fox 1977; Woese et al. 1985). According to Fowler et al. (1986), the first phylogenetic analysis of sulfate-reducing bacteria was performed by the comparison of oligonucleotide catalogs. Based on the sequence comparisons of 16S rRNA, phylogenetic analysis was used for the classification of most SRB genera into distinct lineages (Devereux et al. 1996).

23.3.1 Phylogeny

The phylogenetic analysis of sulfate-reducing prokaryotes (SRP) resulted in a number of species that differ from one publication (120 species) according to Thauer et al. (2007) to another (220 species) according to Wang (2012). Although their classification is still uncertain, five phylogenetic groups (Fig. 23.3) can be characterized:

1. ***Mesophilic delta-proteobacteria*** which represents the largest group including *Desulfobulbus* and the genera *Desulfobacter*, *Desulfobacterium*, and *Desulfovibrio* (Karlin et al. 2006).
2. ***Thermophilic Gram-negative bacteria***, represented by *Thermodesulfovibrio*.
3. ***Gram-positive Peptococcaceae*** with the spore-forming genus *Desulfotomaculum.*
4. ***Autotrophic thermophile*** *Thermodesulfobium narugense* which belongs to the family of *Thermodesulfobiaceae* (Mori et al. 2003).
5. ***A genus of the hyperthermophile Archaea*** including Euryarchaeota with *Archaeoglobus* and Crenarchaeota with *Caldivirga* (Itoh et al. 1999).

About 33 species of SRB have been reported by Sahrani et al. (2008) to be most studied that have been discovered in the previous 20 years.

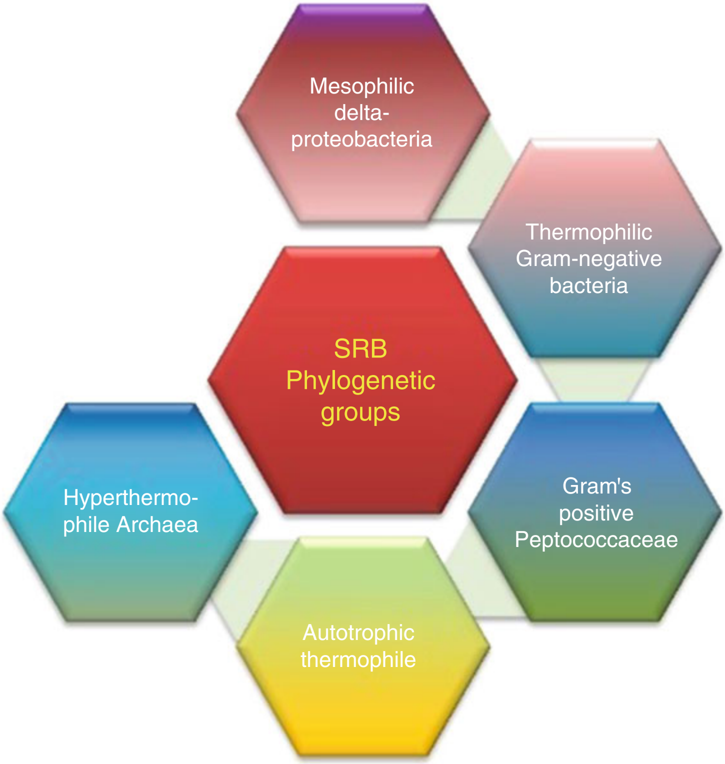

Fig. 23.3 Phylogenetic groups of SRB

23.3.2 SRB Metabolic Activity

The SRB groups differ in their nutritional and morphological characteristics, but all are able to use sulfates, thiosulfates, and sulfites as terminal electron acceptors under anoxic (anaerobic) conditions (de Schulze and Mooney 1993; Muyzer and Stams 2008) producing the reduced forms of sulfur, in particular, hydrogen sulfide (H_2S). Sulfite and thiosulfate are, energetically, preferred as electron acceptors, by SRB, over sulfate. This is because sulfate has to be activated using ATP (requires energy) in the presence of ATP sulfurylase to produce adenosine-5′-phosphosulfate (APS) (Widdel and Hansen 1992) (Eq. 23.5).

$$SO_4^{2-} + ATP \xrightarrow[PPi \rightarrow 2Pi]{ATP\text{-}sulphurylase} APS \xrightarrow[AMP]{2[H]} HSO_3^- \xrightarrow{6[H]} HS^{-1} \quad (23.5)$$

Therefore, these are referred to as "sulfidogenic" microorganisms and have a crucial role in the biogeochemical sulfur cycle (Fig. 23.4a, b) in nature (Barton and Fauque 2009). Throughout the sulfur cycle, many redox intermediate sulfur

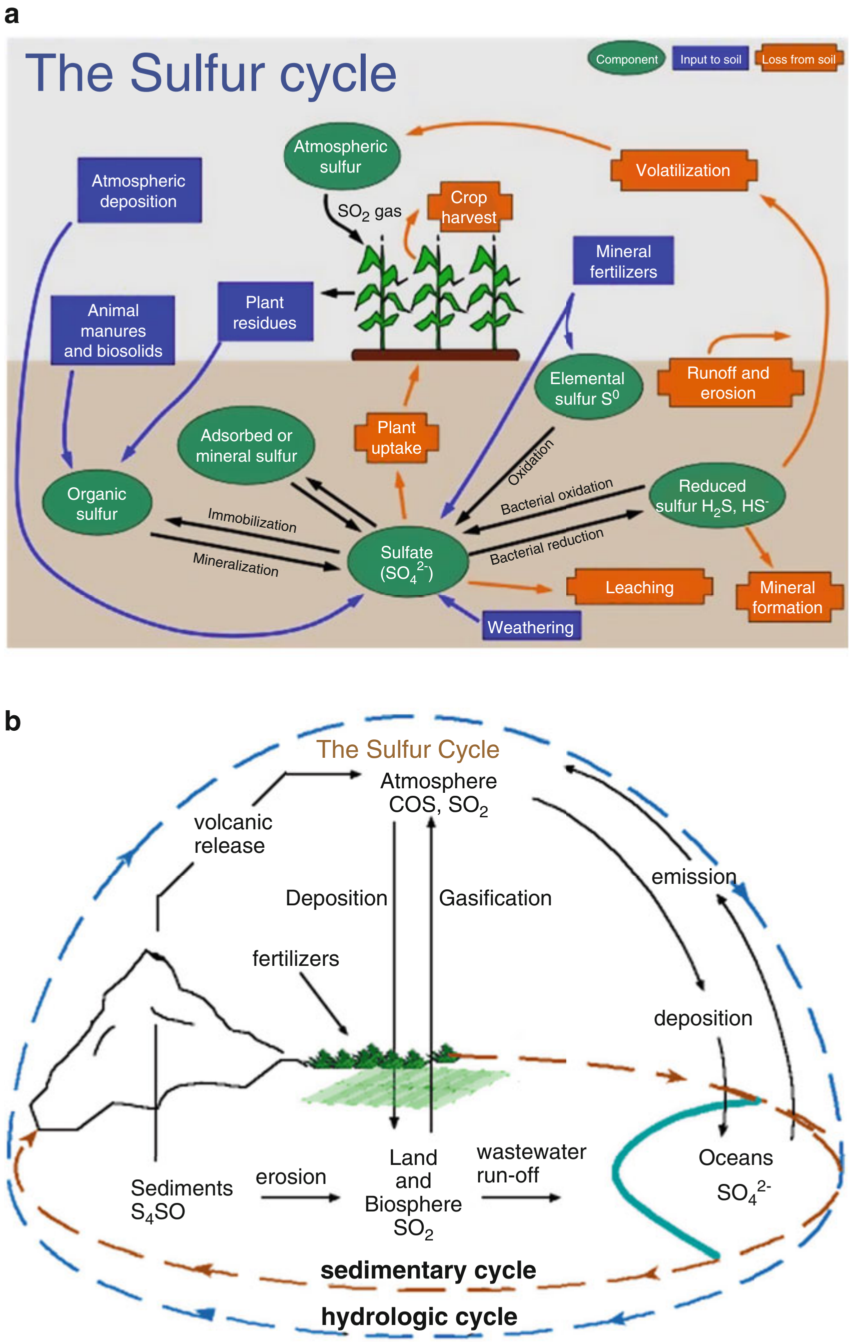

Fig. 23.4 Sulfur cycle

compounds (elemental sulfur, sulfite (Jørgensen and Bak 1991), thiosulfate (Jørgensen 1990), trithionate (Fitz and Cypionka 1990; Sass et al. 1992), and tetrathionate (Wentzien et al. 1994)) have been recognized and taken their positions in the reactions. Although its role is poorly understood in the sulfur cycle, tetrathionate has been detected in some pure SRB culture from different marine sediments (Tuttle et al. 1974; Durand et al. 1994). It was noticed that tetrathionate formation is highly favored in the presence of large concentrations of sulfur and organic matter (Imhoff 1996).

The energy required for these microorganisms' growth is, usually, obtained by oxidation of organic compounds (Rzeczycka and Blaszczyk 2005), according to the following (23.6):

$$2CH_2O + SO_4{}^{2-} \rightarrow H_2S + 2HCO_3{}^{-}(\Delta G = -80\ KJ/mol) \qquad (23.6)$$

Two major types of carbon sources have been recognized and, frequently, used for biotechnological applications. *The first* type includes the complex forms of organic carbon, agricultural, industrial, and food wastes. *The second* is the simple or low molecular weight (LMW) organic compounds, e.g., organic acids (such as acetic, formic, lactic, and pyruvic) and alcohols (such as ethanol and propanol) (Gibson 1990; Fauque et al. 1991; Hao et al. 1996). These compounds are used as carbon sources and preferred by sulfate-reducing microorganisms. Contrarily, it has been concluded that organic acids may result in a complete inhibition of biological sulfate reduction at concentrations (>5 mM) and at pH 3.8 (Gyure et al. 1990).

23.3.3 Mechanism of Sulfate Reduction

Two pathways for sulfate reduction have been identified in bacteria: assimilatory and dissimilatory sulfate reduction (Peck Jr 1961). These pathways differ in the enzymes responsible for catalyzing this reaction.

In the case of assimilatory sulfate reducers, sulfate reduction is catalyzed in the presence of (EC 1.8.4.8), 3′-phosphoadenosine-5′-phosphosulfate-reductase (PAPS reductase), such as *Escherichia coli* and *Salmonella typhimurium*. These are aerobic microbes that reduce sulfate to sulfide, only, to satisfy the sulfur nutritional requirements, i.e., for the synthesis of amino acids (e.g., cysteine) and other sulfur-containing metabolites. In this pathway, sulfate is, enzymatically, converted and actively transported into the cell in the form of adenosine-5′-phosphosulfate (APS) using ATP sulfurylase. APS is further, enzymatically, phosphorylated into 3-′-phosphoadenosine-5′-phosphosulfate (PAPS) by APS kinase. PAPS is then reduced to sulfite by PAPS reductase. The produced sulfite is then reduced to sulfide (S^{2-}) in the presence of sulfite reductase which is then incorporated in metabolic biosynthesis of sulfur-containing compounds (Schwenn 1994; Greene 1996).

On the other hand, in the case of dissimilatory sulfate reducers, sulfate reduction is catalyzed by adenosine-5′-phosphosulfate-reductase (APS reductase) (non-heme iron flavoprotein). These are anaerobic microorganisms that utilize sulfate as a

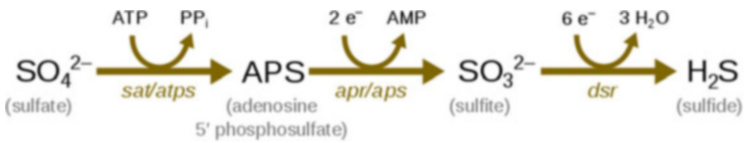

Fig. 23.5 Mechanism of dissimilatory sulfate reduction

terminal electron acceptor, such as *Desulfovibrio desulfuricans*. In the same way, the enzyme adenosine triphosphate (ATP)-sulfurylase activates sulfate into adenosine-5′-phosphosulfate (APS). APS is then reduced by APS reductase to sulfite, and adenosine monophosphate (AMP) is released (Fig. 23.5).

The reductase enzyme of APS (EC 1.8.4.9) has been isolated from several SRB genera (Stille and Trüper 1984) and was reported, in *D. vulgaris* by Bramlett and Peck Jr. (1975), to contain two molecular subunits (72 kD and 20 kD) in addition to other subunit of unknown structure.

Sulfate reduction into hydrogen sulfide requires eight electrons for the reaction to take place, as follows (23.7):

$$SO_4^{2-} + 8e^- + 8H^+ \Leftrightarrow H_2S + 2\,H_2O + 2\,OH^- \qquad (23.7)$$

For this reaction to proceed, a number of intermediate reactions and intermediate products should be produced: sulfite products are the first reduced forms in the sulfate reduction (Madigan et al. 1997) for both the previously mentioned cases. Sulfite is reduced to trithionate in the presence of bisulfite reductase enzyme (LeGall and Fauque 1988). Four types of bisulfite reductase have been identified in dissimilatory reduction of sulfite by SRB: *desulfofuscidin*, *desulforubidin*, P582-type reductase, and *desulfoviridin* (LeGall and Fauque 1988).

23.3.4 Impact of SRB in the Environment

SRB have great economic and pivotal ecological importance originating from their role in the sulfur cycle in nature. Additionally, according to Widdel and Bak (1992), sulfate-reducing bacteria are capable of utilizing a wide range of substrates. *Desulfoarculus baarsii* and *Desulfobotulus sapovorans* are able to utilize long-chain fatty acids (up to C18). *Desulfoarculus baarsii* is able to completely oxidize their substrates such as benzoates to CO_2 (Drzyzga et al. 1993).

23.3.5 Acid Mine Drainage (AMD)

AMD or mining influenced water (MIW) can be defined as a kind of drainage results during mining and metallurgical exploitation of mineral sulfides (Kaksonen et al. 2006) and characterized by its high concentrations of sulfate and metal, low pH, and low content of organic carbons (Sicupira et al. 2015). AMD (Fig. 23.6) is a major

Fig. 23.6 Effect of acid mine drainage (AMD), by Nicholas A. Tonelli (https://pxhere.com/en/photo/115992)

environmental issue in many countries, worldwide, that may cost billions of dollars, yearly (Benner et al. 1999).

In addition to the high dissolved metal content, acidic conditions of acid mine drainage have a destructive effect on the terrestrial and aquatic lives (Sanyahumbi 2003). These characteristics are back to the oxidation of mineral sulfides, when exposed to water, air, or sulfur-oxidizing autochthonous microorganisms, causing serious environmental issues (Sicupira et al. 2015) such as the corrosion of drainage transporting systems including concrete structures. In addition to the AMD, wastewater may contain high sulfate content (reaches 4000 g/m^3) due to various industrial activities and petroleum refinery. The high sulfate content of acid mine drainage and wastewater and their environmental issues can be chemically treated by neutralizing pH conditions using a wide range of chemical agents, e.g., hydrated lime ($Ca(OH)_2$) (Eq. 23.8), calcium oxide (CaO) (Eq. 23.9), limestone ($CaCO_3$), anhydrous ammonia (NH_3), soda ash (Na_2CO_3), anhydrous ammonia (NH_3), magnesium oxide (MgO) and magnesium hydroxide ($Mg(OH)_2$), and caustic soda (NaOH) (Skousen et al. 2000; Johnson and Hallberg 2005). The chemical treatment results in the precipitation of sulfate salts such as $CaSO_4$ according to the following equations:

$$-SO_4^{2-} + Ca(OH)_2 \rightarrow CaSO_4 + -(OH)_2^{2-} \quad (23.8)$$

$$-SO_4^{2-} + CaO \rightarrow CaSO_4 + -O^{2-} \quad (23.9)$$

The precipitation of sulfate salts causes another problem (scale formation) which results in clogging of transporting systems, damage of equipment, and higher labor cost due to further cleaning requirements. Therefore, sulfates must be treated from the industrial wastewater before disposal into the main transportation systems.

Sulfate-reducing bacteria (SRB), isolated from pyritic tailing pond, have been used for AMD treatment by Garcia et al. (2001). They reported 9000 ppm sulfate removal in addition to increase in the medium pH. According to earlier works by Tuttle and coworkers on bio-treatment of acid mine drainage (AMD) using SRB, the drainage was passed through a porous wood dust-made dam along with SRB and cellulolytic microbial consortium, where they noticed a significant reduction in sulfate and raise in the pH value (Tuttle et al. 1968, 1969a, b). In another study by Olem and Unz (1980), the continuous microbiological oxidation of ferrous iron (Fe^{2+}) into ferric iron (Fe^{3+}) was investigated, for the treatment of AMD, before its precipitation and separation using neutralization and rotating disc method, respectively. Since then, many researches have reported successful applications of SRB biotechnology for AMD treatment (Whittington-Jones 2000; Kolmert and Johnson 2001; Foucher et al. 2001; Kaksonen et al. 2003). The addition of nutritional supplements such as energy, carbon, and nitrogen sources may stimulate the metabolic activity and growth of SRB and, subsequently, the sulfate reduction rate. Although most are neutralophiles (grow well at pH 6–8), some SRB isolates are able to grow in moderately acidic conditions (pH 3–4). In 1998, Elliot and coworkers (Elliot et al. 1998) reported 38.3% reduction in the effluent sulfate concentration at pH 3.25 when SRB were applied for the remediation of AMD and wastewater. This tolerance ability may be explained by the formation of biological microenvironments around SRB cells (biofilm formation) that supports their growth and metabolic activities (Ghazy et al. 2013). The treatment of acid mine waters using sulfate-reducing bacteria has been, widely, applied using passive systems, reactive barriers, and SRB anaerobic bioreactors. Waste organic materials (e.g., mushroom compost, manure of household animals, and sawdust, and/or other cheap row organics: e.g., ethanol, methanol, and acetate) play a crucial role in keeping the anaerobic conditions and are utilized for energy and carbon acquisition by SRB.

Sulfate-reducing permeable reactive zones (SR-PRZs) are an anaerobic microbiological system used for the removal of sulfates and heavy metals in AMD

(Lefèvre et al. 2013). This methodology, such as sulfate-reducing bioreactors, anaerobic wetlands, and permeable reactive barriers, represents an efficient method for bio-treatment of acid mine drainage due to low costs and minimal maintenance requirements (Berghorn and Hunzeker 2001; Waybrant et al. 2002). Although their fundamental aspects are poorly understood and have been treated, for many years, as black boxes, SR-PRZs have been well implemented and applied in various spots (Kamolpornwijit et al. 2003).

23.3.6 Bio-Precipitation of Metals

As it has been, previously, mentioned in acid mine drainage, the acidic conditions created in AMD stimulate dissolution of metals causing a serious environmental problem, spread of toxic metals, and destruction of cultivable soils. In addition, seawater is usually supersaturated with calcium and magnesium carbonates causing water hardness. The various inhibiting factors prevent spontaneous precipitation of calcium and magnesium. These factors include the ion pairing with sulfates, high hydration energy (Slaughter and Hill 1991), and organic chelation (Wright and Oren 2005).

Most heavy metal sulfides have very low solubility products; therefore, metal sulfide form is used for potential reduction of metal concentration (Crathorne and Dobbs 1990) to levels below those permitted by the International Environmental Commissions (IEC) (Taylor and McLean 1992). High concentrations of the metals can be produced by this process in levels close to the metallurgical or mining sites (De Vegt et al. 1997). In addition, this process enables the recovery of huge amounts of valuable metal(s) in the form of metal sulfide. Sulfides produced from sulfate reduction may be re-oxidized to sulfate even in suboxic zones in the presence of Fe (III) and Mn(IV) oxides (Jørgensen 1988). This reaction non-enzymatically proceeds to S^0 redox level (Burdige and Nealson 1986) as shown in Eqs. (23.10) and (23.11).

$$HS^{-} + 3\,H^{+} + MnO_2 \rightarrow Mn(II) + S0 + 2\,H_2O \tag{23.10}$$

$$3\,HS^{-} + 3\,H^{+} + 2\,Fe(OH)_3 \rightarrow 2\,FeS + S0 + 6\,H_2O \tag{23.11}$$

Some dissimilatory SRB can re-oxidize sulfides to sulfate in the presence of oxygen or nitrates as electron acceptor (Dannenberg et al. 1992).

During the last few decades, biotechnological applications of biological reduction of sulfate into sulfide using sulfate-reducing bacteria (SRB) were, potentially, used worldwide for the simultaneous and selective removal of heavy metals, toxic and radioactive elements, and sulfates and reduction of environmental and aqueous waste acidity (Gadd and White 1993; White and Gadd 1996). The mechanism of the microbial removal of toxic metals from the environment is, mainly, accomplished by its precipitation in the form of metal sulfides (White et al. 1995; White and Gadd 1996). This process takes place in two stages: *the first stage*, H_2S

production by SRB, and *the second stage*, precipitation of metal(s) by the produced H_2S.

Groudeva et al. (2001) have constructed a system for wastewater passive treatment using SRB. SRB were attributed in the efficient removal of the toxic heavy metals (lead (Pb), cadmium (Cd), manganese (Mn), copper (Cu), and iron (Fe)) to acceptable levels for reuse of the discharges in agriculture and industry (Groudeva et al. 2001).

Uranium is well known for being toxic to cells even at very low concentrations due to its chemical rather than radioactive properties (Ehrlich 1996). It has been reported that uranium is 20 to 40 times more toxic compared with nickel or copper (LeDuc et al. 1997). SRB have a significant role in the geochemistry of uranium and represent a useful tool for decontamination of the environments from uranium (Yun-Juan et al. 2001).

In a study conducted by Bratcova et al. (2002) on uranium (U), arsenic (As), and manganese (Mn), SRB showed high resistance at a concentration of 50 mg/l and produced high concentrations of H_2S along with precipitation of heavy metal sulfides. Uranium was precipitated and removed up to 99%. In this process, the presence of ferrous ions (0.04–2 g/l) stimulates microbial removal of heavy metals at high efficiency. Unfortunately, the presence of high concentrations of nitrate ($>$ 0.5 g/l) may lead to the complete inhibition of SRB activity, which is considered as an important limiting factor during biological treatment of the environment.

Other sulfate-reducing bacteria (SRB), such as *Desulfovibrio* and *Desulfotomaculum*, are able to produce the insoluble sulfide form of uranium (U (IV)) by the enzymatic reduction of its soluble form (U(VI)) (Lovley et al. 1993; Abdelouas et al. 2000).

Tellurium(IV), selenium(IV), chromium(VI), and technetium(VII) were reported by Lloyd et al. (2001) to be reduced by three SRB genera and reported a direct proportion between the growth of *Desulfotomaculum reducens* and metal reduction. The type of electron donors, significantly, affects the reduction of specific metal ions.

The dissolved mercury (Hg) in marine and freshwaters has a great concern related to biological systems, particularly, methylmercury $[CH_3Hg]^+$ (Hosokawa 1995) due to its lipophilic nature. The chronic exposure of biological systems to Hg may result in the biological magnification of mercury in tissues and food chains (Heinz 1974). Due to the cationic nature of $[CH_3Hg]^+$, it has a particular affinity to combine with the sulfur-containing anions (Nolan and Lippard 2008). It has been recorded that about 90–99% of the total environmental mercury present precipitates, while the rest of the percentage accumulates in biological systems, mostly, in $[CH_3Hg]^+$ form (Faust and Osman 1981). Many studies reported that the metabolic activity of SRB is the main reason involved in Hg methylation in sediments (Gilmour et al. 1998; King et al. 1999). Though it is an enzymatic process, the mechanism by which SRB mediate mercury methylation is not, completely, understood. In addition, the potential of different SRB groups for mercury methylation and the ratio between the rates of Hg methylation and sulfate reduction vary widely (King et al. 2000). The main source for human exposure to mercury is the consumption of methylmercury-contaminated sea foods (Environmental Protection Agency 1997. Methylmercury can be readily absorbed in the human gastrointestinal tract and complexes with free cysteine and cysteine-containing proteins and polypeptides (Kerper et al. 1992). This complex is, freely, transported throughout the human body tissues including the placenta where it affects the developing fetus. Methylmercury has a half-life of about 50 days in the human blood (Carrier et al. 2001).

Sulfate-reducing bacterial cells were reported to tolerate copper (Cu) ions up to 150 mg/l. Furthermore, it was concluded that the presence of Cu ions stimulates SRB growth and metabolism and, subsequently, production of hydrogen sulfides and exopolysaccharides (EPS). The metabolic activity of SRB results in the precipitation of copper to very low levels (< 0.1 mg/l) (Jalali and Baldwin 2000). Chen et al. (2000) illustrated the adsorption and precipitation of Cu ions on the biofilm formed by SRB strain (*Desulfovibrio desulfuricans*) and its effect on the bacterial cells.

The geochemical role and metabolic activity of SRB in lithifying microbial mats (Visscher et al. 1992; Reid et al. 2000) were reported to enhance the precipitation of calcium carbonate (Baumgartner et al. 2006). In the lithifying microbial mats, SRB metabolic activity is considered as the natural environmental tool that is used for sustaining carbonate precipitation (Braissant et al. 2007). Calcium carbonate precipitation was shown to be favored in the mere presence of SRB cell which provide sites of heterogeneous nucleation even in the case of inactive form (Bosak and Newman 2003). It has been reported that sulfate-reducing bacteria (SRB) have an obvious role in the precipitation of mineral carbonates, e.g., calcium carbonate ($CaCO_3$), in the lithifying microbial community in several ways:

1. Increasing the environmental alkalinity: the reduction of sulfate results in the significant increase of pH, which subsequently affects the saturation index causing precipitation of mineral carbonate (Visscher and Stolz 2005).
2. The use of organic acids, such as acetate and lactate (low molecular weight molecules) for growth and as energy and electron donors, leads to the consumption of calcium binding carboxylic acids and increase in the available free calcium ions (Dupraz and Visscher 2005; Bosak 2005).
3. The removal of sulfate ions by SRB may alter the kinetic inhibition of mineral carbonate formation (Warthmann et al. 2000; Wright and Wacey 2005).
4. Production of exopolysaccharides (EPS) (Braissant et al. 2007). SRB produces copious amounts of EPS that influences the mineralogy and morphology of mineral carbonate. Due to its ability to interact with minerals, especially calcium, EPS were thought the main mechanism used for precipitation of mineral carbonate.

In laboratory investigations on *Desulfovibrio* sp., the metals nickel, chromium iron, and molybdenum were chelated on the produced EPS (Beech and Cheung 1995; Beech et al. 1999). This fact may explain the presence of other metals such as iron and magnesium in association with calcium carbonate rocks formed by SRB-produced EPS (Braissant et al. 2007). In other studies on cyanobacteria, it was shown that its EPS was capable of binding metal ions such as calcium, mercury,

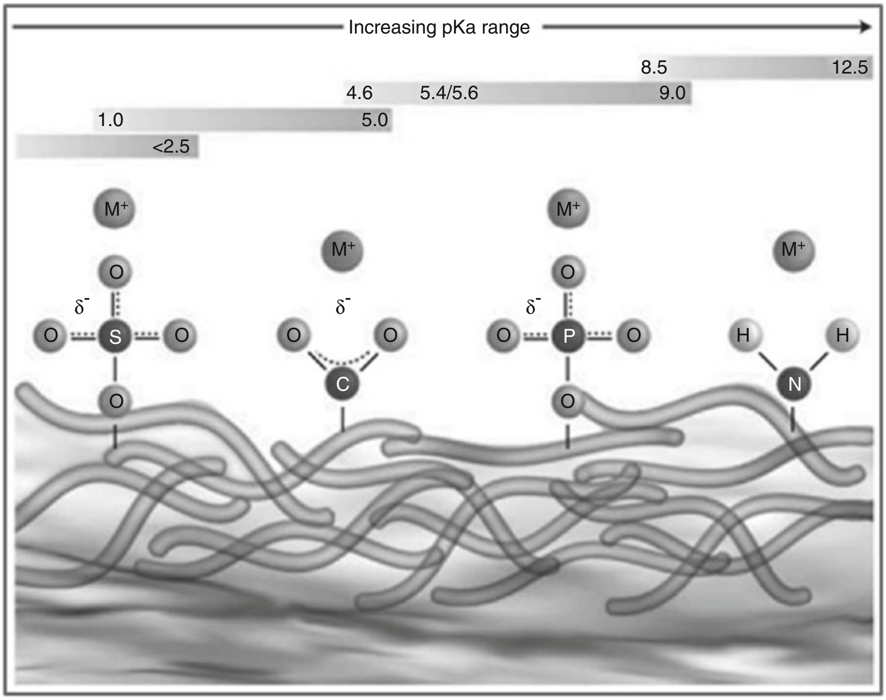

Fig. 23.7 The interaction between EPS and metals (M^+). (Sokolov et al. 2001; Braissant et al. 2007)

cadmium, copper, manganese, calcium, and lead to the various sugar or amino acid functional groups constituting the EPS (Mehta and Gaur 2007). In cyanobacteria, the precipitation and nucleation of calcium carbonate are favored due to the presence of negative charges on its surface. Several functional groups in EPS (such as amino groups and carboxylic acids) were reported to mediate the precipitation of metals. These functional groups provide negative charges to EPS as the pH increases (Socrates 2001; Phoenix et al. 2002; Fig. 23.7).

In addition, the EPS produced by SRB may include non-carbohydrate organic, acidic moieties such as succinate and pyruvate or acidic non-organic functional groups such as phosphate and sulfate (Sutherland 2001). The negative charges of EPS may be attributed to these structures.

For effective and successful use of microorganisms to remove metal as well as other contaminants, they should meet some characteristics including but not limited to: high stability, resistance to high concentrations of heavy metals, sulfate and its reduced forms (e.g., hydrogen sulfide), high metabolic activity (the ability to utilize and convert a broad spectrum of substrates at high rates). The choice of the most suitable carbon and electron donor(s) with respect to their bioavailability and cost plays a key role in the biotechnological applications of SRB in the bioremediation and bio-precipitation of toxic and heavy metals.

23.3.7 Organic Matter Degradation

SRB have an important role in the biodegradation of organic matters (Dexter Dyer 2003). Hence, under anaerobic conditions, the fermenting bacteria use large organic molecules as carbon and energy sources producing smaller compounds that could be utilized by methanogens and acetogens and competed by sulfate-reducing bacteria (Barton 1995). It has been reported by many researchers (Reimers et al. 1992; Canfield et al. 1993; Moeslund et al. 1994) that sulfate-reducing bacteria (SRB) are capable of bio-mineralization of organic compounds present in marine sediments accompanied by reduction of sulfate as a final electron acceptor. Sulfate reduction was, directly, monitored in marine sediment by radio-tracing method (Jørgensen 1978; Fossing and Jørgensen 1989). Although directly monitored, the amount of hydrogen sulfide is usually underestimated due to its rapid rate of re-oxidation in the metal ions' presence (e.g., manganese and iron) or molecular oxygen in the oxidized zones (Lillebæk 1995). The re-oxidation reaction of H_2S is usually mediated by chemical or biological processes (Jørgensen and Bak 1991). Sulfide oxidizers (phototropic sulfur bacteria and colorless sulfur bacteria) such as the members of the family *Chromatiaceae* have been documented as a good example for biologically mediated re-oxidation reaction of H_2S (Lillebæk 1995).

The pathway of organic carbon sources' degradation is highly dependent not only on sulfate-reducing bacteria and the availability of sulfates but also on their interaction with other fermenting bacteria. In addition, the COD/SO_4^{2-} ratio determines the dominant microbial species on the system (Rzeczycka and Blaszczyk 2005). However, up till now, there is no clear interpretation of this phenomenon; it was noted that in wastewater treatment, the COD/SO_4^{2-} ratio lower than 10 causes inhibition of the methanogenic digestive process, while the increase of this ratio has no effect (Rinzema and Lettinga 1988).

Dimethyl Sulfide

Dimethyl sulfide (DMS) (Fig. 23.8) plays a crucial role in the emissions of sulfur to the atmosphere after its degradation.

Aerobic and anaerobic microbial degradation of DMS are well known and have biochemical and ecological aspects in the environment (Wang et al. 2018). Hyphomicrobia or thiobacilli are known for their ability to, aerobically, degrade DMS (Lomans et al. 1999). The pathway of DMS metabolic degradation under

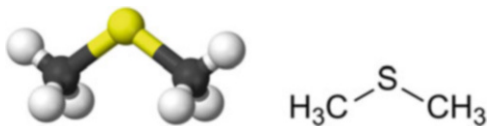

Fig. 23.8 3D structure of dimethyl sulfide (DMS)

anaerobic conditions (Suylen et al. 1986) depends on its oxidation into methanethiol and formaldehyde in the presence of NADH-dependent monooxygenase (which have been purified from *Hyphomicrobium* and *Thiobacillus thioparus*) according to the following (23.12):

$$CH_3SCH_3 + O_2 + NADH + H^+ \rightarrow CH_3SH + HCHO + H_2O + NAD^+ \quad (23.12)$$

Another transformation step depends on methanethiol oxidase which catalyzes oxidation of methanethiol into formaldehyde and hydrogen sulfide as follows (23.13):

$$CH_3SH + O_2 + H_2O \rightarrow HCHO + H_2S + H_2O_2 \quad (23.13)$$

Further oxidation takes place in which H_2S is transformed into sulfate in the presence of H_2O_2. This oxidation process protects the organism against H_2O_2 toxicity, and the excess H_2O_2 is removed by high catalase activity (Smith and Kelly 1988). Anaerobic or O_2-independent mechanism of DMS and CH_3SH degradation (catabolism) is also known, particularly, for methanogenic bacteria (Finster et al. 1992). The alternations between oxic and anoxic conditions are common in various environments (Visscher et al. 1991), so the presence of aerobic and anaerobic mechanisms of DMS degradation is considered to be advantageous.

Aromatic, Nitroaromatic, and Toxic Organic Compounds

Aromatic, nitroaromatic, and toxic organic compounds have been, anaerobically, degraded in the presence of sewage sludge (McCormick et al. 1976, 1981). The presence of nitro-group in the aromatic compounds was proposed as the main cause of their toxicity. The wide use of explosives, pharmaceutics, pesticides, plastics, and many other industrial wastes contributes in the spreading of nitroaromatic compounds to water and soil (Boopathy et al. 1998). Biodegradation of these compounds depends on the utilization of nitrates and sulfates as terminal electron acceptors by SRB (Keith and Herbert 1983). *Desulfovibrio* was documented to be able to degrade 2,4,6-trinitrotoluene (TNT) (up to 100 mg/L) using sulfate group as the terminal electron acceptor and pyruvate as carbon source within 10 days. Many other substrates such as formate, ethanol, and lactate were used to support TNT biodegradation.

Halogenated Organic Compounds

Halogenated organic compounds such as chloroform are proposed to be a threat to public health and a carcinogen. Although chloroform has many applications in various industrial fields and as a solvent, it cannot be, aerobically, biodegraded. Chloroform degradation has been reported by the anaerobic microorganisms, methanogens, and SRB. However, the activity of methanogens is inhibited at chloroform concentrations (>16.74 μM), and SRB are capable to transform chloroform at higher rates without inhibition (Gupta et al. 1996).

Degradation of Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are high molecular weight organic compounds derived from petroleum oil and coal processing (Muckian et al. 2009). They are composed of two or more aromatic benzene rings and pose environmental threats to both animals and humans due to their potential toxic and carcinogenic properties (Delgado-Saborit et al. 2011). In addition, they are recalcitrant, are resistant to biodegradation, have low volatility, and persist in the environment (Varanasi 1989). Generally, the PAHs of high molecular weight ($\geq$ four rings) can be adsorbed to the particles in the environment reducing their environmental adverse effects (Ohura et al. 2004). On the other hand, the PAHs of low molecular weight (two or three rings) occur in vapor form at the atmosphere (Srogi 2007) and are considered as water soluble and mostly transported and spread into the ecosystem through the ground and surface waters. Therefore, the treatment of low molecular weight PAH contaminants is an urgent requirement.

Among the low molecular weight PAHs, fluorine and phenanthrene have been reported to be biodegraded under anaerobic conditions by methanogenic (Natarajan et al. 1999), iron-reducing, nitrate-reducing, and sulfate-reducing bacteria (Rockne and Strand 1998). The highest rate of biodegradation was recorded for sulfate-reducing bacteria. Previously, a correlation between sulfate reduction and PAH biotransformation has been noted as the addition of sodium sulfate (electron acceptor) stimulates the biotransformation process in parallel with the metabolic activity of sulfate-reducing bacteria (Rockne and Strand 1998). Furthermore, the rate and degree of PAH biotransformation are highly linked to the type, density, and activity of microorganisms in the microbial community (ecosystem) during the process (Tsai et al. 2009).

Lignocellulosic Material Degradation

SRB were reported to grow on sulfate-rich wastewater produced from paper and pulp industries. SRB were believed, long ago, to contribute in the breakdown of cellulose material (Bannick and Muller 1952). Although few research papers have reported biodegradation of lignocellulosic materials using SRB, it was two times higher than that under methanogenic conditions (Pareek et al. 1998).

23.3.8 Metal Corrosion

The role of microbes in the corrosion of metals has been known since the early 1900s (Videla and Herrera 2005). The microbial cells are capable of colonizing the metal surfaces resulting in its damage (failure of the system), i.e., gas and crude oil pipeline or even water pipelines (Bachmann and Edyvean 2006). The resulting metal damage is usually referred to as "microbiologically induced corrosion (MIC)" or, simply, bio-corrosion (Beech and Sunner 2004). Microbial corrosion is usually accompanied by significant health and safety, economic, and environmental consequences (Achebe et al. 2012). Although there is no official figure for the estimated cost of

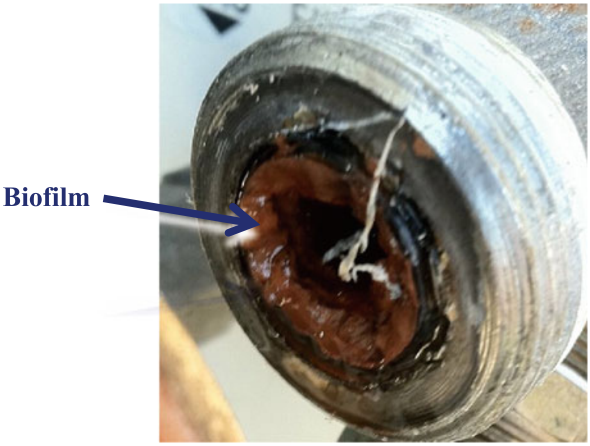

Fig. 23.9 Formation of biofilm on metal surface

microbial corrosion, it has been proposed that it represents about 20–50% of corrosion processes (Jan-Roblero et al. 2004). The cost of microbial corrosion in the industrialized nation has been estimated to reach about 4.9% of the gross national product (GNP).

Many bacterial groups have been reported to be associated with microbial corrosion of cast and mild iron and stainless steel: acid-producing bacteria (APB) (Maruthamuthu et al. 2005), iron-oxidizing/iron-reducing bacteria (IOB and IRB) (Javaherdashti 2008, 2010), manganese-oxidizing bacteria (MOB) (Palanichamy et al. 2002), sulfur-oxidizing bacteria (SOB) (Okabe et al. 2007), exopolysaccharides or slime (biofilm)-forming bacteria (BFB) (Santana et al. 2012), and sulfate-reducing bacteria (SRB) (Wang et al. 2013). One or more of these organisms can coexist in a synergistic community referred to as "biofilm" (consortium) (Alabbas et al. 2013). The activity of SRB is responsible for >75% of the corrosion in productive petroleum oil wells and >50% of the buried steel cable and pipeline damage. In addition, SRB is responsible for the extensive corrosion of big steel structures such as storage tanks and pumping and drilling machineries (Javaherdashti 2008; Wang et al. 2013). Most reports on MIC included sulfate-reducing bacteria (SRB) as a main cause of bio-corrosion (Bento et al. 2005).

Many interpretations (mechanisms) of this bio-process have been discussed by researchers. *One of these mechanisms* is the formation of a gelatin-like matrix, referred to as "biofilm," covering the metal surface (Xu et al. 2002). The biofilm formation (Fig. 23.9) on the metal surface changes the electrochemical balance at the metal-solution interface resulting in cathode and anode formation (Fig. 23.10) and continuous corrosion process (Videla and Herrera 2005).

A second mechanism suggests that the corrosion occurs due to the hydrogen sulfide (H_2S), a toxic and corrosive gas. The production of H_2S was recorded as a cause of a variety of economic and environmental problems including reservoir souring (Hubert et al. 2003). *A third mechanism* proposed the removal of the hydrogen molecules from the metal surface (cathodic depolarization) catalyzed by

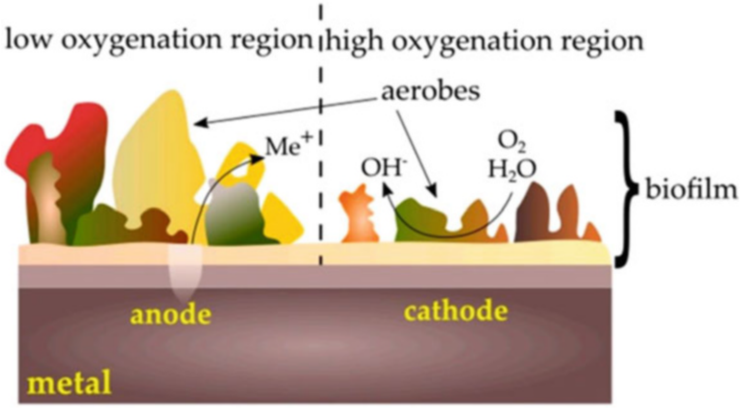

Fig. 23.10 Mechanism and effect of biofilm formation on metal corrosion

hydrogenase enzyme produced by SRB. This enzyme stimulates corrosion process even in the presence or absence of SRB cells (Shi and Xie 2011). *A fourth mechanism* revealed the interaction between sulfide ions and metal to form a non-adhesive layer on the metal surface based on metal sulfide. *In addition* to stimulating corrosion, Salghi et al. (2012) noted that the formed porous layer prevents the natural formation protector oxide film.

23.4 Application of SRB Technology

For environmental application of sulfate-reducing bacteria (SRB), a mixed bacterial culture containing sulfur bacteria is usually used that has been selected and stabilized at the conditions of application site (Davison et al. 1994). This type of cultures has many advantages over the pure counterparts as it represents less liability to contamination by other microorganisms, shows more resistance and adaptability to changes in the surrounding and unfavorable conditions, and prevents the metabolic inhibition of a certain microorganism, due to the formation of utilizable simpler forms of carbon and energy sources and consumption of the produced by-products by the other microorganisms in the consortium (Robin et al. 2001). It is worth mentioning to know that the mixed culture composition modifies in parallel with changing the selective conditions during the growth of the microbial consortium and variation in the microenvironment. For bio-precipitation of heavy metals and mine wastewater treatment using alginate-immobilized mixed SRB culture and methanol as carbon source, an investigation has been conducted by Glombitza (2001) and revealed complete precipitation of heavy metals at 132 mg/l/h sulfate reduction rate, and pH increase from 3 to 6.9. After complete precipitation of heavy metals, the excess sulfide generated was re-oxidized for sulfur production (Glombitza 2001).

Few decades ago, the use of sulfate-reducing bacteria in the technologies of wastewater treatment faced many technical problems that prevented its feasible and economic application. The use of anaerobic process for wastewater treatment was too sensitive and unstable; therefore, it was restricted to manure and sewage water (Visser 1995; Boopathy et al. 1998). In addition, the poor cell retention accompanied by slow SRB growth rate and "clean" waste effluent (wastewater contains metal pollutants and very low or zero concentration of organic matter) exacerbate the problems of continuous bioprocess application of SRB. Most of these problems have been solved by the immobilization of SRB and providing a substrate that represents both electron donor and carbon source or addition of sewage and/or manure to the wastewater (Sanyahumbi 2003).

23.5 Conclusion

In conclusion, SRB are an important group of anaerobic microorganisms that participate in the circulation of sulfur in the environment. In addition, it plays an important role in the precipitation of heavy metals and reduction of COD in aqueous systems which are important processes in the biological treatment of wastewater for its reuse in various fields.

References

Abdelouas A, Lutze W, Gong W, Nuttall EH, Strietelmeier BA, Travis BJ (2000) Biological reduction of uranium in groundwater and subsurface soil. Sci Total Environ 250:21–35

Achebe CH, IAENG Member, Nneke UC, Anisiji OE (2012) Analysis of oil pipeline failures in the oil and gas industries in the Niger delta area of Nigeria. Proceedings of the International Multi Conference of Engineers and Computer Scientists, Vol II

Alabbas FM, Bhola R, Spear JR, Olson DL, Mishra D (2013) Electrochemical characterization of microbiologically influenced corrosion on linepipe steel exposed to facultative anaerobic *Desulfovibrio sp*. Int J Electrochem Sci 8:859–871

Al-Zuhair S, El-Naas MH, Al-Hassani H (2008) Sulfate inhibition effect on sulfate reducing bacteria. J Biochem Technol 1(2):39–44

American Water Works Association [AWWA] (1995) "sulfur bacteria" chapter 3 in AWWA manual m7, AWWA, Denver, Co pp. 671-676

Bachmann RT, Edyvean RGJ (2006) Biofouling: an historic and contemporary review of its causes, consequences and control in drinking water distribution systems. Biofilms 2:197–227

Bannick HF, Muller FM (1952) Utilization of waste liquors from digestion of straw with monosulphite. Anton Leeuw Int J Gen Mol Microbiol 18:45–54

Barton L (1995) Sulfate-reducing bacteria. Springer, Dordrecht

Barton LL, Fauque GD (2009) Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. Adv Appl Microbiol 68:41–98

Baumgartner LK, Reid RP, Dupraz C, Decho AW, Buckley DH, Spear JR, Przekop KM, Visscher PT (2006) Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. Sediment Geol 185:131–145

Beech IB, Cheung WS (1995) Interactions of exopolymers produced by sulphate-reducing bacteria with metal ions. Int Biodeterior Biodegrad 35:59–72

Beech IB, Sunner J (2004) Biocorrosion: towards understanding interactions between biofilms and metals. Curr Opin Biotechnol 15:181–186

Beech IB, Zinkevich V, Tapper R, Gubner R, Avci R (1999) Study of the interaction of sulphate-reducing bacteria exopolymers with iron using X-ray photoelectron spectroscopy and time-of-flight secondary ionisation mass spectrometry. J Microbiol Methods 36:3–10

Beijerinck WM (1895) Ueber Spirillum desulfuricans als Ursache von Sulfat reduction. Zentralbl. Bakteriol. 2. Abt. 1:1–9, 49–59, 104–114

Benner, S.G., D.W. Blowes, W.D. Gould, R.B. Herbert Jr., and C.J Ptacek. 1999. Geochemistry of a permeable reactive barrier for metals and acid mine drainage. Environ Sci Technol 33, 2793–2799

Bento FM, Camargo FAO, Okeke BC, Frankenberger WT (2005) Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresour Technol 96(9):1049–1055

Berghorn GH, Hunzeker GR (2001) Passive treatment alternatives for remediating abandoned-mine drainage. Remediat J:111–127

Boopathy R, Gurgas M, Ullian J, Manning JF (1998) Metabolism of explosive compounds by sulfate reducing bacteria. Curr Microbiol 37:127–131

Bosak T (2005) Laboratory models of microbial biosignatures in carbonate rocks. PhD Thesis, California Institute of Technology, Pasadena, CA, USA, p. 134

Bosak T, Newman DK (2003) Microbial nucleation of calcium carbonate in the Precambrian. Geology 31:577–580

Braissant O, Decho AW, Dupraz C, Glunk C, Przekop KM, Visscher PT (2007) Sulfate-reducing bacteria exopolymeric substances exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals. Geobiology. https://doi.org/10.1111/j.1472-4669.2007.00117.x

Bramlett RN, Peck HD Jr (1975) Some physical and kinetic properties of adenylyl sulfate reductase from *Desulfovibrio vulgaris*. J Biol Chem 250:2979–2986

Bratcova S, Groudev S, Georgiev P (2002) The effect of some essential environmental factors on the microbial dissimilatory sulphate reduction. Annual of the University of Mining and Geology "St. Ivan Rilski" vol. 44–45, part II, Mining and Mineral Processing, Sofia, pp. 123–127

Burdige DJ, Nealson KH (1986) Chemical and microbiological studies of sulfide-mediated manganese reduction. Geomicrobiol J 4:361–387

Canfield DE, Jørgensen BB, Fossing H, Glud R, Gundersen J, Ramsing NB, Thamdrup B, Hansen JW, Nielsen LP, Hall POJ (1993) Pathways of organic carbon oxidation in three continental margin sediments. Mar Geol 113:27–40

Carrier G, Bouchard M, Brunet RC, Caza M (2001) A toxicokinetic model for predicting the tissue distribution and elimination of organic and inorganic mercury following exposure to methyl mercury in animals and humans. II. Application and validation of the model in humans. Toxicol Appl Pharmacol 171(1):50–60

Castro FH, Norris HW, Ogram A (2000) Phylogeny of sulfate-reducing bacteria. FEMS 31:1

Chen BY, Utgikar V, Harmon S, Tabak H, Bishop D, Govind R (2000) Studies on biosorption of Zn (II) and cu(II) on Desulfovibrio desulfuricans. Int Biodeter Biodeg 46:11–18. https://doi.org/10.1016/S0964-8305(00)00054-8

Cocchetto DM, Levy G (1981) Absorption of orally administered sodium sulfate in humans. J Pharm Sci 70:331–333

Crathorne B, Dobbs AJ (1990) Chemical pollution of the aquatic environment by priority pollutants and its control. In: Harrison RM (ed) Pollution: causes, effects and control. The Royal Society of Chemistry, Cambridge, pp 1–18

Cullimore R (2000) Practical atlas for bacterial identification. CRC, Boca Raton

Dannenberg S, Kroder M, Dilling W, Cypionka H (1992) Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. Arch Microbiol 158:93–99

Davison EM, Stukeley MJC, Crane CE, Tay FCS (1994) Invasion of phloem and xylem of woody stems and roots of *Eucalyptus marginata* and *Pinus radiata* by *Phytophthora cinnamomi*. Phytopathology 84:335–340

De Vegt AL, Bayer CJ, Buisman CJ (1997) Biological sulfate removal and metal recovery from mine waters (pp 93–97). SME Annual Meeting, Denver, CO

Delgado-Saborit JM, Stark C, Harrison RM (2011) Carcinogenic potential, levels and sources of polycyclic aromatic hydrocarbon mixtures in indoor and outdoor environments and their implications for air quality standards. Environ Int 37:383–392

Delisle CE, Schmidt JW (1977) The effects of Sulphur on water and aquatic life in Canada. In: Sulphur and its inorganic derivatives in the Canadian environment. Ottawa, Ontario, National Research Council of Canada (NRCC No. 15015)

Deplancke B, Hristova KR, Oakley HA, McCracken VJ, Aminov R, Mackie RI, Gaskins HR (2000) Molecular ecological analysis of the succession and diversity of sulfate-reducing bacteria in the mouse gastrointestinal tract. Appl Environ Microbiol 66:2166–2174

Deveraux R, Stahl D (1992) Phylogeny of sulfate-reducing bacteria. In the sulfate reducing bacteria: contemporary perspectives. In Postgate JR, Odom JM, Singleton R. Contemporary bioscience, 289: 131–160

Devereux R, Hines ME, Stahl DA (1996) S cycling: characterization of natural communities of sulfate-reducing bacteria by 16S rRNA sequence comparisons. Microb Ecol 32:283–292

Dexter Dyer B (2003) A field guide to Bacteria. Comstock Publishing Associates/Cornell University Press

Domagal-Goldman SD, Meadows VS, Claire MW, Kasting JF (2011) Using biogenic sulfur gases as remotely detectable biosignatures on anoxic planets. Astrobiology 11:419–441

Drzyzga O, Küver J, Blotevogel K-H (1993) Complete oxidation of benzoate and 4-hydroxybenzoate by a new sulfate-reducing bacterium resembling Desulfoarculus. Arch Microbiol 159:109–113

Dupraz C, Visscher PT (2005) Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol 13:429–438

Durand P, Benyagoub A, Prieur D (1994) Numerical taxonomy of heterotrophic sulfur-oxidizing bacteria isolated from southwestern Pacific hydrothermal vents. Can J Microbiol 40:690–697

Ehrlich HL (1996) Geomicrobiology. Marcel Dekker, New York

Elliot P, Ragusa S, Catcheside D (1998) Growth of sulphate-reducing bacteria under acidic conditions in an up-flow anaerobic bioreactor as a treatment system for acid mine drainage. Water Res 32(12):3724–3730

Environmental Protection Agency (1997) EPA mercury study report to Congress. Publication EPA-452/R-97-009. Office of Air Quality and Standards and Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC

Fauque G, Legall J, Barton LL (1991) Sulfate-reducing and sulfur-reducing bacteria. In: Shivley JM, Barton LL (eds) Variations in autotrophic life. Academic Press, London, pp 271–337

Faust SD, Osman MA (1981) Chemistry of natural waters. Ann Arbor: Ann Arbor Science Publishers. Mercury, arsenic, lead, cadmium, selenium, and chromium in aquatic environments; pp. 200–225

Finster K, Tanimoto Y, Bak F (1992) Fermentation of methanethiol and dimethylsulfide by a newly isolated methanogenic bacterium. Arch Microbiol 157:425–430

Fitz RM, Cypionka H (1990) Formation of thiosulfate and trithionate during sulfite reduction by washed cells of *Desulfovibrio desulfuricans*. Arch Microbiol 154:400–406

Fossing H, Jørgensen BB (1989) Measurement of bacterial sulfate reduction in sediments: evaluation of a single-step chromium reduction method. Biogeochemistry 8:205–222

Foucher S, Battaglia-Brunet F, Ignatiadis I, Morin D (2001) Treatment by sulphate-reducing bacteria of Chessy acid-mine drainage and metals recovery. Chem Eng Sci 56:1639–1645

Fowler VJ, Widdel F, Pfennig N, Woese CR, Stackebrandt E (1986) Phylogenetic relationships of sulfate- and sulfur-reducing eubacteria. Syst Appl Microbiol 8:32–41

Gadd GM, White C (1993) Microbial treatment of metal pollution—a working technology? Trends Biotechnol 11(353):359

Garcia CSC, Moreno DA, Ballester A, Blázquez ML (2001) Bioremediation of an industrial acid mine water by metal-tolerant sulfate-reducing bacteria. Miner Eng 14(9):997–1008

Ghazy EA, Elmokadem MT, Gadallah M, Mahmoud MN, Abdel Ghany NA, Abo Elsoud MM (2013) Investigation of active corrosion sites of biofilm formed at mild steel electrolyte interface induced by mixed bacterial culture. Int J ChemTech Res 5(1):409–417

Gibson GR (1990) Physiology and ecology of the sulfate-reducing bacteria. J Appl Bacteriol 69:769

Gibson SAW, Gibson GR (1988) A rapid method for determination of viable sulphate-reducing bacteria in human faeces. Lett Appl Microbiol 7(2):33–35

Gilmour CC, Riedel GS, Ederington MC, Bell JT, Benoit JM, Gill GA, Stordal MC (1998) Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry 40:327–345

Glombitza F (2001) Treatment of acid lignite mine flooding water by means of microbial sulfate reduction. Waste Manag 21:197–203

Gomex GG, Sandler RS, Seal E Jr (1995) High levels of inorganic sulfate cause diarrhea in neonatal piglets. J Nutr 125:2325–2332

Greene RC (1996) Biosynthesis of methionine. In: Neidhardt FC (ed) Escherichia coli and Salmonella typhimurium: cellular and molecular biology. ASM Press, Washington, DC, pp 542–560

Greenwood NN, Earnshaw A (1984) Chemistry of the elements. Pergamon Press, Oxford

Groudeva V, Groudev SN, Doycheva AS (2001) Bioremediation of waters contaminated with crude oil and toxic heavy metals. Int J Miner Process 62(1):293–299

Gupta M, Suidan MT, Sayles GD (1996) Modeling kinetics of chloroform cometabolism in methanogenic and sulfate-reducing environments. Water Science & Technology 34 (5–6):403–410

Gyure RA, Konopka A, Brooks A, Doemel W (1990) Microbial sulfate reduction in acidic (pH 3) strip-mine lakes. FEMS Microbiol Lett 73(3):193–201

Hao OJ, Chen JM, Huang LJ, Buglass RL (1996) Sulfate-reducing bacteria. Critical Rev Environ Sci Technol 26:155

Heinz G (1974) Effects of low dietary levels of methylmercury on mallard reproduction. Bull Environ Contam Toxicol 13:554–559

Hosokawa J (1995) Remediation work for hg contaminated bay—experiences of Minamata Bay project. Japan Water Sci Technol 28:338–348

Hubert C, Nemati M, Jenneman G, Voordouw G (2003) Containment of biogenic sulfide production in continuous up-flow packed-bed bioreactors with nitrate or nitrite. Biotechnol Prog 19(2):338–345

Imhoff JF (1996) Variations of the sulfur cycle in marine environments. In: Fischer E, Grieshaber MK (eds) Processes and structures in marine methane and sulfide biotopes. Shaker, Aachen, pp 82–83

Itoh T, Suzuki K, Sanchez PC, Nakase T (1999) Caldivirga maquilingensis gen. Nov., sp. nov., a new genus of rod-shaped crenarchaeote isolated from a hot spring in the Philippines. Int J Syst Bacteriol 49:1157–1163

Jalali K, Baldwin SA (2000) The role of sulfate reducing bacteria in copper removal from aqueous sulphate solutions. Water Res 34(3):797–806

Jan-Roblero J, Romero JM, Amaya M, Le Borgne S (2004) Phylogenetic characterization of a corrosive consortium isolated from a sour gas pipeline. Appl Microbiol Biotechnol 64:862–867

Javaherdashti R (2008) Microbiologically influenced corrosion-an engineering insight. Springer, London. doi: https://doi.org/10.1007/978-1-84800-074-2

Javaherdashti R (2010) MIC and cracking of mild and stainless steels. VDM Verlag Dr Muller Aktiengesellschaft & Co KG

Johnson DB, Hallberg KB (2005) Acid mine drainage remediation options: a review. Sci Total Environ 338:3–14

Jørgensen BB (1978) A comparison of methods for the quantification of bacterial sulfate reduction in costal marine sediments. III Estimation from chemical and bacteriological field data. Geomicrobiol J 1:49–64

Jørgensen BB (1988) Ecology of the Sulphur cycle: oxidative pathways in sediments. Symp Soc Gen Microbiol 42:31–63

Jørgensen BB (1990) The sulfur cycle of freshwater sediments: role of thiosulfate. Lirnnol Oceanogr 35(6):1329–1342
Jørgensen BB, Bak F (1991) Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark). Appl Environ Microbiol 57:847–856
Kaksonen AH, Riekkola-Vanhanen M-L, Puhakka JA (2003) Optimization of metal sulphide precipitation in fluidised-bed treatment of acid wastewater. Water Res 37:255–266
Kaksonen AH, Plumb JJ, Robertson WJ, Riekkola-Vanhanen M, Franzmann PD, Puhakka JA (2006) The performance, kinetics and microbiology of sulfidogenic fluidized-bed treatment of acidic metal- and sulfate-containing wastewater. Hydrometallurgy 83(1–4):204–213
Kamolpornwijit W, Liang L, West OR, Moline GR, Sullivan AB (2003) Preferential flow path development and its influence on long-term PRB performance: column study. J Contam Hydrol 66:161–178
Kanazawa K, Konishi F, Mitsuoka T, Terada A, Itoh K, Narushima S, Kumemura M, Kimura H (1996) Factors influencing the development of sigmoid colon cancer. Bacteriologic and biochemical studies. Cancer 77:1701–1706
Karlin S, Brocchieri L, Mrázek J, Kaiser D (2006) Distinguishing features of delta-proteobacterial genomes. Proc Natl Acad Sci U S A 103:11352–11357
Keith SM, Herbert RA (1983) Dissimilatory nitrate reduction by a strain of *Desulfovibrio desulfuricans*. FEMS Microbiol Lett 18:55–59
Kerper L, Ballatori N, Clarkson TW (1992) Methylmercury transport across the blood–brain barrier by an amino acid carrier. Am J Phys 262(5 Pt 2):R761–R765
King JK, Saunders FM, Lee RF, Jahnke RA (1999) Coupling mercury methylation rates to sulfate reduction rates in marine sediments. Environ Toxicol Chem 18:1362–1369
King JK, Kostka JE, Frischer ME, Saunders FM (2000) Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. Appl Environ Microbiol 66(6):2430–2437
Kolmert A, Johnson DB (2001) Remediation of acidic waste waters using immobilised, acidophilic sulphate-reducing bacteria. J Chem Technol Biotechnol 76:836–843
LeDuc LG, Feroni GD, Trevors JT (1997) Resistance to heavy metals in different strains of Thiobacillus ferrooxidans. World J Microbiol Biotechnol 13:453–455
Lefèvre E, Pereyra LP, Hiibel SR, Perrault EM, De Long SK, Reardon KF, Pruden A (2013) Molecular assessment of the sensitivity of sulfate-reducing microbial communities remediating mine drainage to aerobic stress. Water Res 47(14):5316–5325
LeGall, J. and G. Fauque, 1988. Dissimilatory reduction of sulfur compounds. In: Zehnder AJB (ed) Biology of anaerobic microorganisms, Chapter 11. Wiley, New York
Lillebæk R (1995) Application of antisera raised against sulfate-reducing bacteria for indirect immunofluorescent detection of immunoreactive bacteria in sediment from the German Baltic Sea. Appl Environ Microbiol 61(9):3436–3442
Lloyd JR, Mabbett AN, Williams DR, Macaskie LE (2001) Metal reduction by sulphate-reducing bacteria: physiological diversity and metal specificity. Hydrometallurgy 59:327–337
Logan MV, Ahmann D, Figueroa L, Reardon KF, DuTeau N (2003) Microbial population dynamics in passive mine drainage treatment systems. Billings land reclamation symposium, June 3–6, Billings MT
Lomans BP, Op den Camp HJM, Pol A, Vogels GD (1999) Anaerobic versus aerobic degradation of dimethyl sulfide and methanethiol in anoxic freshwater sediments. Appl Environ Microbiol 65(2):438–443
Lovley DR, Roden EE, Phillips EJP, Woodward JC (1993) Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar Geol 113:41–53
Madigan MT, Martinko JM, Parker J (1997) Brock biology of microorganisms, 8th edn. Prentice Hall International, Inc., New York
Maruthamuthu S, Mohanan S, Rajasekar A, Muthuku-mar N, Ponmarippan P, Subramanian P, Palanis-wamy N (2005) Role of corrosion inhibitor on bacterial corrosion in petroleum product pipelines. Ind J Chem Tech 12(5):567–575

McCormick NG, Feeherry FE, Levinson HS (1976) Microbial transformation of 2,4,6-TNT and other nitroaromatic compounds. Appl Environ Microbiol 31:949–995

McCormick NG, Cornell JH, Kaplan AM (1981) Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. Appl Environ Microbiol 42:817–823

McGuire MJ, Jones RM, Means EG, Izaguirre G, Preston AE (1984) Controlling attached blue-green algae with copper sulphate. J Am Water Works Assoc 76:60

Mehta SK, Gaur JP (2007) Use of algae for removing heavy metal ions from wastewater: progress and prospects. Crit Rev Biotechnol 25:113–152

Moeslund L, Thamdrup B, Jørgensen BB (1994) Sulfur and iron cycling in a costal sediment: radiotracer studies and seasonal dynamics. Biogeochemistry 27:129–152

Mori K, Kim H, Kakegawa T, Hanada S (2003) A novel lineage of sulfate-reducing microorganisms: *Thermodesulfobiaceae fam. nov., Thermodesulfobium narugense, gen. nov., sp. nov.*, a new thermophilic isolate from a hot spring. Extremophiles 7:283–290

Morris ME, Levy G (1983) Absorption of sulfate from orally administered magnesium sulfate in man. J Toxicol Clin Toxicol 20:107–114

Muckian LM, Russell J, Grant RJ, Clipson NJW, Doyle EM (2009) Bacterial community dynamics during bioremediation of phenanthrene- and fluoranthene-amended soil. Int Biodeter and Biodegr 63:52–56

Muyzer G, Stams AJ (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6(6):441–454

Natarajan MR, Wu WM, Sanford R, Jain MK (1999) Degradation of biphenyl by methanogenic microbial consortium. Biotechnol Lett 21:741–745

National Research Council (1977) Drinking water and health. National Academy of Sciences, Washington, DC, pp 425–428

Nieuw Amerongen AV, Bolscher JG, Bloemena E, Veerman EC (1998) *Sulfomucins* in the human body. Biol Chem 379:1–18

Nolan EM, Lippard SJ (2008) Tools and tactics for the optical detection of mercuric ion. Chem Rev 108(9):3443–3480

Odom JM, Rivers Singleton JR (1993) The sulfate-reducing Bacteria: contemporary perspectives. Springer-Verlag, New York

Ohura T, Amagai T, Fusaya M, Matsushita H (2004) Polycyclic aromatic hydrocarbons in indoor and outdoor environments and factors affecting their concentrations. Environ Sci Technol 38: 77–83

Okabe S, Odagiri M, Ito T, Satoh H (2007) Succession of sulfur-oxidizing bacteria in the microbial community on corroding concrete in sewer systems. Appl Environ Microbiol 73(3):971–980

Olem H, Unz RF (1980) Rotating-disc biological treatment of acid mine drainage. J Water Pollut Control Fed 52:257–269

Ouattara AS, Jacq VA (1992) Characterization of sulfate-reducing bacteria isolated from Senegal rice-fields. FEMS Microbiol Lett 101(3):217–228. https://doi.org/10.1111/j.1574-6968.1992.tb05778.x

Palanichamy C, Sundar Babu N, Nadarajan C (2002) Municipal solid waste fueled power generation for India. IEEE Power Engineering Review 22(8):62–63

Pareek S, Kim SK, Matsui S, Shimizu Y (1998) Hydrolysis of (ligno) cellulosic materials under sulphidogenic and methanogenic conditions. Water Sci Technol 38(2):193–200

Parkes RJ, Dowling NJE, White DC, Herbert RA, Gibson GR (1993) Characterization of sulphate-reducing bacterial populations within marine and estuarine sediments with different rates of sulphate reduction. FEMS Microbiol Ecol 10:235–250

Peck HD Jr (1961) Enzymatic basis for assimilatory and dissimilatory sulfate reduction. J Bacteriol 82:933–939

Phoenix VR, Martinez RE, Konhauser KO, Ferris FG (2002) Characterization and implications of the cell surface reactivity of Calothrix sp. strain KC97. Appl Environ Microbiol 68:4827–4834

Postgate JR (1966) Recent advances in the study of sulphate reducing bacteria. Bact Rev 29:425

Reid RP, Visscher PT, Decho AW, Stolz JF, Beboutk BM, Dupraz C, Macintyre IG, Paerl HW, Pinckney JL, Prufert-Beboutk L, Steppe TF, DesMaraisk DJ (2000) The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature 406:989–992

Reimers CE, Jahnke RA, McCorkle DC (1992) Carbon fluxes and burial rates over the continental slope and rise off of Central California with implications for the global carbon cycle. Global Biogeochem Cycles 6:199–244

Rinzema A, Lettinga G (1988) The effect of sulphide on the anaerobic degradation of propionate. Environ Technol Lett 9:83–88

Robin C, Capron G, Desprez-Loustau ML (2001) Root infection by *Phytophthora cinnamomi* in seedlings of three oak species. Plant Pathol 50(6):708–716

Rockne KJ, Strand SE (1998) Biodegradation of bicyclic and polycyclic aromatic hydrocarbons in anaerobic enrichments. Environ Sci Technol 32:3962–3967

Roediger WEW, Moore J, Babidge W (1997) Colonic sulfide in pathogenesis and treatment of ulcerative colitis. Dig Dis Sci 42:1571–1579

Rzeczycka M, Blaszczyk M (2005) Growth and activity of sulphate-reducing bacteria in media containing phosphogypsum and different sources of carbon. Pol J Environ Stud 14(6):891–895

Sahrani FK, Ibrahim Z, Yahya A, Aziz M (2008) Isolation and identification of marine Sulphate-reducing Bacteria, Desulfovibrio sp. and Citrobacter freundii from Pasir Gudang, Malaysia. Sains Malaysiana 37(4):365–371

Salghi R, Luis G, Rubio C, Hormatallah A (2012) Pesticide residues in tomatoes from greenhouses in Souss Massa Valley, Morocco. Bull Environ Contam Toxicol 88(3):358–361

Santana A, Jesusa S, Larrayozb MA, Filho RM (2012) Supercritical carbon dioxide extraction of algal lipids for the biodiesel production. Process Eng 42:1755–1761

Sanyahumbi D (2003) Capsular immobilisation of sulphate-reducing bacteria and application. In Disarticulated Systems. Ph.D. thesis, Rhodes University, South Africa

Sass H, Steuber J, Kroder M, Kroneck PMH, Cypionka H (1992) Formation of thionates by freshwater and marine strains of sulfate-reducing bacteria. Arch Microbiol 158:418–421

de Schulze E, Mooney HA (1993) Biodiversity and ecosystem function. Springer, pp 88–90

Schwenn JD (1994) Photosynthetic sulphate reduction. Z Naturforsch C 49:531–539

Sheahan DG, Jervis HR (1976) Comparative histochemistry of GI mucosubstances. Am J Anat 146: 103–131

Shi X, Xie GJ (2011) Recent progress in the research on microbially influenced corrosion: Abird's eye view through the engineering lens. Recent Patent Corr Sci 1:118–131

Sicupira DC, Silva TT, Ladeira ACQ, Mansur MB (2015) Adsorption of manganese in acid mine drainage effluents using bone char: continuous fixed bed column and batch desorption studies. Braz J Chem Eng 32:577–584

Skousen JG, Sexstone A, Ziemkiewicz PF (2000) Acid mine drainage control and treatment. Agronomy 41:131–168

Slaughter M, Hill RJ (1991) The influence of organic matter in organogenic dolomitization. J Sediment Petrol 61:296–303

Smith NA, Kelly DP (1988) Mechanism of oxidation of dimethyl disulfide by *Thiobacillus thioparus* strain E6. J Gen Microbiol 134:3031–3039

Socrates G (2001) Infrared and Raman characteristic group frequencies: tables and charts. Wiley, New York, p 366

Sokolov I, Smith DS, Henderson GS, Gorby YA, Ferris FG (2001) Cell surface electrochemical heterogeneity of the Fe(III)-reducing bacteria *Shewanella putrefaciens*. Environ Sci Technol 36: 341–347

Srogi K (2007) Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ Chem Lett 5:169–195

Stille W, Trüper HG (1984) Adenylylsulfate reductase in some new sulfate-reducing bacteria. Arch Microbiol 137:145–150

Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147:3–9

Suylen GMH, Stefess GC, Kuenen JG (1986) Chemolithotrophic potential of a Hyphomicrobium species, capable of growth on methylated Sulphur compounds. Arch Microbiol 146:192–198

Taylor MRG, McLean RAN (1992) Overview of clean-up methods for contaminated sites. J Inst Water Environ Mgmt 6:408–417

Thauer RK, Stackebrandt E, Hamilton WA (2007) Energy metabolism and phylogenetic diversity of sulphate-reducing bacteria. Cambridge University Press, pp 1-38. doi:https://doi.org/10.1017/CBO9780511541490.002

Tsai J, Kumar M, Lin J (2009) Anaerobic biotransformation of fluorene and phenanthrene by sulfate-reducing bacteria and identification of biotransformation pathway. J Hazard Mater 164(2):847–855

Tuttle JH, Randles CI, Dugan PR (1968) Activity of micro-organisms in acid mine water. I Influence of acid mine water on aerobic heterotrophs of a normal stream. J Bacteriol 95: 1495–1503

Tuttle JH, Dugan PR, MacMillan CB, Randles CI (1969a) Microbial dissimilatory Sulphur cycle in acid mine water. J Bacteriol 97:594–602

Tuttle JH, Dugan PR, Randles CI (1969b) Microbial sulphate reduction and its potential utility as an acid mine water abatement procedure. J Appl Microbiol 17:297–302

Tuttle JH, Holmes PE, Jannasch HW (1974) Growth rate stimulation of marine pseudomonads by thiosulfate. Arch Microbiol 99:l–14

US EPA (1999a) Health effects from exposure to high levels of sulfate in drinking water study. US Environmental Protection Agency, Office of Water (EPA 815-R-99-001), Washington, DC

US EPA (1999b) Health effects from exposure to high levels of sulfate in drinking water workshop. US Environmental Protection Agency, Office of Water (EPA 815-R-99-002), Washington, DC

Varanasi U (1989) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC, Boca Raton, FL

Videla HA, Herrera LK (2005) Microbiologically influenced corrosion: looking to the future. Int Microbiol 8(3):169–180

Visscher PT, Stolz JF (2005) Microbial mats as bioreactors: populations, processes and products. Palaeo 219:87–100

Visscher PT, Quist P, van Gemerden H (1991) Methylated sulfur compounds in microbial mats: in situ concentrations and metabolism by a colorless sulfur bacterium. Appl Environ Microbiol 57: 1758–1763

Visscher PT, Prins RA, van Gemerden H (1992) Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat. FEMS Microbiol Ecol 86:283–394

Visser A (1995) The anaerobic treatment of sulphate containing wastewater. PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands. pp 1–4, 81–108

Visser TJK, Modise SJ, Krieg HM, Keizer K (2001) The removal of acid sulfate pollution by nanofiltration. Desal 140:79–86

Wang R (2012) Physiological implication of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev 92:791–896

Wang L, Lu X, Ren C, Li X, Chen C (2013) Contamination assessment and health risk of heavy metals in dust from Changqing industrial park of Baoji. NW China Environ Earth Sci 71:2095–2104

Wang S, Maltrud M, Elliott S, Cameron-Smith P, Jonko A (2018) Influence of dimethyl sulfide on the carbon cycle and biological production. Biogeochemistry 138:49–68

Warthmann R, Van Lith Y, Vasconcelos C, McKenzie JA, Karpoff AM (2000) Bacterially induced dolomite precipitation in anoxic culture experiments. Geology 28:1091–1094

Waybrant KR, Ptacek CJ, Blowes DW (2002) Treatment of mine drainage using permeable reactive barriers: column experiments. Environ Sci Technol 36:1349–1356

Wentzien S, Sand W, Albertsen A, Steudel R (1994) Thiosulfate and tetrathionate degradation as well as biofilm generation by *Thiobacillus intermedius* and *Paracoccus versutus* studied by micro-calorimetry, HPLC, and ion-pair chromatography. Arch Microbiol 161:116–125

White C, Gadd GM (1996) A comparison of carbon/energy and complex nitrogen sources for bacterial sulphate reduction: potential applications to bioprecipitation of toxic metals as sulphides. J Ind Microbiol 17(116):123

White PR, Franke M, Hindle P (1995) Integrated solid waste management: a lifecycle inventory. Springer, Berlin

Whittington-Jones K (2000) Sulphide-enhanced hydrolysis of primary sewage sludge: implications for the bioremediation of sulphate-enriched wastewaters. Ph.D. thesis, Rhodes University, South Africa

Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds) The prokaryotes, 2nd edn. Springer, New York, pp 3352–3378

Widdel F, Hansen TA (1992) The dissimilatory sulfate- and sulfur-reducing bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer K-H (eds) The prokaryotes, 2nd edn. Springer, New York, pp 583–624

Willis CL, Cummings JH, Neale G, Gibson GR (1996) In vitro effects of mucin fermentation on the growth of human colonic sulphate-reducing bacteria. Anaerobe 2:117–122

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221–271

Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the three primary kingdoms. Proc Nat Acad Sci U S A 74:5088–5090

Woese CR, Stackebrandt E, Marce TJ, Fox GE (1985) A phylogenetic definition of the major eubacterial taxa. Syst Appl Microbio 6:143–151

Wright DT, Oren A (2005) Non-photosynthetic bacteria and the formation of carbonates and evaporites through time. Geomicrobiol J 22:27–53

Wright DT, Wacey D (2005) Precipitation of dolomite using sulphate-reducing bacteria from the Coorong region, South Australia: significance and implications. Sedimentology 52:987–1008

Xu Y, Keene DR, Bujnicki JM, Höök M, Lukomski S (2002) Streptococcal Scl1 and Scl2 proteins form collagen-like triple helices. J Biol Chem 277:27312–27318

Yun-Juan C, Peacock AD, Long PE, Stephen JR, McKinley JP, Macnaughton SJ, Hussain AKMA, Saxton AM, White DC (2001) Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. Appl Environ Microbiol 67(7):3149–3160

Zehnder JBA (ed) (1988) Biology of anaerobic microorganisms. Wiley, New York

Application of Microbes in Biogas Production

24

Umme Ammara, Faiza Ilyas, Sughra Gulzar, Zeeshan Abid, Munazza Shahid, Raja Shahid Ashraf, and Muhammad Altaf

Abstract

Uncontrolled production of organic waste due to rapid urbanization and growing population has become a global concern. Biogas is an economical, renewable, and eco-friendly source of energy produced by using various groups of microorganisms that work in a synchronized way. Virtually any type of solid organic wastes is transformable into biogas through anaerobic digestion (AD). This chapter discusses the importance of biogas and use of microbes for biogas production. The production processes and parameters influencing the yield are also discussed briefly. In addition, the challenges are faced by enhancement techniques and summarized.

Keywords

Biogas · Microbial community · Solid organic waste · Anaerobic digestion · Parameters · Bioaugmentation

Abbreviations

AD	Anaerobic digestion
C/N	Carbon nitrogen
GHGs	Greenhouse gasses

U. Ammara · F. Ilyas · S. Gulzar · Z. Abid · R. S. Ashraf · M. Altaf (✉)
Government College University, Lahore, Punjab, Pakistan
e-mail: rajashahid@gcu.edu.pk; muhammad.altaf@gcu.edu.pk

M. Shahid
University of Education, Bank Road Campus, Lahore, Punjab, Pakistan

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HPH	Hydrodynamic pressure homogenization
HRT	Hydraulic retention time
MCFC	Molten carbonate fuel cell
MECs	Microbial electrolysis cells
MFCs	Microbial fuel cells
NREAP	National Renewable Energy Action Plan
OLR	Organic load rate
SOFC	Solid oxide fuel cell
SOWs	Solid organic wastes
VFAs	Volatile free fatty acids
WAS	Waste activated sludge

24.1 Introduction

Globally, the demands of energy have been growing gradually. For that reason, there is a need to enhance the growth of renewable and eco-friendly energy sources. The most important energy sources are fossil fuels that provide 80% of the total energy. Although the limited sources of fossil fuel also have some alarming impacts on the environment, it is necessary to reduce the use of fossil fuel because of global warming and other harmful pollutants. Around the world, the limited fossil fuel accessibility and the growing energy demands are the basic reasons that are compelling the governments to pursue the alternatives of renewable energy sources (Hijazi et al. 2016; Chuanchai 2018). Numerous methods including hydropower, solar heat, wind power, and anaerobic digestion (AD) can be used to produce renewable energy. However, bioenergy draws intention as renewable energy due to its viability and less production of CO_2. Generally, biogas consists of carbon dioxide (25–50%), methane (50–75%), water vapors, and some gases, i.e., N_2, H_2S, NH_3, and CO. The general equation of biogas production is as following: (Bo et al. 2014; Lee et al. 2017).

$$CH_3COOH \rightarrow CO_2 + CH_4 \tag{24.1}$$

Conventionally biogas is produced through anaerobic digestion AD process by the microbial decomposition of organic matter. The organic matter including (crop residues, industrial wastes, municipal wastes, and animal manures) decomposed by microorganisms in anaerobic conditions. The AD process has been catalyzed by a wide variety of microbes. These microbes convert the macromolecules into smaller molecules. The first step of the AD process is hydrolysis; various microbial communities can be used for efficient hydrolysis process. Most of the species belong to the class of *Bacilli* and *Clostridia*. Clostridium species are common for degradation under anaerobic conditions. An extensive range of microorganisms such as *Thermomonospora*, *Actinomyces*, *Ralstonia*, *Shewanella*, *Methanobacterium*, and *Methanosarcina* contribute to the degradation and methane production. Recently several species like *Clostridia*-36%, *Bacilli*-11%, both with the members of Mollicutes-3%, Bacteroidia-3%, Actinobacteria-3% and Gammaproteobacteria-3% are reported as the fermented bacteria in the digesters (Khalid et al. 2011; Wirth et al. 2012). Various Archaeal communities identified as methanogens, i.e., *Methanobacterium formicicum, Methanosarcina frisius,* and *Methanosarcina barkeri*. Methanogens are uncultivable microorganisms that increase the production of methane (Goswami et al. 2016), whereas others are the member of thermophilic species (e.g., *Crenarchaea* and *Thermoplasma sp*.). Archaeal 16s rRNA gene clones associated with ArcI taxon have been recovered in large amount from a methanogenic digester to decompose sewage sludge. ArcI is reported as an acetate consumer that plays an important part in acetoclastic methanogenesis. About 16% of rRNA archaeal gene clones have been investigated in a mesophilic methanogenic digester that belongs to *Crenarchaeota* (the subphylum C_2). It also has been observed that by increasing the hydrogenotrophic species the production of methane increases (Chouari et al. 2005; Trzcinski and Stuckey 2010).

The process of AD involves four major steps, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The organic matter is converted into renewable bioenergy by the action of microbes in the presence of enzymes. A large variety of bacterial groups taking part in the AD processes, such as hydrolytic, fermenting, acid-oxidizing, and methanogenic archaea bacteria are used to degrade organic waste (Carballa et al. 2015; Tuesorn et al. 2013). This process is environmentally friendly, requires less energy, economically attractive, and produces high quality of biogas. On the other hand, it also has some limitations, such as low biogas production, destabilizing, and weak degradation of substrates. Various factors can affect the AD process like (temperature, pH, volatile fatty acids, C\N ratio, alkalinity, and substrate characteristics) (Cerrillo et al. 2016). To overcome these problems many physical and chemical methods have been established. Many techniques are used to increase hydrolysis efficiency that is a rate-determining step in the AD process. Currently, several new technologies, e.g., (MECs) and (MFCs) have been introduced to increase the efficiency of anaerobic digesters. These technologies use electric current from microorganisms to improve biogas production. The pretreatment of substrates along with micronutrients also improves gas yield. An improvement of discharge quality is also needed to avoid the adulteration of groundwater by nutrients and pathogens (Lee et al. 2017; Weiland 2010).

24.2 Historical Overview

Recently, one of the major environmental problems is the continuous production of organic material. This organic waste is managed and treated by AD which is a microbial anaerobic (absence of O_2) decomposition process to produce biogas in digesters (airproof reactor tanks). Biogas is a sustainable supply of renewable energy from organic waste. AD has attained global attention to lower the combustion of fossil fuel and to reduce the emission of greenhouse gases (Awe et al. 2017; Hosseini and Wahid 2014).

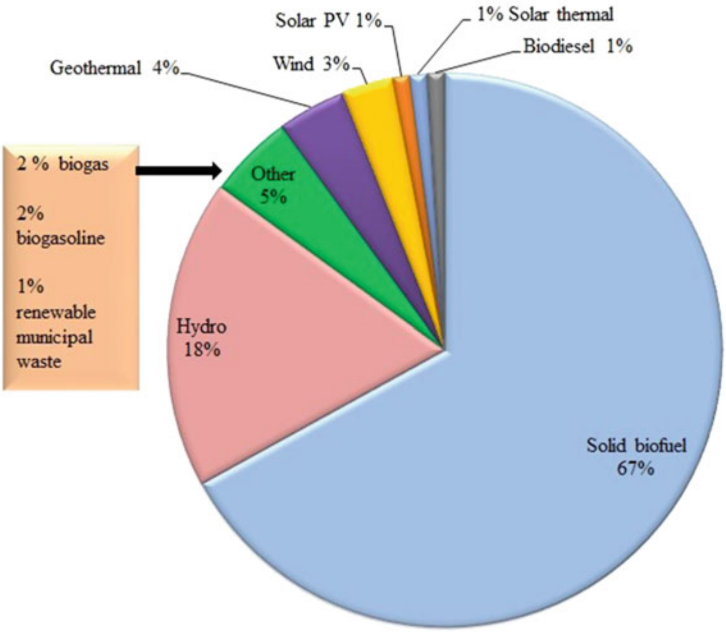

Fig. 24.1 Global energy source in 2013 (Atelge et al. 2018)

In France, Ad was first documented in 1891. In 1895, biogas was produced in the United Kingdom from municipal waste and it was used to harvest heat and light (Gashaw 2014). A comprehensive report in the USA about anaerobic digestion was published in 1936, by Hatfield and Buswell (Wett and Insam 2010). In the middle of the twentieth century, sustainable applications of biogas plants appeared. Currently, AD is a significant treatment of waste (industrial waste, aquatic biomass, sewage solid waste, and energy crops) and produces methane (García-González et al. 2019; Raucci et al. 2019). For years, the production of biogas has been applied in households and farms on a small scale. Since the 1930s, the production of biogas after viable stabilization requirements of sewage sludge became a standard process to treat sludge at large to medium scale treatment plans. In Europe particularly, over the last few years, biogas plant has developed an industrial scale largely by increasing the efficiency of biogas conversion. At the start of the twenty-first century, we came to know that biogas has the potential to eliminate many issues instantly. Taking methane in biogas can provide waste disposal management, reduction of GHGs emissions, and renewable energy production (Chiumenti et al. 2018; Hou and Hou 2019). Biogas is a common renewable energy source in developed countries. On the other hand in developing countries, this trend has not altered. Globally, the production of biogas was reported only 2% as displayed in Fig. 24.1, whereas in the EU, it was extended to 7% in 2013 as shown in Fig. 24.2 (Agency 2016).

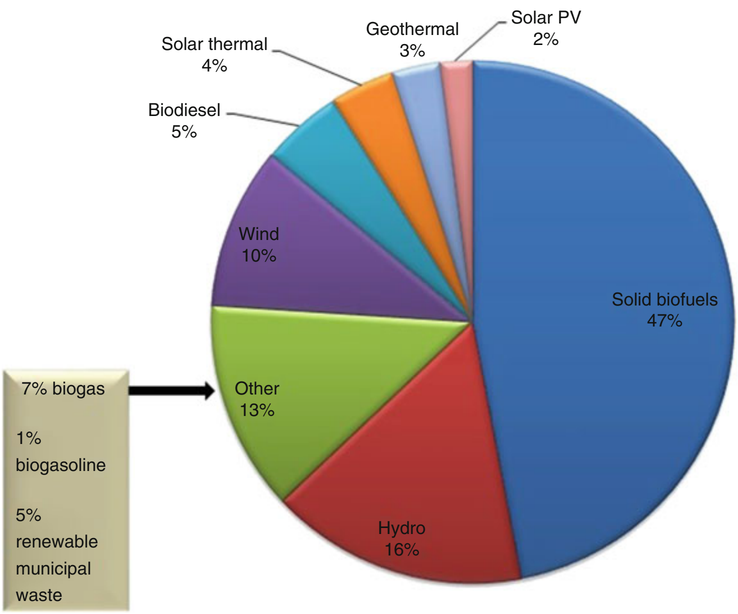

Fig. 24.2 EU-28 level energy source in 2013 (Atelge et al. 2018)

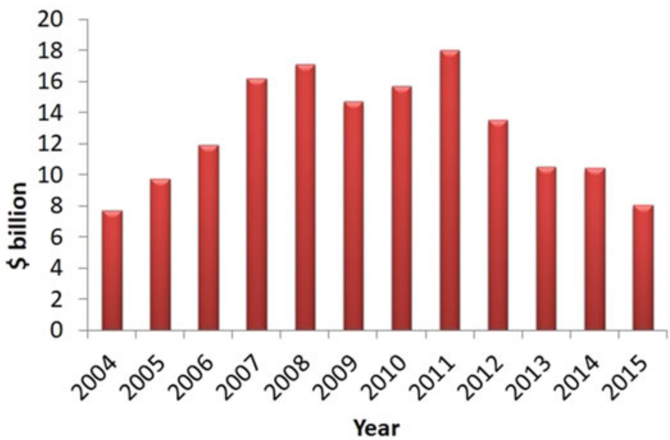

Fig. 24.3 Graphical representation of global investment in biogas production (Atelge et al. 2018)

The continuous increase in the growth of the biogas sector has been supported by the above facts since 1990. The sustainable energy investment trend during the era of 2004–2015 in the world is shown in the following Fig. 24.3.

Figure 24.3 illustrates that there is a continual increase during 2004–2008 where the trend remains relatively constant (Gonzalez-Salazar et al. 2016). The fewer investments made to be constant after 2011. While the rate of growth from

2004–2015 was 2%, the investment in waste and biomass to energy sector was 6 billion dollars in 2015. In developed countries like Denmark, Germany, and England, the energy sector has developed well, therefore investment in waste and biomass to energy lessened in the era of 2011 and 2015 (Solarte-Toro et al. 2018; Edenhofer et al. 2011). Conversely, in developing countries, the investment continues to increase progressively owing to their economic conditions (Offermann et al. 2011). In the EU, to meet the sustainable energy requirements of the National Renewable Energy Action Plan (NREAP), the sustainable energy sector has to develop 4% every year till 2020, to meet the anticipations. In Paris Agreement 2015, the target of the EU for 2050 was the reduction of greenhouse gasses emission to 85–90% from the volume produced in 1990 (Bausch et al. 2017). In the era of 2013–2020, electricity generation from biogas must be enhanced from 46.8 to 63.3 terawatt-hours in the EU to gain their NREAP target. Italy and Germany have achieved their goals because of their numbers of functional biogas plants, whereas other countries require economic investments and policies for the operation and development of more biogas plants (Repele et al. 2017).

Animal waste has been alleviated by AD unless the middle of the 1970s in North America (Abbasi et al. 2012). The biogas plant number with well-developed AD has increased in the USA. Recently, the number of AD plants in operation are around 2100, it is still lesser compare to their model potential (Wang et al. 2019). Japan is also using this technique to manage and treat its waste. At this time, thermophilic AD is used only in Japan in the world. 200 mL biogas was formed in 2006. Many cities in Japan like Kobe, Nagaoka, and Kanazawa are producing biogas from sewage sludge with various capacities, e.g., 800,000, 600,000, and 280,000 m^3/year, respectively (Yolin 2015; Gubaidullina and Kargina 2015). In developing countries, AD has been becoming more suitable and standard technique due to high energy costs compared to developed nations. At present, India and China have a large number of operated biogas plants with 4.7 million and 42.6 million correspondingly as shown in Fig. 24.4 (Tongia and Gross 2018).

Other countries in Asia like Bangladesh, Kenya, Nepal, Cambodia, and Vietnam have installed progressively more domestic biogas plants (Geng et al. 2016). In 2016, the numbers of small scale biogas plants installed in these nations are in the range of 360–15,000. In this year Asia has invested more for AD technologies compared to any other region. African Biogas Partnership Program operated almost 68,000 biogas plants in 2016, in Africa. In developing countries, more than 700,000 plants have been installed in 2015 (Appavou et al. 2017).

24.3 Importance of Biogas

Fossil fuels are the renewable source of energy but their formation process is very slow and current consumption is rapidly draining the reserves. Biogas is formed during the process of anaerobic digestion and is a reliable and flammable gas with short formation time (Hosseini and Wahid 2014). Biogas has versatile applications

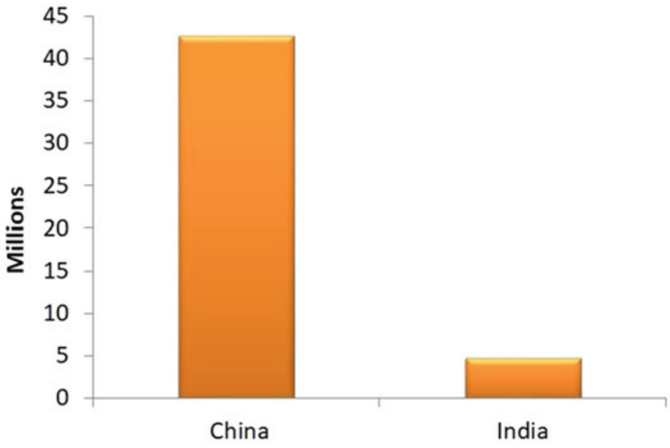

Fig. 24.4 Domestic number of biogas plants established in 2016 by India and China (Atelge et al. 2018)

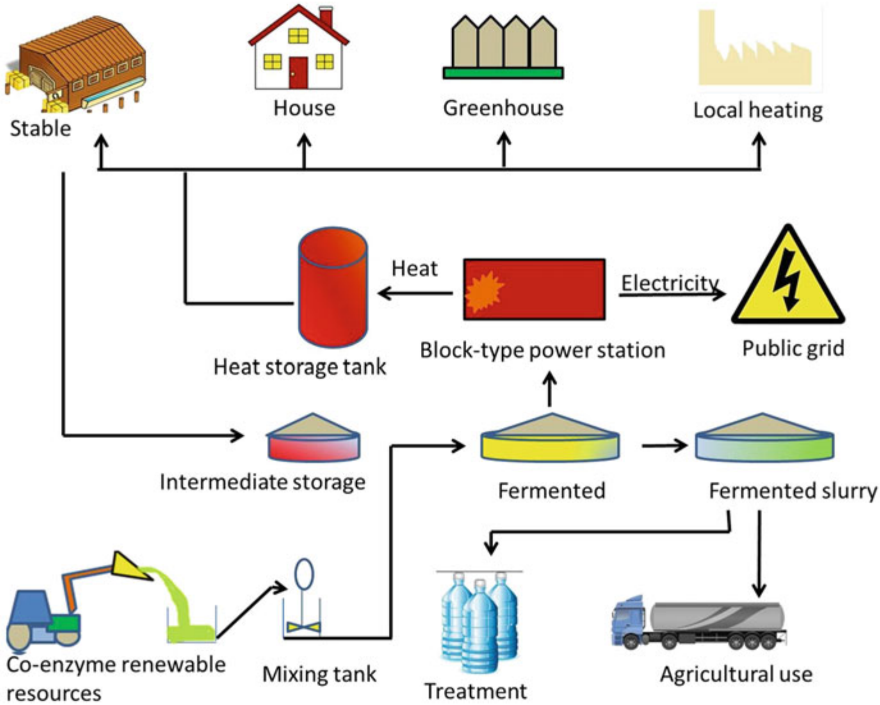

Fig. 24.5 Utilizations of biogas (Ferreira et al. 2019)

as shown in Fig. 24.5, e.g., due to controlled combustion its chemical energy can be transformed into mechanical energy.

Thermal energy can also be generated from biogas when it burns to yield heat energy in boilers. In stationary engines as well as in automotive it is used as fuel. It is a promising source of H_2 that is loaded into fuel cells (Alves et al. 2013).

Use of Biogas for Sludge Treatment The sludge sanitization process can be performed with the help of a boiler worked on biogas and a heated concrete tank. Once a day, the tank might be aided to avoid the necessity of a large gas holder. A heat exchanger is fitted in the tank to heat the sludge for 30 min at 70 °C. Therefore, excess thermal heat (up to 70%) can be used for cooking and water heating (Passos et al. 2020).

Use of Biogas in Fuel Cells The techniques to change H_2 into electrical energy and desirable power levels are near to commercialization. In fuel cells, the direct biogas use is termed as internal reforming (Ohkubo et al. 2010; Membrez and Bucheli 2004). (SOFC) and (MCFC) are high-temperature fuel cells. They have a greater ability of internal reforming (use of biogas directly) due to better capacity of thermal integration and great tolerance of H_2 contaminants. In the literature, various studies indicated that in fuel cells, biogas reforming is used frequently. However, some studies revealed that biogas can be converted into electricity without a humidifier, ancillary fuel, external reformer, and metal catalyst (Shiratori et al. 2008). During internal reforming, CO is also produced which is a poison for fuel cells (Xuan et al. 2009).

Use of Biogas as Biofuels Biogas is a high octane fuel. The components of biogas can be categorized in the following ways:

- Combustible.
- Non-combustible.

The combustible components include CO_2, H_2, and CH_4 while CO and N_2 are non-combustible components. Various factors such as the source of substrates and preparation techniques may change the composition of the biogas. Biofuel is a biomass-based fuel. It has various advantages compared to fossil fuel. Primarily, biofuel is readily available from biomass. Furthermore, biofuel circulates the carbon between the fuel and air, as a result, many problems, i.e., energy scarcity and greenhouse gas emission can be resolved. Thirdly, various kinds of biofuel like ethanol and biodiesel have physicochemical characteristics for combustion in the internal combustion engine (Raheem et al. 2015; Brown and Brown 2013). Similarly, bioethanol (a renewable substitute) has been used for gasoline in the system isolated engine. As compared to natural gas and LPG, biogas has a lower heating value and lower flame speed. Secondly, the autoignition temperature is also greater than that of natural gas and LPG. Their chemical and physical properties have a greater effect on the use of biogas in the spark-ignition engine (Qian et al. 2017) (Table 24.1).

Table 24.1 Shows the contents of organic matter and their theoretical yield (Braun 2007)

Substrates	Biogas(Nm^3/tTS)	CO_2 (%)	CH_4 (%)
Carbohydrates	790–800	50	50
Raw fat	1200–1250	32–33	67–68
Raw protein	700	29–30	70–71
Lignin	0	0	0

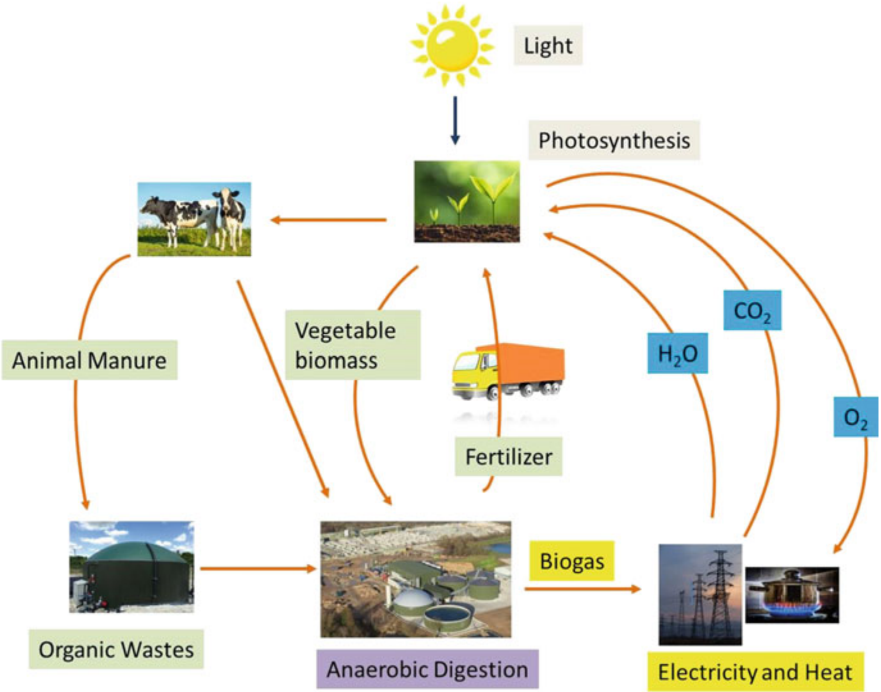

Fig. 24.6 A conventional biogas production cycle (Al Seadi 2001)

24.4 Commonly Used Substrates for Biogas Production

For renewable energy (biogas) biomass is the most commonly used substrate in AD. Some substrates are shown in Fig. 24.6. The biomass consists of proteins, carbohydrates, hemicelluloses, celluloses, and fats. However, some co-substrates are also used to obtain the highest gas yield. These co-substrates are agricultural wastes, food wastes, harvesting residue, i.e., leaves, and top of sugar beet and household municipal wastes. The composition and total yield of biogas depend on the type of feedstock and substrate used in the anaerobic plant to determine the composition and yield of biogas (Braun 2007; Achinas et al. 2017). Contents of organic matter and their theoretical yield are listed in the table.

Methane production from different feedstocks is very difficult to compare. Experimental conditions, i.e., temperature, volatile solids, total solids are analyzed for the maximum performance of particular raw material. Thus it is useful to relate different feedstocks by their methane yield (B_0) (Owen et al. 1979).

Manures are used as the substrate in AD which is an abundant source of organic matter. The use of manures as feedstock also reduces the emission of greenhouse gases. Some biochemical methane potential assays showed that the potential yield of methane differs among livestock types. Various factors take part in the potential of methane, e.g., animal growth stage, type of bedding, species, breed, feed, amount, and any decomposition process (Møller et al. 2004). Farm manures have a high concentration of NH_3 that may be an inhibitory factor in the AD process. The feedstocks having low nitrogen concentration involve high ammonia concentration for effective degradation. Beyond this manures usually consist of recalcitrant fiber that is hard to degrade. The pretreatment of manures gives up to 20% increase in methane production by reducing the particle size (Sung and Liu 2003; Angelidaki and Ahring 2000).

Biomass contains straws from rice, wheat, sorghum, and other waste products of food. It is the most favorable feedstock for the AD process. Their methane yield (B_0) is high. However, the high amount of recalcitrant material usually needs pretreatment to completely comprehend the potential yield. The biogas yield is also affected by the harvesting time (Petersson et al. 2007).

It is the most capricious feedstock because the production of methane is influenced by the location (source of material), sorting method, and time of collection. Cultural values, beliefs, the lifestyle of communities impact their recycling practices and waste disposal approaches (Cho et al. 1995). When the municipal solid waste is not differentiated by source then the process of pretreatment is mandatory to remove metals, glass, plastics. The pretreatment process can be done manually or mechanically, i.e., pressing, screening, and pulping. Sewage sludge is another form of industrial or municipal waste. It has a high methane yield due to the presence of high organic matter for AD (Ward et al. 2008).

Food waste has a high content of volatile solids, low total solids, and its degradation is easy in an anaerobic digester. These substrates during hydrolysis may accumulate acid in the digester and inhibit methanogenesis consequently. In the early 1980s, it was revealed that the various carbohydrate comprising wastes required alkali buffer as well as co-digestion for stable performance (Hills and Roberts 1982; Knol et al. 1978) (Table 24.2).

24.5 Application of Microbes in Biogas Production

24.5.1 Decomposition

It is an incessant and intricate microbial decomposition of complex organic biomass into its mineral forms. Decomposition is categorized by various physical and biological processes like biological fragmentation, respiration, and leaching

Table 24.2 Biogas production feedstocks (Krishania et al. 2012)

Feedstocks	Total waste (Kg/day/head)	Gas yield(m^3/kg)	Requirement of pretreatment
Poultry	0.75	0.46	No
Pig	1.3	0.39	Yes
Sheep	0.75	0.37	No
Cattle	10–15	0.34	No
Kitchen	0.25	0.30	Yes
Night soil	0.75	0.38	No
Wheat straw	3.5	0.41	Yes
Rice straw	1.2	0.61	Yes
Marine algae	3.3	0.40	No
Water hyacinth	5	0.40	Yes

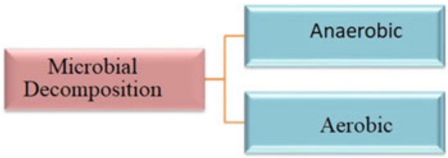

Fig. 24.7 Types of microbial decomposition

(Hahn-Hägerdal et al. 2007; Busing et al. 2008). These processes work synergistically as they are very closely related to each other. Many factors affect decomposition processes such as the concentration of O_2/CO_2, temperature, humidity quality of substrate containing components, species, position, and size. Generally, decomposition has two types: abiotic and biotic. Biotic decomposition is the microbial (fungi, bacteria, and protozoa) disintegration of the complex substrate into simpler units. On the other hand, abiotic decomposition uses physical and chemical methods to breakdown complex organic substrate (Rahman et al. 2013). The microbial decomposition occurs in either anaerobic or aerobic environment as shown in Fig. 24.7.

Anaerobic Decomposition

It is an anaerobic symbiotic microbial conversion of organic waste to biogas, salts, nutrients, refractory organic matter and additional cell matter, etc. It is an environmentally friendly technique.

The main components of the raw biogas are 60% CH_3, 40%CO_2 trace amount of H_2S and water vapors. It is a colorless and odorless gas. When it burns a blue color flame is made which is similar to the flame of LPG gas. Archaea and bacteria are two basic kinds of microbes used for the conversion of biogas strictly in an anaerobic environment (Adekunle and Okolie 2015; Kusch et al. 2012). AD reduces pathogens, organic wastewater solids, and the odor by producing biogas from fractions of volatile solids. The product of this process has not only stabilized solids but also has some nutrients like ammonia-nitrogen. AD is applied in waste management including industrial wastewater, agriculture waste, sludge digester, municipal wastewater, septic tank, and waste treatment (Zhou and Wen 2019). It is used in both domestic and industrial fermentation to produce food and drink products. Different factors influencing biogas conversion may include the nature of substrate, volatile free fatty acids, carbon-nitrogen ratio, temperature, hydraulic retention time, digester design, pH and loading rate (Kusch 2008). It can be either a batch process or a continuous process. The organic waste is added continuously in continuous AD to the reactor. On the other hand, organic biomass is added in the batch process at the start of the process to the reactor.

Aerobic Decomposition

It is a decomposition of organic biomass in the presence of oxygen by microorganisms into $SO^{-}{}_4$, CO_2, $NO^{-}{}_{3,}$ H_2O, etc. It is the most common process that occurred in the forest to produce stable organic compounds from dropping animals and trees. It can also take place in bins, piles, pits, stacks, etc. and insufficient O_2 environment. Some compounds cannot decompose well in an aerobic environment that is the major disadvantage of this process. These unreactive compounds contain insoluble materials which require chemical oxygen demand up to 70%. To overcome these problems versatile AD technique is used to treat organic waste.

24.5.2 The Biochemical Process of Biogas Production

Biogas production through the AD process has significant advantages. It is a versatile source of energy that reduces the emission of greenhouse gases. Various types of organic substrates (agricultural remains, animal wastes, municipal solid wastes, and market wastes) are converted into biogas and digestate (Hijazi et al. 2016; Weiland 2010). The process of anaerobic digestion has been carried out by various independent progressive and biological reactions in anaerobic conditions (Parawira 2012). It is an enzyme-driven process during which organic matter is converted into CH_4 and CO_2. AD process consists of four main steps which are as follows and described in Fig. 24.8: (Weiland 2010).

- Hydrolysis.
- Acidogenesis.
- Acetogenesis/Dehydrogenation.
- Methanogenesis.

Hydrolysis

Hydrolysis is a process that transforms the complex organic macromolecules (lipids, polysaccharides, proteins) into smaller ones with the help of microbes secreted from different enzymes (Cirne et al. 2007). The different degradation steps involve diverse groups of microscopic organisms, which work in a closely related way. Hydrolyzing microorganisms are initially attacking polymers and converting them into long-chain fatty acids, monosaccharides, and amino acids. However, many

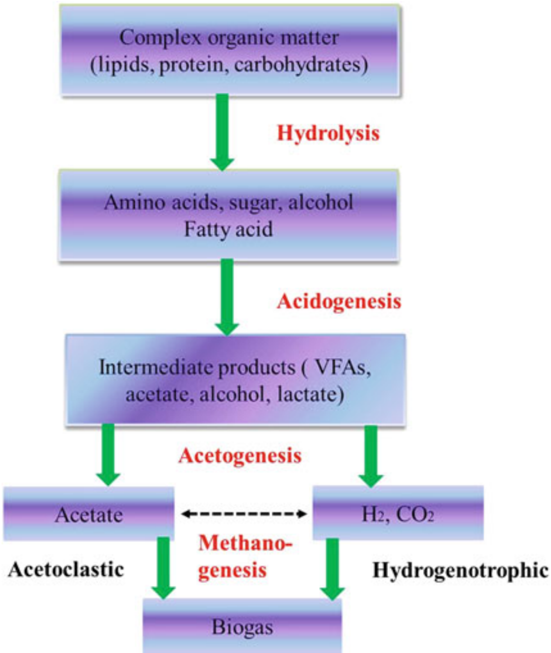

Fig. 24.8 Schematic diagram of the biochemical process of biogas production

hydrolytic enzymes that are secreted by microorganisms, e.g., cellobiose, cellulose, amylase, protease, xylanase, and lipase are taking part in hydrolysis (Weiland 2010; Bagi et al. 2007). Various bacterial groups are also included in the hydrolysis of polysaccharides, most of them are strictly anaerobic, i.e., *Clostridium*, *Bacteroides,* and *Acetivibrio*. The resulted products of hydrolysis are further decomposed by other microorganisms (Heeg et al. 2014).

$$\text{Lipids} \overset{\text{lipase}}{\longrightarrow} \text{Fatty acid} + \text{Glycerol} \tag{24.2}$$

$$\text{Polysaccharides} \overset{\text{cellobiase cellulose, amylase, xylanase}}{\longrightarrow} \text{Monosaccharide} \tag{24.3}$$

$$\text{Protein} \overset{\text{protease}}{\longrightarrow} \text{Amino acids} \tag{24.4}$$

Acidogenesis

In acidogenesis, the final products of hydrolysis, i.e., fatty acids, sugars, and amino acids are further decomposed by fermenting organisms. Some facultative and various hydrolyzing microorganisms (i.e., *Paenibacillus*, *Ruminococcus*, *Streptococci*) are taking part in fermentation (Ziganshin et al. 2013; Zheng et al. 2014). However, microorganisms, e.g., *Acetobacterium*, *Enterobacterium,* and *Eubacterium* along with the hydrolyzing microbes are also included to carry out the fermentation.

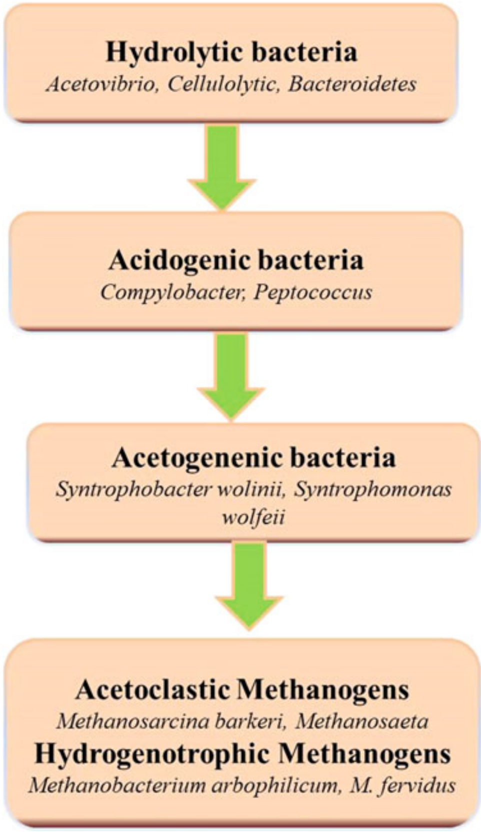

Fig. 24.9 Shows an overall view of bacteria involved in the AD (Goswami et al. 2016)

These fermented bacteria (acidogens) convert the hydrolyzing products into numerous organic acids (i.e., butyric acid, acetic acid, propionic acid, succinic acid, lactic acids), alcohols, NH_3, CO_2, and H_2. The resulting compound depends on the type of microorganism's present, the kind of substrate used, and on environmental conditions (Schnurer and Jarvis 2010).

Acetogenesis

In acetogenesis, the fermented products are further oxidized into methanogenic substrates. The obligate acetogenic hydrogen-producing bacteria convert the high VFAs, amino acids, and alcohol into acetate and hydrogen. *Syntrophus, Clostridium*, *Syntrophomonas*, and *Syntrobacter* are the microorganisms that carried out acetogenesis as shown in Fig. 24.9 (Bagi et al. 2007; McInerney et al. 2008).

Methanogenesis

In the biochemical process, the very last step of AD is methanogenesis in which fermentation of various organic compounds synthesized methane gas. The process of

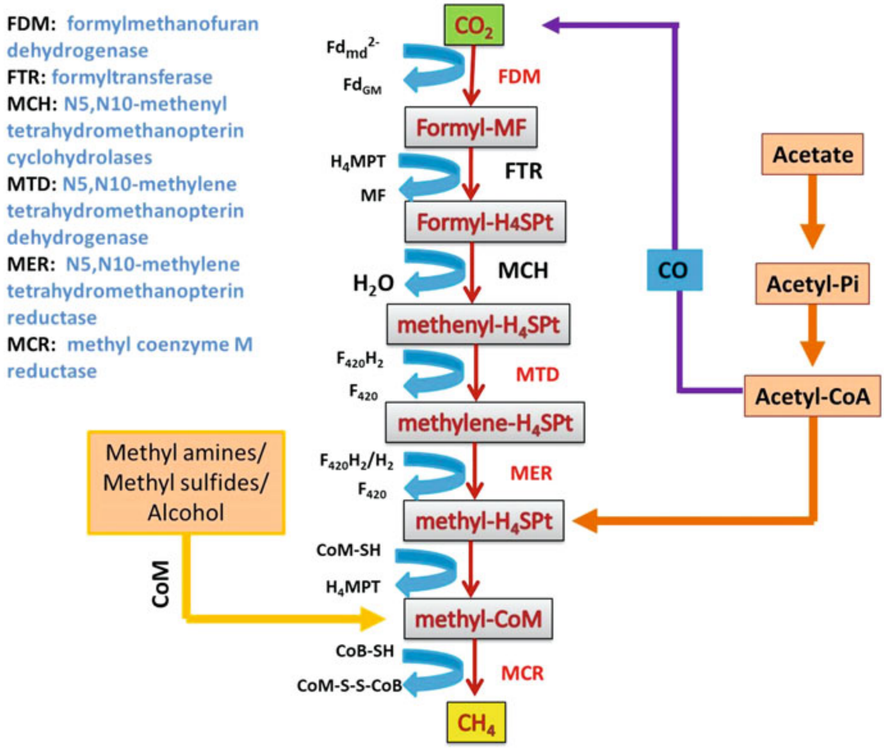

Fig. 24.10 shows the complex process of methanogenesis including three pathways (Goswami et al. 2016)

methanogenesis has been compelled by six different pathways, in which three are the major pathways, i.e., methylotrophic pathway, hydrogenotrophic pathway, and acetoclastic pathway. Every pathway is differentiated by other pathways by the source of energy and nature of the substrate used for methane. These substrates are formic acid, carbon dioxide, methylamine, dimethyl sulfate, and methanol. The common pathway of methanogenesis is the reduction of CO_2 into CH_4. However, according to methanogenic cofactors other five pathways may be assembled into two (Slonczewski and Foster 2013; Garcia et al. 2000). The three basic pathways are described in Fig. 24.10.

Methylotrophic pathway: In this pathway, methane is produced by the decarboxylation of methylamine/methyl sulfides/alcohols.

Hydrogenotrophic pathway: In this pathway, methane is produced by the reduction of CO_2.

Acetoclastic pathway: In this pathway, methane is produced by the decarboxylation of acetate. This pathway has been reported as the major pathway to produce methane in anaerobic conditions. It has been stated that during the AD process of domestic sewage about 70% of the total CH_4 is produced through this process. The

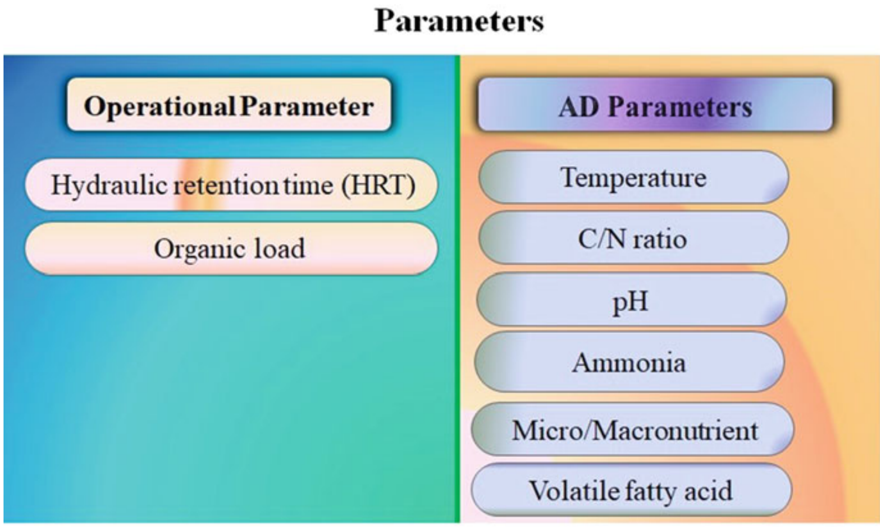

Fig. 24.11 Important parameters in AD

process of methanogenesis is very complex, it requires various substrates and cofactors to take place (Goswami et al. 2016; Lettinga 1995).

24.5.3 Parameters Influencing Microbial Growth and Biogas Yield

There are two main parameters such as operational and AD parameters to enhance biogas yield. These parameters are described in Fig. 24.11.

Anaerobic Digestion Parameters

1. Temperature.

Temperature is a fundamental factor significantly influencing different functions such as hydrolysis rate, biogas conversion, sludge quality, enzyme and its related coenzyme activities in the AD process. In that process, various anaerobic microorganisms work well at different temperature ranges (Yan et al. 2015). Three thermal stages are described in Fig. 24.12.

Enzymes may not show their optimal catalytic activity at very low temperatures, whereas sensitive enzymes may become denature at very high temperatures, as a result, lead to process failure. From literature, we come to know that ammonia accumulation, endergonic metabolic reactions, and biogas yield accelerated at the thermophilic thermal range compared to the mesophilic thermal range (Sikora et al. 2019; Keating 2015). It was also noted that thermophilic thermal conditions could not be promising for exergonic metabolic reactions and specific substrates like co-digestion of sugar beet pulp with sewage waste (Montañés et al. 2015). The

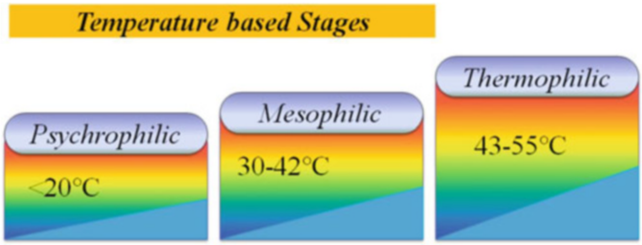

Fig. 24.12 Important thermal stages in AD

thermophilic condition also causes adverse environmental effects. During the digestion, process temperature must be kept constant because temperature fluctuations affect biogas yield negatively. Thermophilic bacteria are more sensitive than mesophilic bacteria.

2. C/N ratio.

This ratio plays a significant role in anaerobic digestion (Mathew et al. 2015). For the growth of anaerobic microorganism in a stable environment, optimum ratio of C/N is required. Commonly, a range of 20–30 C/N ratio is suitable for the AD process (Meegoda et al. 2018). Wang et al. executed anaerobic co-digestion of three substrates (wheat straw, dairy and chicken manure) at a low concentration of free ammonia and ammonium ion and stable pH; as a result, he found that the maximum yield of methane produces at 27.2 C/N ratios (Wang et al. 2012). Zeshan et al. were also found that digestion accomplished well at a C/N ratio of 27 than 32 (Karthikeyan and Visvanathan 2012). Whereas according to modern study, AD performed well at 15–20 C/N ratios. For co-digestion, Zhong et al. found that the most favorable C/N ratio was 20 (Zhong et al. 2013). Anaerobic co-digestion for cattle manure and food waste was done by Zhang et al. at C/N ratios 15.8 (Zhang et al. 2013a). The optimum C/N ratios depend on inoculum and feedstock for the anaerobic digestion process. For a long-term AD operation, suitable C/N ratios are enforced.

3. pH

In the AD process, pH is an indispensable parameter to regulate and stabilize the activities of methanogenic and acidogenic bacteria because their activities are greatly affected by pH changes. Usually, an optimum pH between 6 and 8 is reported for higher biogas yield (Deepanraj et al. 2014; Zhao et al. 2008). Acidogenesis and hydrolysis take place at pH 6.5 and 5.5, respectively. The amount of volatile fatty acids (VFAs) and CO_2 formed during the digestion process affects significantly the pH of matter present in a digester. Typically to ensure fermentation in AD, then the concentration of CH_3COOH and volatile free fatty acid should be <2000 mg/I. In 1998, Mattiasson and Jain reported that the efficiency of methane yield was enhanced >75% at above pH 5. In the co-digestion of two substrates such as dairy manure and cheese whey, when pH was uncontrolled, a two-phase anaerobic digester worked as a single-phase reactor, whereas in the methanogenic phase, when whey pH was controlled, then the digester functioned as a two-phase two-stage reactor (Bertin et al. 2013; Venetsaneas et al. 2009). From previous reports, we come to know that the pH of anaerobic reactor affects VFAs in a great manner, at low pH butyric acid and acetic acids are dominant VFAs however at pH 8 main VFAs are propionic acid and acetic acid (Horiuchi et al. 1999). Similarly, with the help of optimum pH, we should control acidogenic bacteria and their number (Horiuchi et al. 2002).

4. Ammonia.

Ammonia and ammonium ions are obtained by degrading nitrogen-rich organic waste and protein (Yenigün and Demirel 2013; Whelan et al. 2010). Ammonia is a crucial nutrient for bacterial growth but in higher concentrations, it can be very toxic to bacteria (Walker et al. 2011). A recent study revealed that during anaerobic digestion ammonia could increase buffer capacity by neutralizing VFAs (Scherer et al. 2009). Zhang et al. have reported reaction equations between VFAs and ammonia as follows:

$$C_xH_yCOOH \rightleftharpoons C_xH_yCOO^- + H^+ \tag{24.5}$$

$$NH_3.H_2O \rightleftharpoons NH_4^+ + OH^- \tag{24.6}$$

$$C_xH_yCOOH + NH_3 \times H_2O \rightarrow C_xH_yCOO^- + NH_4^+ + H_2O \tag{24.7}$$

In the above equations, C_xH_yCOOH symbolizes VFAs. With the increase of organic load rate (OLR), the amount of VFAs increases which inhibits the AD process therefore to avoid this inhibition NH_3 could react with VFAs and allow enough fatty acids for biogas production. Ammonia is directly proportional to both pH and temperature. It means that free ammonia concentration rises with increasing temperature and pH values such as at 35 °C and pH 7 the amount of free ammonia formed is <1%. Conversely, free ammonia at pH 8 and the same temperature increase to 10% (Fernandes et al. 2012). Bacteria grow at low ammonia concentration, whereas its higher concentration can inhibit bacterial growth. To regulate AD functions various techniques are used to remove excess ammonia such as microwave (Lin et al. 2009a, b), ion exchange (Wirthensohn et al. 2009), electrochemical conversion (Lei and Maekawa 2007), ammonia stripping (Böhm et al. 2011), membrane contractor (Lauterböck et al. 2012) and biological nitrogen elimination processes (Hsia et al. 2008), etc. We can calculate concentration free ammonia from the following formula.

$$[NH_3] = \frac{[T - NH_3]}{\left(1 + \frac{H^+}{K_a}\right)} \tag{24.8}$$

Here, Ka is the dissociation parameter, while $[NH_3]$ and $[T\text{-}NH_3]$ represent free ammonia and total ammonia, respectively.

5. Volatile Fatty Acid.

Valeric acid, acetic acid, butyric acid, and propionic acid are the basic VFAs intermediates that identify the stability of the AD process (Buyukkamaci and Filibeli 2004; Pham et al. 2012). Among these acids, propionic acid and acetic acids are essential for biogas production (Zhang et al. 2013b). During acidogenesis these intermediates are formed with a chain of carbon up to 6 atoms. Mainly, methanogens and acetogenic bacteria converted VFAs finally into CO_2 and CH_4. However, volatile fatty acids are directly proportional to organic load. High organic loading can increase VFAs concentration inside the reactor as a result of pH value drops which inhibit the AD process (Zhang et al. 2013a; Palacio-Barco et al. 2010).

6. Macro- and Micronutrients.

Trace elements such as nickel (Ni), cobalt (Co), molybdenum (Mo), iron (Fe), tungsten (W), selenium (Se) and macronutrient carbon (C), phosphorus (P), sulfur (S), and nitrogen (N) are important equally for the survival and growth of microorganism in anaerobic digestion (Agler et al. 2008). These nutrients not only maintain the activities of enzymes but also help in their synthesis (Moestedt et al. 2013; Facchin et al. 2013). The optimum ratio of microelements S: P: N: C for AD is 1: 5: 15: 600.

Operational Parameters

1. Organic Load Rate.

It is defined as the amount of organic waste fed continuously to anaerobic reactor per day per unit working volume as shown in the equation below:

$$B_R = m \times \frac{c}{V_R} \tag{24.9}$$

where B_R, V_R, c, and m are the organic load (Kg/d*m^3), digester volume (m^3), organic matter concentration (%), and mass of substrate fed per time unit (Kg/d), respectively.

In diverse AD operations, the OLR differs because of variances in feedstock properties, operating temperature, and hydraulic retention time (Divya et al. 2015a). An optimal amount of OLR is required because too high organic load could accumulate VFAs in AD reactors that inhibit bacterial growth resulting in process failure; on the other hand, too low organic load could lead to the malnutrition of fermenting microbes consequently reducing the efficiency of the AD process. Generally, to some extent, OLR is directly proportional to the biogas yield. Various factors influence significantly OLR like operational cost and conditions as well as the type of SOWs fed (Meegoda et al. 2018).

2. Hydraulic Retention Time.

Hydraulic retention time (HRT) is defined as the time (days/hours) required for the complete degradation of SOWs. It is expressed in the following equation:

$$HRT = \frac{V_R}{V} \tag{24.10}$$

In this equation, V and V_R are substrate volume fed per unit time (m^3/day) and digester volume (m^3) correspondingly. It is inversely proportional to the organic load as shown in the above equation. It is a very important parameter influencing microbial growth in anaerobic reactor; therefore, it should be optimized (Mao et al. 2017). In the presence of very low HTR, volatile fatty acids could accumulate that lead to process failure by inhibiting bacteria while a very high HRT could result in insufficient feedstock usage. It depends upon the specific fed feedstock in the digester (Dareioti and Kornaros 2015). For SOWs treatment a 15-30 days HRT is required for AD operation (Mao et al. 2017).

24.6 Current Trends in Biogas Production

The general process of anaerobic digestion to produce biogas still requires intensive research. However, the information about the process has been increasing throughout recent years. The recently achieved methodological and technological advancements in that facet area, i.e., biogas upgrading (Angelidaki et al. 2018), use of new substrates (Vergara-Fernández et al. 2008), ammonia toxicity (Westerholm et al. 2009), process monitoring tools, i.e., VFAs sensors (Boe et al. 2007) and membrane reactors (Vyrides and Stuckey 2009). Reduction in the cost and time required for sequencing techniques played an important role in comprehending the complex microbial AD process. Nowadays various omics tools are used to decode the anaerobic digestion black box (Kougias and Angelidaki 2018).

Use of Pretreatment Techniques To make the AD system economically viable national systems have been supported to use an array of various substrates. However, several studies examined that biogas synthesis is directly affected by various interacted waste streams. So the researchers try to improve the arrangement of different waste streams for the optimal production of biogas also called co-digestion. Advance studies illustrated that the co-digestion of crops, lignocellulosic and sewage sludge wastes give the better quality as well as quantity of biogas. Despite these, the different pretreatment technologies help to improve the biogas yield, speed of the AD process and also provide a wide variety of substrates (Mahanty et al. 2014; Igoni et al. 2008).

Modifications in Biogas Digesters Biogas digesters are the air-tight bioreactors that are used to produce the biogas by the AD process. In the past, the basic model of digesters faced many problems and failures including high cost and unsteady gas pressure. Recently a new digester named puxin digester has been developed by China to contain all the qualities to improve biogas production. By the changing trends, the small household digesters holding the 5000 m^3 capacity have been designed to produce biogas for vehicular fuel (Bharathiraja et al. 2018; Rajendran et al. 2012). On large scales to preclude system failures the biogas plants have been modified to work in a programmed manner. These modifications (i.e., heating accouterments, mechanical agitators, performance monitoring systems, and temperature regulators) in response help to lessen the system failures (Ward et al. 2008).

Biogas Upgradation The conventionally used upgradation methods are pressure swing adsorption, pressure water scrubbing, amine adsorption, and biological methods. Even though, the latest cryogenic upgradation technology is becoming popular day by day. It is designed for the purification and bottling of biogas. In this technology boiling or sublimation points of different gases are used at very low temperature and high pressure. It is a very demanding technology because it yields 99% methane (Petersson and Wellinger 2009; Allegue et al. 2012).

High Pressurized and Multiple Stage AD To increase the efficiency of the AD process various research projects are designed to estimate different formations, i.e., single and multi-stage reactors. According to modern studies, the physical partition of the AD in two phases, i.e., acidogenesis or hydrolysis and methanogenesis or acetogenesis in separate reactors helps to elevate the organic matter decomposition into methane. The configuration of multiple bioreactors plays an important role to increase efficiency and process stability (Yu et al. 2017). Blonskaja et al. studied that the use of a two-stage scheme gives high growth of methanogens which respond in high gas production (Blonskaja et al. 2003). Similarly, Kim et al. referred that by using the four stages anaerobic digestion system the digestion activity enhanced rather than the single-stage Scheme (Kim et al. 2011). Furthermore, Nasr et al. suggested that the two-stage technology enriched the efficiency and performance of the process (Nasr et al. 2012). A recent technique is developed which works at high pressure (100 bars) and it gives the methane content about 95%. Previous studies also showed that working at high pressure (up to 90 bars) affect the microbial processes and provide enriched methane. However further analysis is required to find the microbial pressure-dependent techniques (Lindeboom et al. 2011).

24.7 Challenges, Approaches, and Enhancement Techniques

24.7.1 A Gap between Biotech and Commercialization Research

Lignocellulosic biomass, i.e., forestry residues, municipal wastes, and crop wastes are the high potential and sustainable feedstocks for the production of biofuels worldwide. The production of biogas from lignocellulose requires further research efforts for developments. It is due to the technical problems and lack of understanding of reactor operations involved in the process. The complications of the AD process and the threat to the technologies' strength are the notable problems (Himmel et al. 2007; Weber et al. 2010). To identify the research-biotech gaps, it is important to evaluate the impacts on economical, technical, and ecological barriers. For example, to reduce the cost, it is compulsory to determine the critical stages (e.g., use of enzymes or the investment on multi-stage AD systems) which affect the economy impressively. Once these standards are analyzed, they will help to indicate the costs, benefits, and research issues for improvement (Lynd et al. 2005). The finding of economically sustainable pretreatment processes has been identified as the major hurdle for the commercialization of biofuels (Philbrook et al. 2013). The amount and type of biocatalyst and microorganisms used for degradation affect the process stability and conversion rate but their cost is very high. So recent research initiatives have pay attention to the improvement of biocatalysts or microorganisms with better characteristics, low production cost, and wider applications. Recent studies also suggested the combination of high pressure and multi-stage technologies. These technologies will improve process efficiency (Blanch 2012; Banerjee et al. 2010). The research gap and scheme for the bio-industry are displayed in Fig. 24.13.

24.7.2 Biogas Future in a Green/Circular Economy

One of the renewable energy sources is biogas, and it does not generate CO_2. However, CO_2 is absorbed from the atmosphere during the biochemical process in AD and it is released with energy. When the CO_2 and minor constituents are taking away, then 100% methane is obtained. This is a zero-carbon source that is compatible with any ancillary natural gas that makes it a perfect fuel (Bharathiraja et al. 2018). Biogas has many industrial, household applications and gradually is finding as a vehicular fuel. Many efforts have been made to enhance the methane content through the optimization of techniques (i.e., pretreatment and multi-stage AD system). Various new technologies are used to improve biogas production but the challenges are still present. These challenges are (1) hydrolysis as the rate-limiting step, (2) lignocellulosic biomass particle size, lignin content, and crystallinity of cellulose. The enzyme pretreatment method helps to increase the lignocellulosic digestibility. In recent times, the biogas generates from SOWs may satisfy nearly 20% of the total natural gas. Extensive research is in progress to diversify the technological advancements and low-cost energy sources. Although to complement

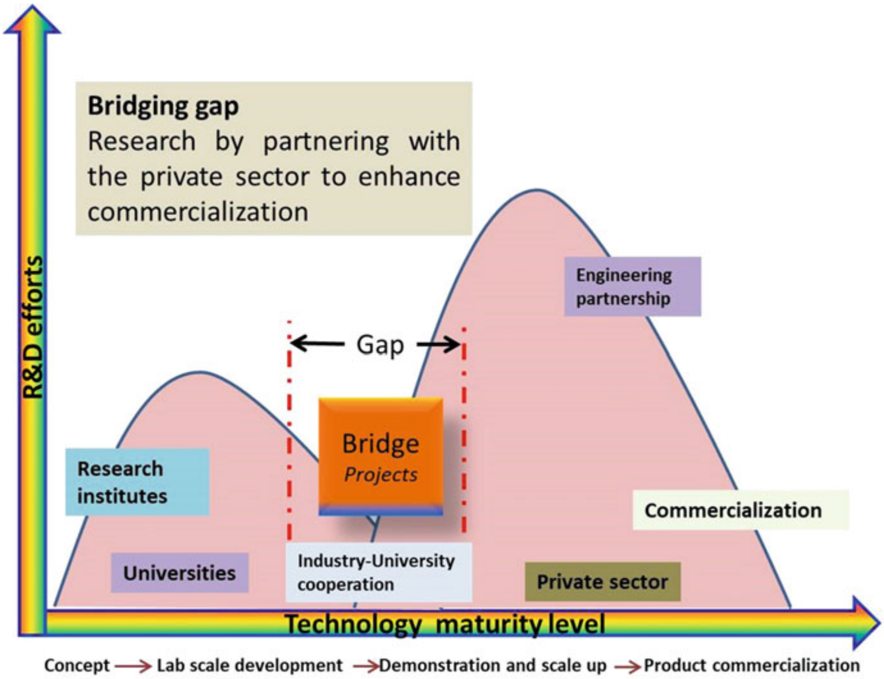

Fig. 24.13 Research gap and scheme for bio-industry (Bharathiraja et al. 2018)

the existing and developing technology, there is a sustainable management scheme for the future (Christy et al. 2014).

24.7.3 Pretreatment Techniques to Enhance Biogas Production

The treatment of solid organic wastes (SOWs) mainly agriculture waste and yard waste is very essential to expose cellulose and hemicellulose for bacterial attacks and enzymatic hydrolysis (Hu et al. 2015; Ravindran and Jaiswal 2016). Pretreatment has been classified into three main types as shown in Fig. 24.14.

Chemical Pretreatment

Chemical pretreatment of SOWs uses ionic liquids, alkalies, oxidizing agents and strong acids, etc. The reactions involved in this pretreatment are electrochemical, hydrolysis and oxidation reactions, etc. Some chemical pretreatment methods are shown in Table 24.3. It received more attention compared to physical pretreatment owing to its sound performance in enhancing methane yield. It increases organic waste's surface area and lowers the cellulose crystallinity and degree of polymerization. Despite a larger enhancement in biogas synthesis, but only alkali hydrolysis has found its practical application in the industry particularly for SOWs containing low

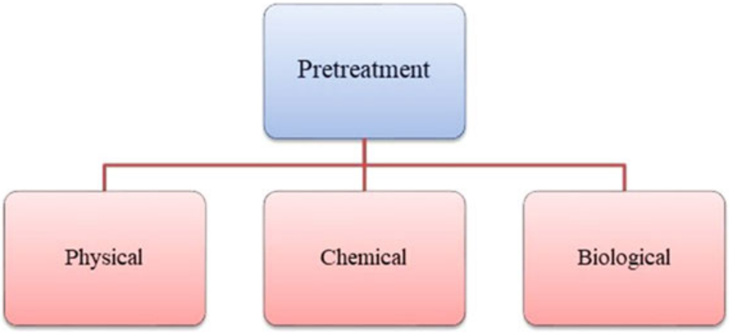

Fig. 24.14 Classification of pretreatment

lignin content (Shah et al. 2015). Whereas the main disadvantages of NaOH pretreatment are Na^+ ions that not only inhibit methane formation but also cause detrimental environmental impacts like soil salinization as well as water pollution (Zheng et al. 2014).

Modern research is trying to find eco-friendly chemicals for the maximum biogas yield.

Physical Pretreatment

The physical treatment technique does not use microorganisms or chemicals. It is used for the anaerobic conversion of SOWs to biogas as shown in Table 24.4.

Table 24.4 shows that the highest yield of biogas is produced as a result of hydrodynamic pressure homogenization (HPH) treatment of SOWs. The HPH is an environmentally friendly technique that produces a high quantity of CH_3 without any chemical in a very short time at room temperature. The lignocellulosic networks of biomass are destroyed in this technique due to sudden expansion (Yusaf and Al-Juboori 2014; Fang et al. 2015). It is also used in pharmaceutical and food industries for cell distraction and food emulsification consequently (Zhang et al. 2013c). Another physical method such as milling or comminution not only decreased the degree of polymerization and crystallinity of cellulose but also increased the surface area of feedstock by decreasing its particle size. The ultrasonic process uses high-frequency waves to obliterate the complex polymerization network in SOWs that facilitate enzymatic degradation efficiency (Ormaechea et al. 2017). Microwave is an irradiation technique that generates intense heating by applying an electromagnetic field to water comprising substances.

The steam explosion method consumes efficiently wheat straw as a substrate to increase the yield of biogas production. It is a commercial-scale process, but it yields a smaller amount of methane than HPH. It is a favorable choice for more industrial installation due to its number of benefits, for example, low energy input, commercially available tools, and low pollution tendency (Bauer et al. 2010; Forgács et al. 2012).

Table 24.3 Some chemical pretreatment approaches for SOWs

Reaction type	Conditions	Chemicals	Substrates	CH_3% yield (enhanced)	References
Ionic liquid treatment	1-15 h, 50–55% W/W NMNO, 120–130 °C	(NMNO) N-methyl-morpholine-N-oxide	Birchwood, oil palm bunch, rice straw, Ssoftwood spruce, etc.	47–1200%	Goshadrou et al. (2013)
Oxidation	3 h, pH 7–9, H_2O_2/COD: 0.05–0.25, 20 ± 2 °C	H_2O_2	Olive mill waste	> 1000 °C (77% COD reduction)	Siciliano et al. (2016), Travaini et al. (2016)
	15-120 min, 100–200 °C, 200 rpm, 6-12 bar	O_2 (air)	Distilleries effluents	280% (biogas)	Travaini et al. (2016)
	1 h, gas flow rate 12 L/min, 0.6–1% O_3	O_3	Wheat straw	45%	Padoley et al. (2012)
Acid hydrolysis	60 min, 50% (V/V) an organic solvent, 190 °C	CH_3COOH	Forest residue	500%	(Monlau et al. (2013))
	1–5% (W/W), 170 °C	H_2SO_4	Sunflower oil	48%	Kabir et al. (2015)
Electrochemical treatment	2 h, 0.5 M, 110 °C,	Na_2CO_3	Rice straw	125%	Dehghani et al. (2015)
	40 min, 2 cm electrode	Hypochlorite	Waste activated sludge (WAS)	63.40%	Iskander et al. (2016)
Alkali hydrolysis	10-240 min, 0.2–1% (W/W), 50–121 °C	Sodium hydroxide	Sugarcane bagasse, wheat straw, YW	30–78% or 250%	Bolado-Rodríguez et al. (2016)
	2.0% ca $(OH)_2$ and 0.5% KOH,	$Ca(OH)_2$ or KOH	Corn Stover	77%	Li et al. (2015a)
	10 bar, 0–30.8%	NH_4OH	Wheat straw	56% (biogas)	Li et al. (2015b)

(continued)

Table 24.3 (continued)

Reaction type	Conditions	Chemicals	Substrates	CH_3% yield (enhanced)	References
	(W/V),6-48 h, 20–80 °C				
	72 h, 8–10% (W/W), 25 °C	Calcium hydroxide	Rice straw	34.3–36.7% (biogas)	Gu et al. (2015)

Table 24.4 Physical treatment of Solid Organic Wastes to produce biogas

Substrate	Conditions	Methods	CH_3% yield (enhanced)	References
Wheat straw	60 minutes, 140 °C	Steam explosion	4–30%	Bauer et al. (2010)
Wheat straw	6–33 mm particle length	Milling or comminution	11–13%	Dumas et al. (2015)
Wheat straw and waste activated sludge	96 KJ/kg sludge specific energy	Microwave	20–28%	Jackowiak et al. (2011)
Organic residues and waste activated sludge	96–3380 KJ/kg total waste specific energy	Ultrasonic	27–71%	Cesaro et al. (2014)
Yard waste and wheat straw (aqueous suspension containing 5% wt.)	170 °C, 20 minutes	Expansion	41%	Kuttner et al. (2015)
Wheat straw	2300–2700 rpm rotor speed	Hydrodynamic cavitation	144%	Patil et al. (2016)
Yard waste	10 MPa	High-pressure homogenization	250%	Jin et al. (2015)
Yard waste Sewage sludge	0.5–15 h 70–121 °C	Thermal treatment	20–88%	Ruffino et al. (2015)

The major drawback of hydrothermal waste pretreatment is the high temperature required to heat liquid water present in the waste substrate. Globally, it is an effective advantageous technique compared to both chemical and biological pretreatments.

Biological Pretreatment

Generally, biological pretreatment uses fungal species or biological agents to produce biodegradable enzymes that help in SOWs degradation (Yıldırım et al. 2017). The main advantages of this technique are described in the following:

1. Minimum input energy due to its low operational cost.
2. Environmental friendly.
3. No expensive consumption of chemical.

The objective of this method is to remove lignin with fewer carbohydrates that can be obtained from the SOWs (Zhang et al. 2014). Some common types of this method such as fungal, sludge, bacterial, and enzymatic pretreatment are shown in Table 24.5. Among these types, bacterial and fungal pretreatment improves both biodegradable efficiency and biogas conversion of corn straw (Zhong et al. 2011). This has not been applied effectively on a large scale due to the slow microbial growth rate and enzymatic reaction rate (Shah et al. 2015). Enzymatic treatment improves only 13–19% biogas yield.

More suitable SOWs substrates for chemical, physical and the combination of both of these techniques are agriculture and yard waste. However, simple physical process such as milling, animal manure, and food waste are preferred to reduce their particle size. The physical process breaks down capably large granules of WAS into smaller particles.

Table 24.5 Different categories of biological pretreatment for SOWs

Category of biological pretreatment	Substrates	Active constituent	Conditions	Biogas % yield (enhanced)	References
Fungi	Tall wheatgrass; Miscanthus	*Ceriporiopsis ubvermispora* and *Flammulina velutipes*	4 weeks and 28 °C	120%	Lalak et al. (2016)
The liquid fraction of digestate pretreatment	Corn Stover	Mixed microorganisms	3 days, 17.6% of TS content, 20 ± 1 °C	70%	Wei et al. (2015)
Bacteria	Corn straw and organic sludge	*Thermophilic aerobic* bacteria	pH 5.0–8,5, 20 °C, or 60–70 °C 0.01% (W/W) dose of microbial agent for 15 days	30–150%	Zhong et al. (2011)
Enzymatic pretreatment	Spent hops and sugar beet pulp	Xylanase, endoglucanase and pectinase	24 h, 0.03–0.75FUP/g enzymatic dose, 50 °C	13–19%	Ziemiński and Kowalska-Wentel (2015); Passos et al. (2016); Kiran et al. (2015)

24.7.4 Genetic Engineering

Recently, genetic engineering plays an important role to improve biogas yield by either integrating particular DNA fragments or manipulating specific genes into desirable species (Lim et al. 2018; Han et al. 2017). A yeast strain was genetically engineered in 2010 to generate its own enzymes for cellulose digestion. Nowadays, 205 Eubacterial and 21 Archaeal genomes have been sequenced. Almost 80% of genomes of Archaebacteria are methanogens that were insulated from sludge as well as from other anaerobic environments. In the same way, many acidogenic bacterial genomes are sequenced too. The genome of *Methanobacterium thermoautotrophicum H* (thermophilic bacteria) is fully sequenced which was segregated from municipal solid waste (Zhu et al. 2017; Kougias et al. 2017).

24.7.5 Bioaugmentation

As it is discussed earlier the AD process requires microorganisms for each step. The disturbance in microorganism balance may cause bioreactor instability and lead to inhibition of methane production (Christy et al. 2014). This disturbance is due to various inhibitory factors, e.g., high level of sulfate, ammonia, phosphate, and metal ions. Some other parameters, i.e., pH variation, temperature, and resistance of feedstock also became the reason to decrease AD efficiency (Mao et al. 2015; Divya et al. 2015b). So to overcome these limitations, bioaugmentation as an alternate strategy might be used. Bioaugmentation is the addition of efficient stress-resistant microscopic species into a community of bacteriological to improve the efficacy of methane production (Lebeau et al. 2008). Some of the bioaugmentation examples are as follows:

Upgrading of Hydrolysis, Acetogenesis, and Acidogenesis In AD the very first phase is hydrolysis in which the feedstock is converted into simpler compounds. Cellulose, lignin, and hemicellulose containing substrates are among the most commonly used substrates. Though the major drawback of feedstock is the hydrolysis resistance to produce desirable products for fermentation. Different pretreatment techniques are used to improve hydrolysis but they have their limitations, i.e., partial hydrolysis and high cost (Carlsson et al. 2012). To overcome these problems various microorganisms are added that enhance the hydrolysis process due to their greater ability to break molecules (Mshandete et al. 2005). Coll and Weiss used a hemicellulolytic microbes group on the sludge obtained from the maize silage digesting plant. The outcomes displayed a 53% increase in methane production as compare to non-bioaugmented culture (Weiß et al. 2010). Similarly, Zhang and Coll suggested a pretreatment method for cassava residue. To achieve these thermophilic microorganisms enriched with cellulose and hemicellulose were used for the pretreatment of cassava residues. The outcomes showed a 97% growth in methane production (Zhong et al. 2011).

Role of H_2 in the AD The hydrogen produced in acetogenesis is used for the reduction of CO_2. Generally, methanogenesis does not carry out at a low H_2 level. So the high level of H_2 leads to enhancing methane production (Pap et al. 2015). Through bioaugmentation, the thermophilic Caldiecellulosiruptor saccharolyticus is used to convert hemicelluloses, cellulose, and pectin to acetate, H_2, and CO_2 (Bagi et al. 2007). In 2010 it is evaluated that the C. saccharolyticus species uses cellulose to produce H_2 (Herbel et al. 2010). Similarly, the bioaugmentation Acetobacteroides hydrogenigenes on corn straw and biogas slurry give a high yield of acetate and H_2. The outcomes showed a 23% increase in methane production (Zhang et al. 2015). It is evident from literature that the concentration of H_2 smaller than 10^{-4} is thermodynamically unfavorable for methane production. On the other hand, the high concentration of H_2 ($>10^{-8}$) acts as an inhibitory factor to hydrogenotroph. So it is very important to maintain a suitable concentration of H_2 to produce CH_4 (Kovacs et al. 2004).

Overcoming Ammonium Inhibition The obtainability of nitrogen is persistent with the cell growth which is obtained from nitrogenous matter. In aqueous solution, the inorganic nitrogen is present in the form of NH_3 and NH_4+. It is showed that the high concentration of ammonia inhibited the AD process because nitrogen is diffused into cells, causing potassium deficiency and proton imbalance (Chen et al. 2008). High temperature and high pH values produce free NH_3 in a higher concentration that increases toxicity. To overcome the toxicity of ammonia various methods have been studied, i.e., addition of NH_3 binding ions, high C/N ratios and low temperature of digester that reduces NH_3 toxicity (Nielsen and Angelidaki 2008). Fotidis and Coll suggested the bioaugmentation with an archaea species, i.e., hydrogenotroph Methanoculleus bourgensis can tolerate high ammonia levels (Fotidis et al. 2014).

Overcoming Low Temperature To enhance the AD process temperature is another significant parameter. Generally, by increasing the temperature the metabolic rate also increases which leads to high methane production. For example, when the mesophilic microorganisms are revealed to low temperature, the overall yield of biogas decreased (Appels et al. 2008). However, at low temperatures when the AD process is operating, the bioaugmentation with psychrophilic species increases the methane production. Consequently, the decrease of methane production due to low temperature can be overcome by using microorganisms that work more effectively at low temperatures (Akila and Chandra 2010).

Overcoming O_2 Produced Toxicity The O_2 present in the reactor leads to the amassing of H_2 by decreasing methanogens as a result methane production decreases. Under these conditions, exogenous methanogens accumulation helps to restore methane yield. Schauer-Gimenez and Coll used a group of H_2 amassing methanogens for bioaugmentation of the bioreactor. The outcomes showed a 60% increase in methane production (Schauer-Gimenez et al. 2010).

24.8 Conclusion

The energy crisis has been increasing day by day and the resources of renewable energy would be enough to meet the 50% global energy needs by 2050. So, biogas production attains a strategic location in the global market. The stability and performance of AD to produce biogas are primarily dependent on various groups of microscopic organisms and in turn, their functions and networks are influenced by operational parameters as well as properties of substrates. The anaerobic waste treatment process is an efficient technique to lessen the mass of the organic waste. Microbes play a very important role in the biochemical process of biogas production. In this era, it is necessary to implement the better acceptance technologies such as biotechnological advancements and investigations are needed to discover the effective feedstocks, effectiveness, and competency of the microbes and substrates for pretreatment. In recent times, the obtainability of efficient and genetically modified microbes, preparation of enzymes that are substrate-specific, microbial growth understanding, and cost reduction would be a challenge for scientists. However, the multi-stage digester designs, biological pretreatment techniques, genetic engineering, and bioaugmentation are the outstanding options used for the sustainable development of AD performance in biogas generation.

References

Abbasi T, Tauseef S, Abbasi S (2012) A brief history of anaerobic digestion and “biogas”. In: Biogas energy. Springer, Dordrecht. p. 11–23

Achinas S, Achinas V, Euverink GJW (2017) A technological overview of biogas production from biowaste. Engineering 3(3):299–307

Adekunle KF, Okolie JA (2015) A review of biochemical process of anaerobic digestion. Adv Biosci Biotechnol 6(03):205

Agency EE (2016) Renewable energy in Europe 2016: recent growth and knock-on effects. EEA Report 4/2016

Agler MT et al (2008) Thermophilic anaerobic digestion to increase the net energy balance of corn grain ethanol. Environ Sci Technol 42(17):6723–6729

Akila G, Chandra T (2010) Stimulation of biomethanation by Clostridium sp. PXYL1 in coculture with a Methanosarcina strain PMET1 at psychrophilic temperatures. J Appl Microbiol 108 (1):204–213

Al Seadi T (2001) Good practice in quality management of AD residues from biogas production: task 24 og AEA technology environment

Allegue LB, Hinge J, Allé K (2012) Biogas and bio-syngas upgrading. Danish Technological Institute. p. 5–97

Alves HJ et al (2013) Overview of hydrogen production technologies from biogas and the applications in fuel cells. International Journal of Hydrogen Energy 38(13):5215–5225

Angelidaki I, Ahring BK (2000) Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Water Sci Technol 41(3):189–194

Angelidaki I et al (2018) Biogas upgrading and utilization: current status and perspectives. Biotechnol Adv 36(2):452–466

Appavou F, et al. (2017) Renewables 2017 global status report. Renewable energy policy network for the 21st century. REN21, Paris

Appels L et al (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Prog Energy Combust Sci 34(6):755–781
Atelge MR, Krisa D, Kumar G, Eskicioglu C, Nguyen DD, Chang SW, Atabani AE, Al-Muhtaseb A, Unalan S (2018) Biogas production from organic waste: recent progress and perspectives. Waste Biomass Valoriz 11:1019–1040. https://doi.org/10.1007/s12649-018-00546-0
Awe OW et al (2017) A review of biogas utilisation, purification and upgrading technologies. Waste and Biomass Valorization 8(2):267–283
Bagi Z et al (2007) Biotechnological intensification of biogas production. Appl Microbiol Biotechnol 76(2):473–482
Banerjee S et al (2010) Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. Biofuels, Bioproducts and Biorefining: Innovation for a sustainable economy 4(1):77–93
Bauer A et al (2010) Analysis of methane yields from energy crops and agricultural by-products and estimation of energy potential from sustainable crop rotation systems in EU-27. Clean Techn Environ Policy 12(2):153–161
Bausch C, Görlach B, Mehling M (2017) Ambitious climate policy through centralization? Evidence from the European Union. Climate Policy 17(sup1):S32–S50
Bertin L et al (2013) Innovative two-stage anaerobic process for effective codigestion of cheese whey and cattle manure. Bioresour Technol 128:779–783
Bharathiraja B et al (2018) Biogas production–a review on composition, fuel properties, feed stock and principles of anaerobic digestion. Renew Sust Energ Rev 90(C):570–582
Blanch HW (2012) Bioprocessing for biofuels. Curr Opin Biotechnol 23(3):390–395
Blonskaja V, Menert A, Vilu R (2003) Use of two-stage anaerobic treatment for distillery waste. Adv Environ Res 7(3):671–678
Bo T et al (2014) A new upgraded biogas production process: coupling microbial electrolysis cell and anaerobic digestion in single-chamber, barrel-shape stainless steel reactor. Electrochem Commun 45:67–70
Boe K, Batstone DJ, Angelidaki I (2007) An innovative online VFA monitoring system for the anerobic process, based on headspace gas chromatography. Biotechnol Bioeng 96(4):712–721
Böhm K, Tintner J, Smidt E (2011) Modelled on nature-biological processes in waste management. Integrated Waste Management 1:153–178
Bolado-Rodríguez S et al (2016) Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse. Bioresour Technol 201:182–190
Braun R (2007) Anaerobic digestion: a multi-faceted process for energy, environmental management and rural development. In Improvement of crop plants for industrial end uses. Springer, Dordrecht. p. 335–416
Brown TR, Brown RC (2013) A review of cellulosic biofuel commercial-scale projects in the United States. Biofuels Bioprod Biorefin 7(3):235–245
Busing R, et al. (2008) Hurricane disturbance in a temperate deciduous forest: patch dynamics, tree mortality, and coarse woody detritus. In Forest ecology. Springer, Dordrecht. p. 351–363
Buyukkamaci N, Filibeli A (2004) Volatile fatty acid formation in an anaerobic hybrid reactor. Process Biochem 39(11):1491–1494
Carballa M, Regueiro L, Lema JM (2015) Microbial management of anaerobic digestion: exploiting the microbiome-functionality nexus. Curr Opin Biotechnol 33:103–111
Carlsson M, Lagerkvist A, Morgan-Sagastume F (2012) The effects of substrate pre-treatment on anaerobic digestion systems: a review. Waste Manag 32(9):1634–1650
Cerrillo M, Viñas M, Bonmatí A (2016) Overcoming organic and nitrogen overload in thermophilic anaerobic digestion of pig slurry by coupling a microbial electrolysis cell. Bioresour Technol 216:362–372
Cesaro A et al (2014) Enhanced anaerobic digestion by ultrasonic pretreatment of organic residues for energy production. J Clean Prod 74:119–124

Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: a review. Bioresour Technol 99(10):4044–4064
Chiumenti A et al (2018) Biogas from fresh spring and summer grass: effect of the harvesting period. Energies 11(6):1466
Cho JK, Park SC, Chang HN (1995) Biochemical methane potential and solid state anaerobic digestion of Korean food wastes. Bioresour Technol 52(3):245–253
Chouari R et al (2005) Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. Environ Microbiol 7(8):1104–1115
Christy PM, Gopinath L, Divya D (2014) A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. Renew Sust Energ Rev 34:167–173
Chuanchai A, R. Ramaraj (2018) Sustainability assessment of biogas production from buffalo grass and dung: biogas purification and bio-fertilizer. 3 Biotech 8(3): 151
Cirne D et al (2007) Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. J Appl Microbiol 103(3):516–527
Dareioti MA, Kornaros M (2015) Anaerobic mesophilic co-digestion of ensiled sorghum, cheese whey and liquid cow manure in a two-stage CSTR system: effect of hydraulic retention time. Bioresour Technol 175:553–562
Deepanraj B, Sivasubramanian V, Jayaraj S (2014) Biogas generation through anaerobic digestion process-an overview. Research Journal of Chemistry and Environment 18:5
Dehghani M, Karimi K, Sadeghi M (2015) Pretreatment of rice straw for the improvement of biogas production. Energy Fuel 29(6):3770–3775
Divya D, et al (2015a) Enhancement of biogas production through sustainable feedstock utilization by co-digestion. 5(3). Coden: IJPAJX-CAS-USA, Copyrights@ 2015 ISSN-2231-4490 Received: 20 th May-2015 Revised: 20 th June-2015 Accepted: 24 th June-2015
Divya D, Gopinath L, Christy PM (2015b) A review on current aspects and diverse prospects for enhancing biogas production in sustainable means. Renew Sust Energ Rev 42:690–699
Dumas C et al (2015) Effects of grinding processes on anaerobic digestion of wheat straw. Ind Crop Prod 74:450–456
Edenhofer O et al (2011) Renewable energy sources and climate change mitigation: special report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge
Facchin V et al (2013) Batch and continuous mesophilic anaerobic digestion of food waste: effect of trace elements supplementation. Chem Eng 32:457–462
Fang W et al (2015) Physicochemical properties of sewage sludge disintegrated with high pressure homogenization. Int Biodeterior Biodegradation 102:126–130
Fernandes TV et al (2012) Effect of ammonia on the anaerobic hydrolysis of cellulose and tributyrin. Biomass Bioenergy 47:316–323
Ferreira A, et al (2019) Combining microalgae-based wastewater treatment with biofuel and bio-based production in the frame of a biorefinery. In Grand challenges in algae biotechnology. Springer, Dordrecht. p. 319–369
Forgács G et al (2012) Methane production from citrus wastes: process development and cost estimation. J Chem Technol Biotechnol 87(2):250–255
Fotidis IA et al (2014) Bioaugmentation as a solution to increase methane production from an ammonia-rich substrate. Environ Sci Technol 48(13):7669–7676
Garcia J-L, Patel BK, Ollivier B (2000) Taxonomic, phylogenetic, and ecological diversity of methanogenic archaea. Anaerobe 6(4):205–226
García-González MC et al (2019) Positive impact of biogas chain on GHG reduction. In: Improving biogas production. Springer, Dordrecht, pp 217–242
Gashaw A (2014) Anaerobic co-digestion of biodegradable municipal solid waste with human excreta for biogas production: a review. American Journal of Applied Chemistry 2(4):55–62
Geng W et al (2016) China′ s new energy development: status, constraints and reforms. Renew Sust Energ Rev 53:885–896

Gonzalez-Salazar MA et al (2016) A general modeling framework to evaluate energy, economy, land-use and GHG emissions nexus for bioenergy exploitation. Appl Energy 178:223–249
Goshadrou A, Karimi K, Taherzadeh MJ (2013) Ethanol and biogas production from birch by NMMO pretreatment. Biomass Bioenergy 49:95–101
Goswami R et al (2016) An overview of physico-chemical mechanisms of biogas production by microbial communities: a step towards sustainable waste management. 3 Biotech 6(1):72
Gu Y, Zhang Y, Zhou X (2015) Effect of ca (OH) 2 pretreatment on extruded rice straw anaerobic digestion. Bioresour Technol 196:116–122
Gubaidullina MS, Kargina A (2015) Theoretical analysis of the energy efficiency policy concept: Japan and Germany's experience to Kazakhstan. International Relations and International Law Journal 72:4
Hahn-Hägerdal B et al (2007) Towards industrial pentose-fermenting yeast strains. Appl Microbiol Biotechnol 74(5):937–953
Han G et al (2017) A comparative study on the process efficiencies and microbial community structures of six full-scale wet and semi-dry anaerobic digesters treating food wastes. Bioresour Technol 245:869–875
Heeg K et al (2014) Microbial communities involved in biogas production from wheat straw as the sole substrate within a two-phase solid-state anaerobic digestion. Syst Appl Microbiol 37 (8):590–600
Herbel Z et al (2010) Exploitation of the extremely thermophilic Caldicellulosiruptor saccharolyticus in hydrogen and biogas production from biomasses. Environ Technol 31 (8–9):1017–1024
Hijazi O et al (2016) Review of life cycle assessment for biogas production in Europe. Renew Sust Energ Rev 54:1291–1300
Hills D, Roberts D (1982) Conversion of tomato, peach and honeydew solid waste into methane gas. Transactions of the ASAE 25(3):820–0826
Himmel ME et al (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315(5813):804–807
Horiuchi JI et al (1999) Dynamic behavior in response to pH shift during anaerobic acidogenesis with a chemostat culture. Biotechnology Techniques 13(3):155–157
Horiuchi J-I et al (2002) Selective production of organic acids in anaerobic acid reactor by pH control. Bioresour Technol 82(3):209–213
Hosseini SE, Wahid MA (2014) Development of biogas combustion in combined heat and power generation. Renew Sust Energ Rev 40:868–875
Hou J, Hou B (2019) Farmers' adoption of low-carbon agriculture in China: an extended theory of the planned behavior model. Sustainability 11(5):1399
Hsia T-H et al (2008) PVA-alginate immobilized cells for anaerobic ammonium oxidation (anammox) process. J Ind Microbiol Biotechnol 35(7):721–727
Hu Y et al (2015) Promoting anaerobic biogasification of corn Stover through biological pretreatment by liquid fraction of digestate (LFD). Bioresour Technol 175:167–173
Igoni AH et al (2008) Designs of anaerobic digesters for producing biogas from municipal solid-waste. Appl Energy 85(6):430–438
Iskander SM et al (2016) Resource recovery from landfill leachate using bioelectrochemical systems: opportunities, challenges, and perspectives. Bioresour Technol 201:347–354
Jackowiak D et al (2011) Optimisation of a microwave pretreatment of wheat straw for methane production. Bioresour Technol 102(12):6750–6756
Jin S et al (2015) Comparative study of high-pressure homogenization and alkaline-heat pretreatments for enhancing enzymatic hydrolysis and biogas production of grass clipping. Int Biodeterior Biodegradation 104:477–481
Kabir MM et al (2015) Experimental and economical evaluation of bioconversion of forest residues to biogas using organosolv pretreatment. Bioresour Technol 178:201–208
Karthikeyan OP, Visvanathan C (2012) Effect of C/N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester. Bioresour Technol 113:294–302

Keating C (2015) Hydrolysis, methanogenesis and bioprocess performance during low-temperature anaerobic digestion of dilute wastewater. PhD thesis, National University of Ireland

Khalid A et al (2011) The anaerobic digestion of solid organic waste. Waste Manag 31 (8):1737–1744

Kim J, Novak JT, Higgins MJ (2011) Multistaged anaerobic sludge digestion processes. J Environ Eng 137(8):746–753

Kiran EU, Trzcinski AP, Liu Y (2015) Enhancing the hydrolysis and methane production potential of mixed food waste by an effective enzymatic pretreatment. Bioresour Technol 183:47–52

Knol W, Van Der Most MM, De Waart J (1978) Biogas production by anaerobic digestion of fruit and vegetable waste. A preliminary study. J Sci Food Agric 29(9):822–830

Kougias PG, Angelidaki I (2018) Biogas and its opportunities—a review. Front Environ Sci Eng 12 (3):14

Kougias PG et al (2017) A novel archaeal species belonging to Methanoculleus genus identified via de-novo assembly and metagenomic binning process in biogas reactors. Anaerobe 46:23–32

Kovacs KL et al (2004) Improvement of biohydrogen production and intensification of biogas formation. Rev Environ Sci Biotechnol 3(4):321–330

Krishania M et al (2012) Opportunities for improvement of process technology for biomethanation processes. Green Processing and Synthesis 1(1):49–59

Kusch S (2008) Key success factors in discontinuously operated dry digestion. International Biogas and Bioenergy Competence Centre

Kusch S, Oechsner H, Jungbluth T (2012) Effect of various leachate recirculation strategies on batch anaerobic digestion of solid substrates. Int J Environ Waste Manag 9(1–2):69–88

Kuttner P et al (2015) Examination of commercial additives for biogas production. Agron Res 13 (2):337–347

Lalak J et al (2016) Effect of biological pretreatment of *Agropyron elongatum* 'BAMAR'on biogas production by anaerobic digestion. Bioresour Technol 200:194–200

Lauterböck B et al (2012) Counteracting ammonia inhibition in anaerobic digestion by removal with a hollow fiber membrane contactor. Water Res 46(15):4861–4869

Lebeau T, Braud A, Jézéquel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ Pollut 153(3):497–522

Lee B et al (2017) Microbial communities change in an anaerobic digestion after application of microbial electrolysis cells. Bioresour Technol 234:273–280

Lei X, Maekawa T (2007) Electrochemical treatment of anaerobic digestion effluent using a Ti/Pt–IrO2 electrode. Bioresour Technol 98(18):3521–3525

Lettinga G (1995) Anaerobic digestion and wastewater treatment systems. Antonie Van Leeuwenhoek 67(1):3–28

Li L et al (2015a) Pretreatment of corn Stover for methane production with the combination of potassium hydroxide and calcium hydroxide. Energy Fuel 29(9):5841–5846

Li Y et al (2015b) Optimization of ammonia pretreatment of wheat straw for biogas production. J Chem Technol Biotechnol 90(1):130–138

Lim JW, Ge T, Tong YW (2018) Monitoring of microbial communities in anaerobic digestion sludge for biogas optimisation. Waste Manag 71:334–341

Lin L et al (2009a) Removal of ammonia nitrogen in wastewater by microwave radiation. J Hazard Mater 161(2–3):1063–1068

Lin L et al (2009b) Removal of ammonia nitrogen in wastewater by microwave radiation: a pilot-scale study. J Hazard Mater 168(2–3):862–867

Lindeboom R et al (2011) Autogenerative high pressure digestion: anaerobic digestion and biogas upgrading in a single step reactor system. Water Sci Technol 64(3):647–653

Lynd LR et al (2005) Consolidated bioprocessing of cellulosic biomass: an update. Curr Opin Biotechnol 16(5):577–583

Mahanty B et al (2014) Optimization of co-digestion of various industrial sludges for biogas production and sludge treatment: methane production potential experiments and modeling. Waste Manag 34(6):1018–1024

Mao C et al (2015) Review on research achievements of biogas from anaerobic digestion. Renew Sust Energ Rev 45:540–555
Mao C et al (2017) Linkage of kinetic parameters with process parameters and operational conditions during anaerobic digestion. Energy 135:352–360
Mathew AK et al (2015) Biogas production from locally available aquatic weeds of Santiniketan through anaerobic digestion. Clean Techn Environ Policy 17(6):1681–1688
McInerney MJ et al (2008) Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. Ann N Y Acad Sci 1125(1):58–72
Meegoda JN et al (2018) A review of the processes, parameters, and optimization of anaerobic digestion. Int J Environ Res Public Health 15(10):2224
Membrez Y, Bucheli O (2004) Biogas as a fuel source for SOFC co-generators. J Power Sources 127(1–2):300–312
Moestedt J et al (2013) Biogas production from thin stillage on an industrial scale—experience and optimisation. Energies 6(11):5642–5655
Møller HB, Sommer SG, Ahring BK (2004) Methane productivity of manure, straw and solid fractions of manure. Biomass Bioenergy 26(5):485–495
Monlau F et al (2013) Enhancement of methane production from sunflower oil cakes by dilute acid pretreatment. Appl Energy 102:1105–1113
Montañés R, Solera R, Pérez M (2015) Anaerobic co-digestion of sewage sludge and sugar beet pulp lixiviation in batch reactors: effect of temperature. Bioresour Technol 180:177–184
Mshandete A et al (2005) Enhancement of anaerobic batch digestion of sisal pulp waste by mesophilic aerobic pre-treatment. Water Res 39(8):1569–1575
Nasr N et al (2012) Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. Bioresour Technol 111:122–126
Nielsen HB, Angelidaki I (2008) Strategies for optimizing recovery of the biogas process following ammonia inhibition. Bioresour Technol 99(17):7995–8001
Offermann R et al (2011) Assessment of global bioenergy potentials. Mitig Adapt Strateg Glob Chang 16(1):103–115
Ohkubo T, Hideshima Y, Shudo Y (2010) Estimation of hydrogen output from a full-scale plant for production of hydrogen from biogas. Int J Hydrog Energy 35(23):13021–13027
Ormaechea P et al (2017) Influence of the ultrasound pretreatment on anaerobic digestion of cattle manure, food waste and crude glycerine. Environ Technol 38(6):682–686
Owen W et al (1979) Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res 13(6):485–492
Padoley K et al (2012) Wet air oxidation as a pretreatment option for selective biodegradability enhancement and biogas generation potential from complex effluent. Bioresour Technol 120:157–164
Palacio-Barco E et al (2010) On-line analysis of volatile fatty acids in anaerobic treatment processes. Anal Chim Acta 668(1):74–79
Pap B et al (2015) Temperature-dependent transformation of biogas-producing microbial communities points to the increased importance of hydrogenotrophic methanogenesis under thermophilic operation. Bioresour Technol 177:375–380
Parawira W (2012) Enzyme research and applications in biotechnological intensification of biogas production. Crit Rev Biotechnol 32(2):172–186
Passos F et al (2016) Improving biogas production from microalgae by enzymatic pretreatment. Bioresour Technol 199:347–351
Passos F et al (2020) Potential applications of biogas produced in small-scale UASB-based sewage treatment plants in Brazil. Energies 13(13):3356
Patil PN et al (2016) Intensification of biogas production using pretreatment based on hydrodynamic cavitation. Ultrason Sonochem 30:79–86
Petersson A, Wellinger A (2009) Biogas upgrading technologies–developments and innovations. IEA bioenergy 20:1–19

Petersson A et al (2007) Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. Biomass Bioenergy 31(11–12):812–819

Pham TN et al (2012) Volatile fatty acids production from marine macroalgae by anaerobic fermentation. Bioresour Technol 124:500–503

Philbrook A, Alissandratos A, Easton CJ (2013) Biochemical processes for generating fuels and commodity chemicals from lignocellulosic biomass. Environ Biotechnol 12:39–63

Qian Y et al (2017) Review of the state-of-the-art of biogas combustion mechanisms and applications in internal combustion engines. Renew Sust Energ Rev 69:50–58

Raheem A et al (2015) Thermochemical conversion of microalgal biomass for biofuel production. Renew Sust Energ Rev 49:990–999

Rahman MM et al (2013) Lignin and its effects on litter decomposition in forest ecosystems. Chem Ecol 29(6):540–553

Rajendran K, Aslanzadeh S, Taherzadeh MJ (2012) Household biogas digesters—A review. Energies 5(8):2911–2942

Raucci D, Agostinone S, Carnevale M (2019) Technical and economic evaluation of renewable energy production in the Italian agricultural firm: financing a biogas plant investment. World Review of Entrepreneurship, Management and Sustainable Development 15(4):513–538

Ravindran R, Jaiswal AK (2016) A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: challenges and opportunities. Bioresour Technol 199:92–102

Repele M, Udrene L, Bazbauers G (2017) Support mechanisms for biomethane production and supply. Energy Procedia 113:304–310

Ruffino B et al (2015) Improvement of anaerobic digestion of sewage sludge in a wastewater treatment plant by means of mechanical and thermal pre-treatments: performance, energy and economical assessment. Bioresour Technol 175:298–308

Schauer-Gimenez AE et al (2010) Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. Water Res 44(12):3555–3564

Scherer P et al (2009) Application of a fuzzy logic control system for continuous anaerobic digestion of low buffered, acidic energy crops as mono-substrate. Biotechnol Bioeng 102 (3):736–748

Schnurer A, Jarvis A (2010) Microbiological handbook for biogas plants. Swedish Waste Management U 2009:1–74

Shah FA et al (2015) Co-digestion, pretreatment and digester design for enhanced methanogenesis. Renew Sust Energ Rev 42:627–642

Shiratori Y, Oshima T, Sasaki K (2008) Feasibility of direct-biogas SOFC. Int J Hydrog Energy 33 (21):6316–6321

Siciliano A, Stillitano M, De Rosa S (2016) Biogas production from wet olive mill wastes pretreated with hydrogen peroxide in alkaline conditions. Renew Energy 85:903–916

Sikora A, et al. (2019) Searching for metabolic pathways of anaerobic digestion: a useful list of the key enzymes

Slonczewski JL, Foster JW (2013) Microbiology: an evolving science: Third international student edition. WW Norton & Company

Solarte-Toro JC, Chacón-Pérez Y, Cardona-Alzate CA (2018) Evaluation of biogas and syngas as energy vectors for heat and power generation using lignocellulosic biomass as raw material. Electron J Biotechnol 33:52–62

Sung S, Liu T (2003) Ammonia inhibition on thermophilic anaerobic digestion. Chemosphere 53 (1):43–52

Tongia R, Gross S (2018) Working to turn ambition into reality: the politics and economics of India's turn to renewable power

Travaini R et al (2016) Ozonolysis: an advantageous pretreatment for lignocellulosic biomass revisited. Bioresour Technol 199:2–12

Trzcinski AP, Stuckey DC (2010) Treatment of municipal solid waste leachate using a submerged anaerobic membrane bioreactor at mesophilic and psychrophilic temperatures: analysis of recalcitrants in the permeate using GC-MS. Water Res. 44(3):671–680

Tuesorn S et al (2013) Enhancement of biogas production from swine manure by a lignocellulolytic microbial consortium. Bioresour Technol 144:579–586
Venetsaneas N et al (2009) Using cheese whey for hydrogen and methane generation in a two-stage continuous process with alternative pH controlling approaches. Bioresour Technol 100 (15):3713–3717
Vergara-Fernández A et al (2008) Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Biomass Bioenergy 32(4):338–344
Vyrides I, Stuckey D (2009) Saline sewage treatment using a submerged anaerobic membrane reactor (SAMBR): effects of activated carbon addition and biogas-sparging time. Water Res 43 (4):933–942
Walker M et al (2011) Ammonia removal in anaerobic digestion by biogas stripping: an evaluation of process alternatives using a first order rate model based on experimental findings. Chem Eng J 178:138–145
Wang X et al (2012) Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour Technol 120:78–83
Wang Q et al (2019) Consumer support and willingness to pay for electricity from solar, wind, and cow manure in the United States: evidence from a survey in Vermont. Energies 12(23):4467
Ward AJ et al (2008) Optimisation of the anaerobic digestion of agricultural resources. Bioresour Technol 99(17):7928–7940
Weber C et al (2010) Trends and challenges in the microbial production of lignocellulosic bioalcohol fuels. Appl Microbiol Biotechnol 87(4):1303–1315
Wei Y et al (2015) Mesophilic anaerobic co-digestion of cattle manure and corn Stover with biological and chemical pretreatment. Bioresour Technol 198:431–436
Weiland P (2010) Biogas production: current state and perspectives. Appl Microbiol Biotechnol 85 (4):849–860
Weiß S et al (2010) Enhancement of biogas production by addition of hemicellulolytic bacteria immobilised on activated zeolite. Water Res 44(6):1970–1980
Westerholm M, et al (2009) Changes in the acetogenic population in a mesophilic anaerobic digester in response to increasing ammonia concentration. Microbes and environments. p. 1107250321–1107250321
Wett B, Insam H (2010) Biogas technology–Controlled gas flow for enhanced mixing, heating, and desulfurization. In: Microbes at Work. Springer, Dordrecht, pp 79–91
Whelan M, Everitt T, Villa R (2010) A mass transfer model of ammonia volatilisation from anaerobic digestate. Waste Manag 30(10):1808–1812
Wirth R et al (2012) Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. Biotechnol Biofuels 5(1):41
Wirthensohn T et al (2009) Ammonium removal from anaerobic digester effluent by ion exchange. Water Sci Technol 60(1):201–210
Xuan J et al (2009) A review of biomass-derived fuel processors for fuel cell systems. Renew Sust Energ Rev 13(6–7):1301–1313
Yan Z et al (2015) The effects of initial substrate concentration, C/N ratio, and temperature on solid-state anaerobic digestion from composting rice straw. Bioresour Technol 177:266–273
Yenigün O, Demirel B (2013) Ammonia inhibition in anaerobic digestion: a review. Process Biochem 48(5–6):901–911
Yıldırım E et al (2017) Improvement of biogas potential of anaerobic digesters using rumen fungi. Renew Energy 109:346–353
Yolin C (2015) Waste management and recycling in Japan opportunities for European companies (SMEs focus). EU-Japan Center for Industrial Cooperation, Tokyo, Japan
Yu L et al (2017) Two-stage anaerobic digestion systems wherein one of the stages comprises a two-phase system. Google Patents
Yusaf T, Al-Juboori RA (2014) Alternative methods of microorganism disruption for agricultural applications. Appl Energy 114:909–923

Zhang C et al (2013a) The anaerobic co-digestion of food waste and cattle manure. Bioresour Technol 129:170–176

Zhang C, Su H, Tan T (2013b) Batch and semi-continuous anaerobic digestion of food waste in a dual solid–liquid system. Bioresour Technol 145:10–16

Zhang Y et al (2013c) Sewage sludge solubilization by high-pressure homogenization. Water Sci Technol 67(11):2399–2405

Zhang Z et al (2014) Impact of pretreatment on solid state anaerobic digestion of yard waste for biogas production. World J Microbiol Biotechnol 30(2):547–554

Zhang J et al (2015) Bioaugmentation with an acetate-type fermentation bacterium Acetobacteroides hydrogenigenes improves methane production from corn straw. Bioresour Technol 179:306–313

Zhao M et al (2008) The influence of pH adjustment on biogas production from kitchen wastes by anaerobic fermentation. Chin J Bioprocess Eng 6(4):45–49

Zheng Y et al (2014) Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog Energy Combust Sci 42:35–53

Zhong W et al (2011) Effect of biological pretreatments in enhancing corn straw biogas production. Bioresour Technol 102(24):11177–11182

Zhong W et al (2013) Enhanced methane production from Taihu Lake blue algae by anaerobic co-digestion with corn straw in continuous feed digesters. Bioresour Technol 134:264–270

Zhou H, Wen Z (2019) Solid-state anaerobic digestion for waste management and biogas production. Solid State Fermen Res Indust Appl:147–168

Zhu X et al (2017) Characterization of the planktonic microbiome in upflow anaerobic sludge blanket reactors during adaptation of mesophilic methanogenic granules to thermophilic operational conditions. Anaerobe 46:69–77

Ziemiński K, Kowalska-Wentel M (2015) Effect of enzymatic pretreatment on anaerobic co-digestion of sugar beet pulp silage and vinasse. Bioresour Technol 180:274–280

Ziganshin AM et al (2013) Microbial community structure and dynamics during anaerobic digestion of various agricultural waste materials. Appl Microbiol Biotechnol 97(11):5161–5174

Applications of Microbes in Antibiotics

25

Sinazo Zezezethu Zongeziwe Cobongela

Abstract

The discovery of antibiotics is one of the most successful therapies to ever occur in the history of medicine. They have saved millions of lives throughout the world by treating numerous bacterial infectious diseases in humans, animals, and to a lesser extent, plants. They have found use in food preservation, animal nutrition, etc. In contrast to microorganisms being known for causing diseases, they are also the major and primary source of antibiotics. In a large and diverse population, microorganisms such as fungi, bacteria, and actinomycetes produce antibiotics as a natural defense system against other microorganisms occupying the same vicinity. Microbes, especially soil microbial inhabitants, do this as a form of adaptive strategy for survival and successful reproduction in a large variety of biotic and abiotic conditions. Therefore, it is to no surprise that microbes mutate to avoid being wiped out by the antibiotics or develop resistance to the antibiotics they produce. Antibiotic research and production have become the most promising field as there is a rising need to combat infections and mitigate the exponential growth in antibiotic resistance with the forever evolving microbial family.

Keywords

Actinomycetes · Antibiotic-producing · Antibiotics · Aspergillus · Bacillus · Bacteria · Cephalosporin · Enzymes · Fermentation · Fungi · Gene cluster ·

S. Z. Z. Cobongela (✉)
Advanced Materials Division, Nanotechnology Innovation Centre, Randburg, South Africa

Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Johannesburg, South Africa

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Microbes · Microorganism · Penicillin · Penicillium · Resistance · Soil bacteria · Streptomyces · Streptomycin

25.1 Introduction

The existence of microorganisms plays a huge role in the ecosystem. Though they are notorious for causing life-threatening infections, they have a wide range of environmental benefits. Their benefits range from oxygen production vital for humans to decomposition that yields nutrients in the environment for use by plants and animals. The list of microorganisms includes bacteria, fungi, algae, protozoa, and viruses. Microorganisms, especially bacteria, also form symbiotic relationships with plants and animals, with about thousands of bacteria existing in the human digestive system (Thursby and Juge 2017). In approximately 1.5 million identified bacteria, only less than a hundred are deemed pathogenic to humans. Bacterial diseases are classified as communicable as they can be transmitted from one source to another. Unlike viruses, bacteria and other microbes can reproduce on their own.

Bacterial infections contribute to major causes of death worldwide. Harmful infections caused by bacteria include tuberculosis (TB) which is one of the top ten causes of death globally (WHO 2018). TB is caused by a slow-growing *Mycobacterium tuberculosis* which mainly affects the lungs (Smith 2003). Other bacterial based infections include pneumonia caused by *Pseudomonas* species (sp.) (Hatchette et al. 2000; Fujisawa et al. 2001), meningitis caused by pneumococcal bacteria (McCormick and Molyneux 2011; Mook-Kanamori et al. 2011), food poisoning by *Shigella* sp. (Nygren et al. 2013), *Campylobacter* sp. (Epps et al. 2013), and *Salmonella* sp. (Hardy 2004), some sexually transmitted infections caused by *Neisseria gonorrhoeae* (Lenz and Dillard 2018), *Treponema pallidum* (Zinsser et al. 1916), and numerous others.

The pathophysiology of bacteria in the host largely lies around the production of endotoxins and exotoxins. These toxins in turn damage tissues and disturb homeostasis. During reproduction, bacteria use tissues as nutrients for growth and multiplication, incurring physical damage to tissues. Examples of bacteria notorious for causing infections consist of *Staphylococcus* sp., *Streptococcus* sp., and *E. coli*. Usually, the body's immune system fights off bacterial infections easily before they can cause any damage or illness. However, they can escape the immune system, generally accelerated by underlying immuno-compromising conditions. In such cases, treatment interventions are required with drugs classified as bactericidal and/or bacteriostatic that are chiefly known as antibiotics.

25.2 Antibiotics

Antibiotics are low molecular mass drugs/agents. About 85% of antibiotics are produced by actinomycetes, while 11% and 4% are produced by fungi and bacteria, respectively (Benedict 1953). They are primarily produced as secondary metabolites during reproduction. The metabolites have found use in various activities such as antiviral, anticancer, and antimicrobial agents. They are usually produced during the late log phase of reproduction and are non-essential for the growth of the producing microorganism. Antibiotics have found use in killing or restricting growth in both Gram-negative and Gram-positive different strains of pathogens.

Antibiotics induce cell death or inhibit growth upon interaction with bacterial cells. They are classified according to their mechanism of action by which they kill pathogens. The most popular modes of action are inhibiting bacterial cell wall biosynthesis, inhibiting bacterial protein synthesis, and interference with DNA replication and transcription. Other antibiotics focus on disrupting cell membranes, inhibition of folic acid metabolism, and inhibiting DNA replication and RNA synthesis. Aminoglycosides, macrolides, etc., are known to inhibit the biosynthesis of proteins that are essential for bacterial cell homeostasis thus causing cell death. The conventional mode of action for most antibiotics, such as glycopeptides, is to target the bacterial cell wall biosynthesis. Penicillin is an example of β-lactam antibiotics that kill bacteria by specifically inhibiting the transpeptidase that catalyzes the final step in cell wall biosynthesis and the cross-linking of peptidoglycan (Smanski et al. 2009). Table 25.1 contains a list of common classes of antibiotics naturally produced by microbes and their mode of action in killing pathogens (Waksman et al. 1946, 1949; Crawford et al. 1952; McGuire et al. 1952; Darken et al. 1960; Ahmed and Vining 1983; Birnbaum et al. 1985; Dhillon et al. 1989; Vilches et al. 1990; Balakrishnan and Pandey 1996; Chopra and Roberts 2001, 2001; Laich et al. 2002; Levine 2006; Koběrská et al. 2008; Niewerth et al. 2011; Borghi et al. 2014; Fernández-Martínez et al. 2014; Salvaggio et al. 2016; Khan 2017; Petković et al. 2017).

25.2.1 Soil Microorganisms and Antibiotic Production

To this day, the majority of the antibiotics currently used are mainly secondary metabolites produced by several microorganisms (Rolain et al. 2016). Most of these microbes' antagonists to disease-producing bacteria are from soil cultures. Antibiotics can now also be produced via semi-synthesis and/or chemically synthesis of analogues based on the natural structures from natural products. The soil provides a composite and diverse environment for antibiotic-producing microbes. In 1904, Frost (Waksman and Woodruff 1940) was among the very first researchers to produce a detailed study of the role of soil microorganisms in suppressing/destructing the development of pathogens. The study was motivated by a "disappearance" of pathogens when in the soil. Microbes, especially from the soil, produce antibiotics to kill or inhibit the growth of other microorganisms that compete with

Table 25.1 Commonly used antibiotic classes produced by microbes

Classes	Mode of action	Examples	Producing species	References
β-Lactams	Inhibits bacterial cell wall synthesis	Penicillin	*Penicillium* sp.	Balakrishnan and Pandey (1996); Laich et al. (2002); Pham et al. (2019)
		Cephalosporin	*Acremonium* sp. (preciously known as *Cephalosporium* sp.)	Crawford et al. (1952); Khan (2017)
β-Lactams (Carbapenems)	Inhibits bacterial cell wall synthesis	Thienamycin	*Streptomyces cattleya*	Birnbaum et al. (1985)
Tetracyclines	Inhibits protein synthesis	Chlortetracycline	*Streptomyces aureofaciens*	Darken et al. (1960); Chopra and Roberts (2001); Borghi et al. (2014)
		Oxytetracycline	*Streptomyces rimosus*	Chopra and Roberts (2001); Borghi et al. (2014); Petković et al. (2017)
Quinolones	Interferes with replication and transcription of bacterial DNA		*Pseudomonas* sp	Niewerth et al. (2011); Salvaggio et al. (2016)
Lincosamide	Inhibits bacterial protein synthesis	Lincomycin	*Streptomyces lincolnensis*	Kobĕrská et al. (2008)
Macrolides	Inhibits bacterial protein synthesis	Erythromycin	*Saccharopolyspora erythraea* (formerly known as *Streptomyces erythraeus*)	McGuire et al. (1952); Dhillon et al. (1989)
			Arthrobacter sp.	McGuire et al. (1952)
		Oleandomycin		

(continued)

Table 25.1 (continued)

Classes	Mode of action	Examples	Producing species	References
			Streptomyces antibioticus	Vilches et al. (1990)
Glycopeptides	Inhibits bacterial cell wall synthesis	Vancomycin	*Amycolatopsis orientalis* (formerly known as *Streptomyces orientalis*)	Levine (2006)
Aminoglycoside	Inhibits bacterial protein synthesis, leading to cell death	Streptomycin	*Streptomyces griseus*	Waksman et al. (1946)
		Neomycin	*Streptomyces fradiae*	Waksman et al. (1949)
Chloramphenicol	Inhibits bacterial protein synthesis, leading to stagnant growth	Chloramphenicol	*Streptomyces venezuelae*	Ahmed and Vining (1983); Fernández-Martínez et al. (2014)

them for food, water, and nutrients necessary for their growth. About hundreds of millions to one billion different bacterial species can be found in just a teaspoon of soil. Approximately 60% of available antibiotics drugs in the market are derived from soil microorganisms (Molinari 2009). Essentially, microbes produce antibiotics as their survival strategy. This strategy has been adopted as a major therapy to combat infectious diseases that are a threat to animals, plants, and mostly, humans.

25.3 The History of Antibiotics

It is widely known that penicillin was the first antibiotic discovered in 1928 by Sir Alexander Flemings (Fleming 1929) and it was only introduced into clinical practice in the 1930s (Tan and Tatsumura 2015; Rolain et al. 2016). Actually, in 1899 Emmerich and Low discovered pyocyanase (Caltrider 1967), which would now be called an antibiotic, previously thought to be an enzyme. Pyocyanase extracted from *Pseudomonas aeruginosa,* a Gram-negative bacterium, was active against several pathogenic bacteria. It was the first antibiotic drug to be used clinically to treat various diseases. Unfortunately, pyocyanase was abandoned due to inconsistent treatment and its preparation was quite toxic to humans (Aminov 2010). This strain also produced pyocyanase, a pigment possessing antimicrobial properties.

On the other hand, spore-forming *Bacillus* species have been investigated for their antimicrobial activities since 1907 by M. Nicolle (Nicolle 1907). They studied the antimicrobial activity of an enzyme-like substance produced by *Bacillus subtilis.* This was followed through work done by E. Pringsheim on *Bacillus mycoides* in1920. Around the same period (1923), a group led by Dr. Waksman (Waksman and Starkey 1923) at the Rutgers Agriculture School also studied soil microbiology focusing on antibiotics. They found out that the actinomycetes were killing most of the resident bacterial (Waksman 1937; Waksman and Foster 1937; Waksman and Hutchings 1937).

Actinomycetes bacteria are mostly Gram-positive and are known for their great contribution to the soil system for agricultural economic benefit. About 14 years later they discovered four antibiotics (actinomycin, streptothricin, fumigacin, and clavacin), which turned out to be toxic to animals (Woodruff 2014). Their research seemed to be futile until they observed the production of an antibiotic by *Streptomyces griseus* strain. The antibiotic was named streptomycin and it was the very first antibiotic from this group that was non-toxic to animals. In today's research, streptomycin is still used as a standard to measure against new antibiotic discoveries. In 1944 (Feldman and Hinshaw 1944), streptomycin was initially tested as an anti-tuberculosis in *Mycobacterium tuberculosis* infected guinea pigs and later used as the first successful anti-tuberculosis drug to ever been used in humans (Pfuetze and Pyle 1949). This discovery led to Dr. Waksman awarded a Nobel Prize in 1952 for Physiology or Medicine. He is also the inventor of the now popular term antibiotic, which was introduced to microbial literature in 1942.

Tyrothricin and gramicidin were the first antimicrobial peptides (AMP) isolated by R.J Dubos from *Bacillus brevis* in 1939 (Dubos 1939b, c). These peptides exhibited a bactericidal effect against a wide range of Gram-positive bacteria in both in vitro and in vivo and later successfully treated infected wounds in guinea-pig skin. They were also used to cure a staphylococcal infection, however, they are used as topical antibiotics because of their toxicity (Dubos 1939a). In recent studies, 2012, Tawiah and colleagues discovered antibiotic-producing microbes from water bodies (river, lake, and sea) (Tawiah et al. 2012). They isolated a variety of microbes including bacteria, actinomycetes, and fungi. The antibiotics from these microbes were active against several pathogenic bacteria such as *Enterococcus faecalis*, *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, etc. For the longest time, the majority of antibiotics discovered were mostly from Actinomycetes.

25.3.1 Antibiotics Produced by Actinomycetes

Actinomycetes belong to the Actinobacteria phylum. They are Gram-positive, mostly anaerobic, and filamentous bacteria behaving like fungi. They are found in nature and wildly distributed in soil, water, and the natural or man-made environment. Actinomycetes are known to produce a variety of bioactive secondary metabolites with high commercial value. *Actinomyces*, *Nocardia*, and *Streptomyces* are examples of strains under the Actinomycetes class. Actinomycetes have

Table 25.2 Actinomycetes produced antibiotics

Actinomycetes	Antibiotics	References
Streptomyces kanamyceticus	Kanamycin	Gao et al. (2017)
Amycolatopsis mediterranei	Rifamycin	Zhao et al. (2010); Verma et al. (2011)
Micromonospora purpurea and Micromonospora echinospora	Gentamycin	Weinstein et al. (1963); Chang et al. (2019)
Streptomyces roseosporus	Daptomycin	Li et al. (2013)
Steptomyces platensis	Platensimysin	Smanski et al. (2009)
	Platencin	
Kocuria	PM181104	Mahajan et al. (2013)

produced a wide range of antibiotics in the nineteenth century which include streptothricin, tetracyclines, erythromycin, etc. (Mahajan and Balachandran 2012; Venkataswamy 2018). They have continued to strive even in the twentieth century to produce some successful antibiotics such as daptomycin, thienamycin, epirubicin, and others (Venkataswamy 2018). *Streptomyces* sp. is the most significant genus of Actinomycetes, producing about two-thirds of antibiotics including the well-known streptomycin antibiotic (Waksman et al. 1946). Chloramphenicol and neomycin are examples of other clinically used and naturally produced Streptomyces antibiotics. Table 25.1 also shows most of the clinically used antibiotics are from the *Streptomyces* sp. Table 25.2 lists some of the discovered antibiotics from Actinomycetes (Weinstein et al. 1963; Smanski et al. 2009; Zhao et al. 2010; Li et al. 2013; Mahajan et al. 2013; Gao et al. 2017; Chang et al. 2019). Bacillus, like Actinomycetes, has also been intensely researched with several novel antibiotics produced.

25.3.2 Antibiotics Produced by *Bacillus* Species

Soil is rich in a variety of microorganisms that can be classified according to their shapes as cocci, spirilli, and bacilli. Amongst other genus, *Bacillus* species (spp.) are the most abundant strains found in the soil and can produce a variety of antibiotics (Hussein and AL-Janabi 2006). It is a bacterium belonging to the domain of Eubacteria. *Bacillus* spp. are spore-producing, rod-shaped Gram-positive bacteria with aerobic or facultative anaerobic respiration. They can survive for long under harsh conditions in the soil because of their ability to form spores and antimicrobial compounds. For this reason, they have been employed in food bio-preservation and crop protection. For instance, *Bacillus subtilis* (*B. subtilis*) produced an antimicrobial substance that was found to be effective on several pathogenic and bacteria that are notorious for food spoilage such as *Listeria monocytogenes*, *Salmonella enteritidis*, and methicillin-resistant *Staphylococcus* sp. (Tabbene et al. 2009).

Bacillus spp. produce the most biologically active secondary metabolites (Lisboa et al. 2006) with pharmaceutical and biotechnological importance (Hassan et al. 2014). Table 25.3 lists some of the *Bacillus* producing antibiotics (Dubos and

Table 25.3 *Bacillus* produced antibiotics

Species	Antibiotics	References
B. cereus	Zwittermicin	Stabb et al. (1994)
	Cerexin	Shoji et al. (1975)
B. brevis	Tyrothricin	Dubos and Hotchkiss (1941); Okuda et al. (1963)
	Gramicidin	Okuda et al. (1963); Vandamme and Demain (1976)
B. circulans	Circulin	Murray et al. (1949)
B. Licheniformis	Bacitracin	Bernlohr and Novelli (1960), (Haavik 1974)
B. Laterosporus	Laterosporin	Barnes (1949)
B. Polymyxa	Polymyxin	Gupta et al. (2009); Poirel et al. (2017)
	Colistin	Gupta et al. (2009)
B. subtilis	Bacitracin	Johnson et al. (1945)
	Polymyxin	Park et al. (2012)
	Difficidin	Wu et al. (2015)
	Bacilysin	Wu et al. (2015)
	Subtilin	Klein and Entian (1994); Bongers et al. (2005)
	Mycobacillin	Majumdar and Bose (1958)
B. pumilus	Pumilin	Bhate (1955)

Hotchkiss 1941; Johnson et al. 1945; Barnes 1949; Murray et al. 1949; Bhate 1955; Majumdar and Bose 1958; Bernlohr and Novelli 1960; Okuda et al. 1963; Haavik 1974; Shoji et al. 1975; Vandamme and Demain 1976; Klein and Entian 1994; Stabb et al. 1994; Bongers et al. 2005; Gupta et al. 2009; Park et al. 2012; Wu et al. 2015; Poirel et al. 2017). The *Bacillus* sp. produces a wide variety of antibiotics that are most active against Gram-positive pathogens (Ming and Epperson 2002). In addition, *Bacillus* sp. mostly produce soluble antibiotics that are cheaper, more effective, and for that reason, they are preferable for commercial production.

25.3.3 Antibiotics Produced by Fungi

It is assumed that on earth there are about 1.5 million fungi species with about 95% of these not yet discovered (Dictionary of the Fungi 2020). Fungi produce the most structurally diverse metabolites used in pharmaceuticals. They are one of the microbes serving as a source for the best antibiotics available in the markets to date. Like soil bacteria, fungi also produce antibiotics to compete against a variety of soil microbiota. About 20% of fungi produced antibiotics are from soil fungi (Bérdy 2005). Fungi produced what is claimed to be the very first antibiotic, penicillin. Penicillin was initially discovered in a *Penicillium* (*P*) mold. Scientists have identified some of the *Penicillium* sp. to be *P. chrysogenum*, *P. nalgiovense* (Laich et al. 2002), *P. notatum* (Pham et al. 2019). Later, the higher-yielding *Penicillium* sp. is *P. chrysogenum* (Balakrishnan and Pandey 1996).

Besides penicillin, fungi produce cephalosporin antibiotics. They are also β-lactam antibiotics like penicillin and have a similar mode of action. Cephalosporin

Table 25.4 Fungi produced antibiotics

Species	Antibiotics	References
Penicillium griseofulvum	Patulin	Torres et al. (1987); Banani et al. (2016)
	Griseofulvin	
Aspergillus fumigatus	Aspergillin	Soltys (1944)
	Fumagillin	Hanson and Eble (1949)
Aspergillus Niger	Rubrofusarin	Song et al. (2004)
Aspergillus awamori	Emodin	Chang et al. (2010); Ismaiel et al. (2016)
Aspergillus sp.	Xanthoascin	Zhang et al. (2015)
Mucor ramannianus	Ramycin	Van Dijck and De Somer (1958)
Psalliota campestris	Campestrin	Bose (1955)

is a broad-spectrum antibiotic produced by *Acremonium chrysogenum* also known as *Cephalosporium acremonium* (Demain and Zhang 1998; Khan 2017). *Acremonium chrysogenum* is abundant in soil matrices. Especially in a humid environment. Cephalosporin acts by inhibiting bacterial cell wall synthesis. Some of the fungi produced antibiotics are listed in Table 25.4 most of which are from *Aspergillus* sp. (Soltys 1944; Hanson and Eble 1949; Bose 1955; Van Dijck and De Somer 1958; Torres et al. 1987; Song et al. 2004; Chang et al. 2010; Zhang et al. 2015; Banani et al. 2016; Ismaiel et al. 2016).

25.4 Biochemical and Genetic Aspects of Antibiotic Production

In a screening of antibiotic-producing microbes, highly selective procedures are employed in detecting and isolating microorganisms of interest from a large pool of other microorganisms. The survival of microbes is largely dependent on environmental factors such as nutrient availability, temperature, moisture content, etc. It is also vital to be cognizant of these factors when growing the selected microbes. Improvement of antibiotic production is enhanced by the advances in microbial molecular genetics. One of the technologies involved in the modification of genes includes mutagenesis aided by ultraviolet radiation, x-rays, and mutagenic chemicals. The use of resistant mutation is another approach used to improve the microbial production of antibiotics (Cundliffe and Demain 2010).

The DNA of antibiotic-producing microbes is clustered with genes encoding for enzymes involved in antibiotic biosynthesis. They also encode genes that express resistance to the antibiotic they produce to avoid antibiotic autotoxicity. The expression of the resistant gene should be linked to the expression of the gene encoding the antibiotic production. In most cases, the resistant gene is activated by the presence of the antibiotic or the presence of transformation compounds involved in antibiotic biosynthesis. Sometimes, the resistant gene is expressed regardless of the antibiotic-related gene is being expressed. For example, the erythromycin-resistant gene is expressed in the absence of erythromycin gene expression (Bibb et al. 1985). The resistance and defense mechanism are through various strategies, i.e. modification of drug receptors, metabolic shielding to prevent drug target reaction, etc. (Cundliffe and Demain 2010). Additionally, antibiotic-producing microbes also produce antibiotic inactivation enzymes.

The enzymes involved in some antibiotic biosynthesis include and are not limited to *N*-acetyl transferases, *O*-phosphotransferases, and *O*-adenyltransferases (Peterson and Kaur 2018). A well-studied streptomycin-producing strain *Streptomyces griseus* co-produces a modification enzyme. This modification enzyme is streptomycin-6-phosphotransferase which converts active streptomycin to inactive streptomycin-6-phosphate (Shinkawa et al. 1985). Streptomycin is produced by the bacteria A-factor signaling cascade secreted by the γ-butyrolactone signaling molecule (Horinouchi 2002). It binds to a member of the TetR-family of repressors, the Arp protein to release adpA, a target promoter (Ohnishi et al. 2005; Cuthbertson and Nodwell 2013). The adpA being the main secondary metabolite regulator together with the str gene cluster which is specific to the biosynthesis of streptomycin (Ohnishi et al. 2005). On the str gen cluster, adpA targets the aphD promoter on the strR-aphD operon. This results in activation of the biosynthetic gene and streptomycin resistant gene by the expression of a transcription factor StrR and AphD, respectively (Vujaklija et al. 1991, 1993). The AphD gene encodes for the inactivation enzyme streptomycin-6-phosphotransferase. The inactive phosphorylated streptomycin can be reactivated by removing the phosphate group using StrK phosphatase (Mansouri and Piepersberg 1991; Beyer et al. 1996).

The mechanism of antibiotic is complex and tightly linked to the resistant gene. Antibiotic biosynthesis in nature does not occur at the same time and this may lead to inter-strain toxicity. However, non-producing cells within the same strain get resistance via cell-cell signaling. Another important and beneficial technique used by antibiotic-producing microbes is antibiotic efflux. Microbes use this technique to pump the antibiotic out of the cell to decrease intracellular toxicity while inducing toxicity to the neighboring pathogens. Unlike the inactivation technique, efflux is useful in the industrial production of antibodies. The inactivation technique requires activation of the antibiotic before use while the efflux technique readily excretes the active antibiotic.

25.5 Application of Microbes in Industrial Antibiotic Production

Due to the high demand and importance of antibiotics, industrial microbiology came up with ways to increase production. Gene amplification is a technology employed in the overexpression of genes. The amplification process multiplies these genes and inserts them back to the microorganisms using vectors such as phage and plasmid. It involves the amplification of the gene coding for enzymes involved in antibiotic production. In research and development, antibiotic-producing microbes are grown in petri dishes and tubes which can accommodate only less than 100 ml of growth medium. Industrially, the source microorganisms are grown in containers with more than 100,000 L of growth medium. The large-scale production is achieved by a process called fermentation. Oftentimes, microbial strains used in fermentation are genetically modified to increase antibiotic yields. High yielding strains are a prerequisite in antibiotic production, hence the constant strain improvement for better production.

25.5.1 Fermentation

The process of fermentation involves isolation of the desired source microorganism and while maintaining sterile conditions to avoid any form of contamination by other microbes. Glycerol yeast extract media and Saboraud dextrose agar medium are used for the isolation of bacteria and fungi, respectively. The optimum production depends on oxygen concentration, temperature, pH, nutrients, and controlling population size on the growth media. This is often done through a conventional operation such as batch, fed-batch, or continuous culture fermentations. Here, the reproducing microorganisms are in a solution and submerged in the media they are grown in. This requires intense downstream processing.

Fermentation post-production of antibiotics includes vital steps to extract and purify the desired by-products. Processes such as crystallization, ion exchange, adsorption, and chemical precipitation are employed. These are usually time consuming and expensive. To mitigate this problem, scientists have come up with solid-state fermentation (Robinson et al. 2001). The principles of these techniques are the same. However, in solid-state fermentation, the microorganisms are immobilized on the surface of the fermenting reactor. This helps with improving the downstream processes and purification. Also, solid-state formation improves the stability of the desired product. In most cases, the desired antibiotics are obtained through the aforementioned techniques. Sometimes, antibiotics are altered to maximize their activity. For example, penicillin is produced by fermentation, and the addition of functional groups such as the amino group and two methoxy groups further produces two antibiotics, namely ampicillin (Kawamori et al. 1983) and methicillin (Stapleton and Taylor 2002), respectively. The resultant antibiotics have a broader spectrum than penicillin and are of great use in pathogens that are resistant to penicillin.

25.6 Future Aspects and Recent Advancement

The discovery of new antibiotics was at a peak from this era to the late 1960s. It became harder to unearth new and effective antibiotic drugs due to the development of resistant pathogens. The emergence of new pathogens and the rise in pathogen resistance to current antibiotics have increased the demand for new and effective antibiotics. The new pathogen strains cause life-threatening diseases that may become a major public health concern. Scientific technology advancements over the years have led to conditions yielding better antibiotic production. In the stagnant era on the antibiotic discovery, developments in technologies especially genomic sequencing played a huge role in improving the existing antibiotic production. Genomic engineering and direct cloning in a study done by Du and colleagues

(2015) have already shown promising results in improving antibiotic production. Pharmaceutical companies and research institutes have done very little research and developments of new drug discovery due to the high costs associated with this field. This could cause a major fallout in the public health sector worldwide as some of the disease-causing bacteria are continuously mutating and becoming resistant to the existing antibiotics. This enforces the field of drug discovery, especially new antibiotic discovery and development to be one of the ongoing and unceasing research.

25.7 Conclusion

Microbes have been known to cause major infectious diseases for over a century. However, they have played a pivotal role in antibiotic discovery which in turn has saved millions of lives globally. Research has proven that the toxicity and adverse side effects of naturally produced antibiotics in humans have been a bottleneck in coming up with effective antibiotic drugs. The concern has also been about natural intrinsic or acquired resistance besides the use and misuse/abuse of antibiotics are continuing to be a contributing factor to the rising antibiotic resistance of microorganisms. This observation has led to genetic manipulation of the microbes producing antibiotics. Concurrently, manipulation of growth media improves the production of these antibiotics. Both the modification of antibiotic structure and manipulation of growth media have led to improved activity with fewer side effects while increasing production. It is also observed that antibiotic-producing microbes are resistant to the antibiotics they produce and other broad-spectrum antibiotics such as tetracycline, chloramphenicol, streptomycin, etc.

To this day, there is still ongoing research on new antimicrobial production by soil microbes (Armalytė et al. 2019; Cycoń et al. 2019). However, out of about 5000 antibiotics identified, approximately 100 antibiotics were potent and some lost potency due to pathogens becoming resistant to these antibiotics. To solve this problem, old antibiotics and antibiotic-producing antibiotics are being manipulated and chemically modified to create new and effective analogues. Also, screening of new antibiotics, selection of new and rare antibiotic-producing microorganisms are still a need.

References

Ahmed ZU, Vining LC (1983) Evidence for a chromosomal location of the genes coding for chloramphenicol production in Streptomyces venezuelae. J Bacteriol 154:239–244. https://doi.org/10.1128/JB.154.1.239-244.1983

Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbiol:1. https://doi.org/10.3389/fmicb.2010.00134

Armalytė J, Skerniškytė J, Bakienė E, Krasauskas R, Šiugždinienė R, Kareivienė V, Kerzienė S, Klimienė I et al (2019) Microbial Diversity and antimicrobial resistance profile in microbiota from soils of conventional and organic farming systems. Front Microbiol 10. https://doi.org/10.3389/fmicb.2019.00892

Balakrishnan K, Pandey A (1996) Production of biologically active secondary metabolites in solid state fermentation. J Sci Indust Res 55:365–372

Banani H, Marcet-Houben M, Ballester A-R, Abbruscato P, González-Candelas L, Gabaldón T, Spadaro D (2016) Genome sequencing and secondary metabolism of the postharvest pathogen Penicillium griseofulvum. BMC Genomics 17. https://doi.org/10.1186/s12864-015-2347-x

Barnes EM (1949) Laterosporin a and Laterosporin B antibiotics produced by B. laterosporus. Br J Exp Pathol 30:100–104

Benedict RG (1953) Antibiotics produced by actinomycetes. Botan Rev 19:229–320

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26. https://doi.org/10.1038/ja.2005.1

Bernlohr RW, Novelli GD (1960) Some characteristics of bacitracin production by Bacillus licheniformis. Arch Biochem Biophys 87:232–238. https://doi.org/10.1016/0003-9861(60)90166-1

Beyer S, Distler J, Piepersberg W (1996) Thestr gene cluster for the biosynthesis of 5-′-hydroxystreptomycin inStreptomyces glaucescens GLA.0 (ETH 22794): new operons and evidence for pathway-specific regulation by StrR. Mol Gen Genet MGG 250:775–784. https://doi.org/10.1007/BF02172990

Bhate DS (1955) Pumilin, a new antibiotic from *Bacillus pumilus*. Nature 175:816–817. https://doi.org/10.1038/175816a0

Bibb MJ, Janssen GR, Ward JM (1985) Cloning and analysis of the promoter region of the erythromycin resistance gene (ermE) of Streptomyces erythraeus. Gene 38:215–226. https://doi.org/10.1016/0378-1119(85)90220-3

Birnbaum J, Kahan FM, Kropp H, Macdonald JS (1985) Carbapenems, a new class of beta-lactam antibiotics: discovery and development of imipenem/cilastatin. Am J Med 78:3–21. https://doi.org/10.1016/0002-9343(85)90097-X

Bongers RS, Veening J-W, Van Wieringen M, Kuipers OP, Kleerebezem M (2005) Development and characterization of a Subtilin-regulated expression system in Bacillus subtilis: strict control of gene expression by addition of Subtilin. Appl Environ Microbiol 71:8818–8824. https://doi.org/10.1128/AEM.71.12.8818-8824.2005

Borghi AA, Palma MSA, Borghi AA, Palma MSA (2014) Tetracycline: production, waste treatment and environmental impact assessment. Braz J Pharm Sci 50:25–40. https://doi.org/10.1590/S1984-82502011000100003

Bose SR (1955) Campestrin, the antibiotic of Psalliota campestris. Nature 175:468–468. https://doi.org/10.1038/175468a0

Caltrider PG (1967) Pyocyanine. In: Gottlieb D, Shaw PD (eds) Antibiotics: volume I mechanism of action. Springer, Berlin, pp 117–121. https://doi.org/10.1007/978-3-662-38439-8_7

Chang M, Wang J, Tian F, Zhang Q, Ye B (2010) Antibacterial activity of secondary metabolites from Aspergillus awamori F12 isolated from rhizospheric soil of Rhizophora stylosa Griff. Acta Microbiologica Sinica 50:1385–1391

Chang Y, Chai B, Ding Y, He M, Zheng L, Teng Y, Deng Z, Yu Y et al (2019) Overproduction of gentamicin B in industrial strain Micromonospora echinospora CCTCC M 2018898 by cloning of the missing genes genR and genS. Metab Eng Commun 9:e00096. https://doi.org/10.1016/j.mec.2019.e00096

Chopra I, Roberts M (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 65:232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001

Crawford K, Heatley NG, Boyd PF, Hale CW, Kelly BK, Miller GA, Smith N (1952) Antibiotic production by a species of Cephalosporium. J Gen Microbiol 6:47–59. https://doi.org/10.1099/00221287-6-1-2-47

Cundliffe E, Demain AL (2010) Avoidance of suicide in antibiotic-producing microbes. J Ind Microbiol Biotechnol 37:643–672. https://doi.org/10.1007/s10295-010-0721-x

Cuthbertson L, Nodwell JR (2013) The TetR family of regulators. Microbiol Mol Biol Rev 77:440–475. https://doi.org/10.1128/MMBR.00018-13

Cycoń M, Mrozik A, Piotrowska-Seget Z (2019) Antibiotics in the soil environment—degradation and their impact on microbial activity and Diversity. Front Microbiol 10:12. https://doi.org/10.3389/fmicb.2019.00338

Darken MA, Berenson H, Shirk RJ, Sjolander NO (1960) Production of tetracycline by Streptomyces aureofaciens in synthetic media. Appl Microbiol 8:46–51

Demain AL, Zhang J (1998) Cephalosporin C production by Cephalosporium acremonium: the methionine story. Crit Rev Biotechnol 18:283–294. https://doi.org/10.1080/0738-859891224176

Dhillon N, Hale RS, Cortes J, Leadlay PF (1989) Molecular characterization of a gene from Saccharopolyspora erythraea (Streptomyces erythraeus) which is involved in erythromycin biosynthesis. Mol Microbiol 3:1405–1414. https://doi.org/10.1111/j.1365-2958.1989.tb00123.x

Dictionary of the Fungi (2020). *CABI.org*. https://www.cabi.org/bookshop/book/9781845939335/. Accessed July 16

Du D, Wang L, Tian Y, Liu H, Tan H, Niu G (2015) Genome engineering and direct cloning of antibiotic gene clusters via phage ϕBT1 integrase-mediated site-specific recombination in Streptomyces. Sci Rep 5:8740. https://doi.org/10.1038/srep08740

Dubos RJ (1939a) Bactericidal effect of an extract of a soil Bacillus on gram positive cocci. Proc Soc Exp Biol Med 40:311–312. https://doi.org/10.3181/00379727-40-10395P

Dubos RJ (1939b) Studies on a bactericidal agent extracted from a soil BACILLUS. J Exp Med 70:1–10

Dubos RJ (1939c) Studies on a bactericidal agent extracted from a soil BACILLUS. J Exp Med 70:11–17

Dubos RJ, Hotchkiss RD (1941) The production of bactericidal substances by aerobic sporulating Bacilli. J Exp Med 73:629–640

Epps SVR, Harvey RB, Hume ME, Phillips TD, Anderson RC, Nisbet DJ (2013) Foodborne campylobacter: infections, metabolism, pathogenesis and reservoirs. Int J Environ Res Public Health 10:6292–6304. https://doi.org/10.3390/ijerph10126292

Feldman WH, Hinshaw HC (1944) Effects of streptomycin on experimental tuberculosis in Guinea pigs. Proc Staff Meet 19:593–599

Fernández-Martínez LT, Borsetto C, Gomez-Escribano JP, Bibb MJ, Al-Bassam MM, Chandra G, Bibb MJ (2014) New insights into chloramphenicol biosynthesis in Streptomyces venezuelae ATCC 10712. Antimicrob Agents Chemother 58:7441–7450. https://doi.org/10.1128/AAC.04272-14

Fleming A (1929) On the antibacterial action of cultures of a Penicillium, with special reference to their use in the isolation of B. influenzæ. Br J Exp Pathol 10:226–236

Fujisawa M, Hirai H, Nishida T (2001) Degradation of polyethylene and Nylon-66 by the laccase-mediator system 9: 6

Gao W, Wu Z, Sun J, Ni X, Xia H (2017) Modulation of kanamycin B and kanamycin a biosynthesis in Streptomyces kanamyceticus via metabolic engineering. PLoS One 12. https://doi.org/10.1371/journal.pone.0181971

Gupta S, Govil D, Kakar PN, Prakash O, Arora D, Das S, Govil P, Malhotra A (2009) Colistin and polymyxin B: a re-emergence. Ind J Crit Care Med 13:49–53. https://doi.org/10.4103/0972-5229.56048

Haavik HI (1974) Studies on the formation of bacitracin by Bacillus licheniformis: effect of glucose. Microbiology 81:383–390. https://doi.org/10.1099/00221287-81-2-383

Hanson FR, Eble TE (1949) An Antiphage agent isolated from Aspergillus SP. J Bacteriol 58:527–529

Hardy A (2004) Salmonella: a continuing problem. Postgraduate Med J 80:541–545. https://doi.org/10.1136/pgmj.2003.016584

Hassan SA, Hanif E, Zohra RR (2014) Isolation and screening of soil bacteria for potential antimicrobial activity. FUUAST J Biol 4:217–219

Hatchette TF, Gupta R, Marrie TJ (2000) *Pseudomonas aeruginosa* community-acquired pneumonia in previously healthy adults: case report and review of the literature. Clin Infect Dis 31:1349–1356. https://doi.org/10.1086/317486

Horinouchi S (2002) A microbial hormone, A-factor, as a master switch for morphological differentiation and secondary metabolism in Streptomyces griseus. Front Biosci 7:d2045–d2057

Hussein AA, AL-Janabi S (2006) Identification of bacitracin produced by local isolate of *Bacillus licheniformis*. Afr J Biotechnol:5. https://doi.org/10.4314/ajb.v5i18.55802

Ismaiel AA, Rabie GH, El-Aal MAA (2016) Antimicrobial and morphogenic effects of emodin produced by a spergillus awamori WAIR120. De Gruyter, Biologia

Johnson BA, Anker H, Meleney FL (1945) Bacitracin: a new antibiotic produced by a member of the B. Subtilis Group. Science 102:376–377. https://doi.org/10.1126/science.102.2650.376

Kawamori M, Hashimoto Y, Katsumata R, Okachi R, Takayama K (1983) Enzymatic production of amoxicillin by β-lactamase-deficient mutants of *Pseudomonas melanogenum* KY 3987. Agric Biol Chem 47:2503–2509. https://doi.org/10.1080/00021369.1983.10865984

Khan NT (2017) Cephalosporin C production from Acremonium chrysogenum. Enzyme Engineering 06. https://doi.org/10.4172/2329-6674.1000159

Klein C, Entian KD (1994) Genes involved in self-protection against the lantibiotic subtilin produced by Bacillus subtilis ATCC 6633. Appl Environ Microbiol 60:2793–2801. https://doi.org/10.1128/AEM.60.8.2793-2801.1994

Kobĕrská M, Kopecký J, Olšovská J, Jelínková M, Ulanova D, Man P, Flieger M, Janata J (2008) Sequence analysis and heterologous expression of the lincomycin biosynthetic cluster of the type strain Streptomyces lincolnensis ATCC 25466. Folia Microbiol 53:395–401. https://doi.org/10.1007/s12223-008-0060-8

Laich F, Fierro F, Martín JF (2002) Production of penicillin by Fungi growing on food products: identification of a complete penicillin gene cluster in Penicillium griseofulvum and a truncated cluster in Penicillium verrucosum. Appl Environ Microbiol 68:1211–1219. https://doi.org/10.1128/AEM.68.3.1211-1219.2002

Lenz JD, Dillard JP (2018) Pathogenesis of Neisseria gonorrhoeae and the host defense in ascending infections of human fallopian tube. *Frontiers in Immunology* 9. Frontiers. https://doi.org/10.3389/fimmu.2018.02710

Levine DP (2006) Vancomycin: a history. Clin Infect Dis 42:S5–S12. https://doi.org/10.1086/491709

Li L, Ma T, Liu Q, Huang Y, Hu C, Liao G (2013) Improvement of daptomycin production in streptomyces roseosporus through the acquisition of pleuromutilin resistance. BioMed Res Int 2013. https://doi.org/10.1155/2013/479742

Lisboa MP, Bonatto D, Bizani D, Henriques JAP, Brandelli A (2006) Characterization of a bacteriocin-like substance produced by *Bacillus amyloliquefaciens* isolated from the Brazilian Atlantic forest. Int Microbiol 9:111–118

Mahajan GB, Balachandran L (2012) Antibacterial agents from actinomycetes - a review. Front Biosci. https://doi.org/10.2741/e373

Mahajan G, Thomas B, Parab R, Patel ZE, Kuldharan S, Yemparala V, Mishra PD, Ranadive P et al (2013) In vitro and in vivo activities of antibiotic PM181104. Antimicrob Agents Chemother 57:5315–5319. https://doi.org/10.1128/AAC.01059-13

Majumdar SK, Bose SK (1958) Mycobacillin, a new antifungal antibiotic produced by B. subtilis. Nature 181:134–135. https://doi.org/10.1038/181134a0

Mansouri K, Piepersberg W (1991) Genetics of streptomycin production in Streptomyces griseus: nucleotide sequence of five genes, strFGHIK, including a phosphatase gene. Mol Gen Genet 228:459–469. https://doi.org/10.1007/BF00260640

McCormick DW, Molyneux EM (2011) Bacterial meningitis and *Haemophilus influenzae* type b conjugate vaccine, Malawi. Emerg Infect Dis 17:688–690. https://doi.org/10.3201/eid1704.101045

McGuire JM, Bunch RL, Anderson RC, Boaz HE, Flynn EH, Powell HM, Smith JW (1952) Ilotycin, a new antibiotic. Antibiot Chemother 2:281–283

Ming L-J, Epperson JD (2002) Metal binding and structure–activity relationship of the metalloantibiotic peptide bacitracin. J Inorg Biochem 91:46–58. https://doi.org/10.1016/S0162-0134(02)00464-6
Molinari G (2009) Natural products in drug discovery: present status and perspectives. Adv Exp Med Biol 655:13–27. https://doi.org/10.1007/978-1-4419-1132-2_2
Mook-Kanamori BB, Geldhoff M, van der Poll T, van de Beek D (2011) Pathogenesis and pathophysiology of pneumococcal meningitis. Clin Microbiol Rev 24:557–591. https://doi.org/10.1128/CMR.00008-11
Murray FJ, Tetrault PA, Kaufmann OW, Koffler H, Peterson DH, Colingsworth DR (1949) Circulin an antibiotic from an organism resembling Bacillus Circulans12. J Bacteriol 57:305–312. https://doi.org/10.1128/JB.57.3.305-312.1949
Nicolle M (1907) Action du "Bacillus Studies on the role of plakin. Med. J. subtilis" sur diverses bacttries. Ann Inst Osaka Univ 3:293–311
Niewerth H, Bergander K, Chhabra SR, Williams P, Fetzner S (2011) Synthesis and biotransformation of 2-alkyl-4(1H)-quinolones by recombinant Pseudomonas putida KT2440. Appl Microbiol Biotechnol 91:1399–1408. https://doi.org/10.1007/s00253-011-3378-0
Nygren BL, Schilling KA, Blanton EM, Silk BJ, Cole DJ, Mintz ED (2013) Foodborne outbreaks of shigellosis in the USA, 1998–2008. Epidemiol Infect 141:233–241. https://doi.org/10.1017/S0950268812000222
Ohnishi Y, Yamazaki H, Kato J-Y, Tomono A, Horinouchi S (2005) AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in Streptomyces griseus. Biosci Biotechnol Biochem 69:431–439. https://doi.org/10.1271/bbb.69.431
Okuda K, Edwards GC, Winnick T (1963) Biosynthesis of gramicidin and tyrocidine in the dubos strain of Bacillus Brevis I. Experiments with growing cultures. J Bacteriol 85:10
Park S-Y, Choi S-K, Kim J, Oh T-K, Park S-H (2012) Efficient production of Polymyxin in the surrogate host Bacillus subtilis by introducing a foreign ectB gene and Disrupting the abrB gene. Appl Environ Microbiol 78:4194–4199. https://doi.org/10.1128/AEM.07912-11
Peterson E, Kaur P (2018) Antibiotic resistance mechanisms in Bacteria: relationships between resistance determinants of antibiotic producers, environmental Bacteria, and clinical pathogens. Front Microbiol 9. https://doi.org/10.3389/fmicb.2018.02928
Petković, H., T. Lukežič, and J. Šušković. 2017. Biosynthesis of Oxytetracycline by Streptomyces rimosus: past, present and future directions in the development of tetracycline antibiotics. Food Technol Biotechnol 55: 3–13. doi:https://doi.org/10.17113/ftb.55.01.17.4617
Pfuetze KH, Pyle MM (1949) Streptomycin in the treatment of tuberculosis. J Am Med Assoc 139:634–639. https://doi.org/10.1001/jama.1949.02900270018005
Pham JV, Yilma MA, Feliz A, Majid MT, Maffetone N, Walker JR, Kim E, Cho HJ et al (2019) A review of the microbial production of bioactive natural products and biologics. Front Microbiol 10:12. https://doi.org/10.3389/fmicb.2019.01404
Poirel L, Jayol A, Nordmann P (2017) Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 30:557–596. https://doi.org/10.1128/CMR.00064-16
Robinson T, Singh D, Nigam P (2001) Solid-state fermentation: a promising microbial technology for secondary metabolite production. Appl Microbiol Biotechnol 55:284–289. https://doi.org/10.1007/s002530000565
Rolain J-M, Abat C, Jimeno M-T, Fournier P-E, Raoult D (2016) Do we need new antibiotics? Clin Microbiol Infect 22:408–415. https://doi.org/10.1016/j.cmi.2016.03.012
Salvaggio F, Hodgkinson JT, Carro L, Geddis SM, Galloway WRJD, Welch M, Spring DR (2016) The synthesis of quinolone natural products from Pseudonocardia sp. Eur J Org Chem 2016:434–437. https://doi.org/10.1002/ejoc.201501400
Shinkawa H, Sugiyama M, Nimi O, Nomi R (1985) Molecular cloning and expression in Streptomyces lividans of a streptomycin 6-phosphotransferase gene from a streptomycin-producing microorganism. FEBS Lett 181:385–389. https://doi.org/10.1016/0014-5793(85)80298-2

Shoji J, Hinoo H, Wakisaka Y, Koizumi K, Mayama M (1975) Isolation of two new related peptide antibiotics, cerexins a and B (studies on antibiotics from the genus Bacillus. I). J Antibiot 28:56–59. https://doi.org/10.7164/antibiotics.28.56

Smanski MJ, Peterson RM, Rajski SR, Shen B (2009) Engineered streptomyces platensis strains that overproduce antibiotics platensimycin and platencin. Antimicrob Agents Chemother 53:1299–1304. https://doi.org/10.1128/AAC.01358-08

Smith I (2003) *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. Clin Microbiol Rev 16:463–496. https://doi.org/10.1128/CMR.16.3.463-496.2003

Soltys MA (1944) Antibiotic action of Aspergillus fumigatus against *Mycobacterium tuberculosis*. Nature 154:550–551. https://doi.org/10.1038/154550b0

Song YC, Li H, Ye YH, Shan CY, Yang YM, Tan RX (2004) Endophytic naphthopyrone metabolites are co-inhibitors of xanthine oxidase, SW1116 cell and some microbial growths. FEMS Microbiol Lett 241:67–72. https://doi.org/10.1016/j.femsle.2004.10.005

Stabb EV, Jacobson LM, Handelsman J (1994) Zwittermicin A-producing strains of Bacillus cereus from diverse soils. Appl Environ Microbiol 60:4404

Stapleton PD, Taylor PW (2002) Methicillin resistance in Staphylococcus aureus. Sci Prog 85:57–72

Tabbene O, Slimene IB, Djebali K, Mangoni M-L, Urdaci M-C, Limam F (2009) Optimization of medium composition for the production of antimicrobial activity by Bacillus subtilis B38. Biotechnol Prog 25:1267–1274. https://doi.org/10.1002/btpr.202

Tan SY, Tatsumura Y (2015) Alexander Fleming (1881–1955): discoverer of penicillin. Singapore Med J 56:366–367. https://doi.org/10.11622/smedj.2015105

Tawiah AA, Gbedema SY, Adu F, Boamah VE, Annan K (2012) Antibiotic producing microorganisms from river Wiwi, Lake Bosomtwe and the Gulf of Guinea at Doakor Sea beach, Ghana. BMC Microbiol 12:234. https://doi.org/10.1186/1471-2180-12-234

Thursby E, Juge N (2017) Introduction to the human gut microbiota. Biochem J 474:1823–1836. https://doi.org/10.1042/BCJ20160510

Torres M, Canela R, Riba M, Sanchis V (1987) Production of patulin and griseofulvin by a strain of Penicillium griseofulvum in three different media. Mycopathologia 99:85–89. https://doi.org/10.1007/BF00436910

Van Dijck PJ, De Somer P (1958) Ramycin: a new antibiotic. Microbiology 18:377–381. https://doi.org/10.1099/00221287-18-2-377

Vandamme EJ, Demain AL (1976) Nutrition of Bacillus brevis ATCC 9999, the producer of gramicidin S1. Antimicrob Agents Chemother 10:265–273

Venkataswamy M (2018) Development of pharmaceutical drugs from microbial diversity. Unpublished. doi:https://doi.org/10.13140/RG.2.2.12456.67843

Vilches C, Mendez C, Hardisson C, Salas JA (1990) Biosynthesis of oleandomycin by Streptomyces antibioticus: influence of nutritional conditions and development of resistance. J Gen Microbiol 136:1447–1454. https://doi.org/10.1099/00221287-136-8-1447

Vujaklija D, Ueda K, Hong S-K, Beppu T, Horinouchi S (1991) Identification of an A-factor-dependent promoter in the streptomycin biosynthetic gene cluster of Streptomyces griseus. Mol Gen Genet MGG 229:119–128. https://doi.org/10.1007/BF00264220

Vujaklija D, Horinouchi S, Beppu T (1993) Detection of an A-factor-responsive protein that binds to the upstream activation sequence of strR, a regulatory gene for streptomycin biosynthesis in Streptomyces griseus. J Bacteriol 175:2652–2661

Waksman SA (1937) Associative and antagonistic effects of microorganisms. I. Historical review of antagonistic relationships. Soil Sci 43:51–68

Waksman SA, Foster JW (1937) Associative and antagonistic effects of microorganisms. II. Antagonistic effects of microorganisms grown on artificial substrates 43: 69–76

Waksman SA, Hutchings IJ (1937) Associative and antagonistic effects of microorganisms. III. Associative and antagonistic relationships in the decomposition of plant residues. Soil Sci 43:77–92

Waksman SA, Starkey RL (1923) Partial sterilization of soil, microbiological activities and soil fertility. Soil Sci 16:343–358

Waksman SA, Woodruff HB (1940) The soil as a source of microorganisms antagonistic to disease-producing Bacteria*1. J Bacteriol 40:581–600. https://doi.org/10.1128/JB.40.4.581-600.1940

Waksman SA, Reilly HC, Johnstone DB (1946) Isolation of streptomycin-producing strains of Streptomyces griseus12. J Bacteriol 52:393–397

Waksman SA, Lechevalier HA, Harris DA (1949) Neomycin—production and antibiotic properties 123. J Clin Investig 28:934–939. https://doi.org/10.1172/JCI102182

Weinstein MJ, Luedemann GM, Oden EM, Wagman GH, Rosselet JP, Marquez JA, Coniglio CT, Charney W et al (1963) Gentamicin,1 a new antibiotic complex from Micromonospora. J Med Chem 6:463–464. https://doi.org/10.1021/jm00340a034

WHO (2018) The top 10 causes of death

Woodruff HB (2014) Selman A. Waksman, winner of the 1952 Nobel prize for physiology or medicine. Appl Environ Microbiol 80:2–8. https://doi.org/10.1128/AEM.01143-13

Wu L, Wu H, Chen L, Yu X, Borriss R, Gao X (2015) Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against Xanthomonas oryzae rice pathogens. Sci Rep 5. https://doi.org/10.1038/srep12975

Zhang W, Wei W, Shi J, Chen C, Zhao G, Jiao R, Tan R (2015) Natural phenolic metabolites from endophytic Aspergillus sp. IFB-YXS with antimicrobial activity. Bioorg Med Chem Lett 25:2698–2701. https://doi.org/10.1016/j.bmcl.2015.04.044

Zhao W, Zhong Y, Yuan H, Wang J, Zheng H, Wang Y, Cen X, Xu F et al (2010) Complete genome sequence of the rifamycin SV-producing Amycolatopsis mediterranei U32 revealed its genetic characteristics in phylogeny and metabolism. Cell Res 20:1096–1108. https://doi.org/10.1038/cr.2010.87

Zinsser H, Hopkins JG, McBurney M (1916) Studies on TREPONEMA pallidum and syphilis. J Exp Med 23:341–352

Applications of Microbes in Fuel Generation 26

Mohd Imran Ahamed and Naushad Anwar

Abstract

Microbes have been considered as the best career of natural products in which few of them have been extensively used in drugs, trade good and specialty chemicals, polymers, fuels and many more biological materials. Recent works have been extensively focused to develop the microbial systems for the production of biofuels which is a promising approach in the development of executable process for the generation of these fuel materials in various applications from sustainable resource. Regarding these approachable works, the researchers and scientific community have been found that one of the microbes, namely Escherichia coli (*E. coli*) has become a prognosticating host microorganism in the production microbial fuels due to the ease at which this microorganism can be influenced. A number of well-known processes such as synthetic biology and metabolic engineering, *E. coli* acts as a biocatalyst to yield a large variety of potential biofuels from a number of biomass constituents. Like *E. coli*, many more microbes have been extensively utilized for the microbial transformation of waste materials using novel bioremediation strategies like microbial fuel cells (MFCs) for the production of energy are considered as an efficient and environmentally gracious approach. In this chapter we have discussed about the nature of microbes which may be employed for bioenergy production, power output, their major applications and future challenges that will be helpful for new comers in academia as well as in industrial and technology in near future.

Keywords

Microorganism · Biofuel · Biohydrogen · Bioenergy · Applications

M. I. Ahamed · N. Anwar (✉)
Faculty of Science, Department of Chemistry, Aligarh Muslim University, Aligarh, UP, India

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26.1 Introduction

Few past decades, the continue fall back in the denseness of the fossil fuels and the pioneering global demands of various forms of energies have required the generation of their substitutes which are most efficient and low cost effective fuel to discards the conventional fuel which have been retort to increase the accruement of greenhouse gases in an environment that accompanied to appreciable environmental changes. These appreciable variations could lead in the harmful effects in present and/or near future such as rise in temperatures and sea levels (Panwar et al. 2011; Marousek et al. 2012, 2014; Yilanci et al. 2009). From IPCC survey, the use of fossil fuels in the generation of heat and electricity and also for transferral accounts for 14% and 25% of the all emissions of greenhouse gases (Slate et al. 2019). Therefore, in the present time, the world's highest energy demands in the production of eco-friendly and economically feasible renewable energy fuels that communicates the potential to concurrent replacement of conventional fossil fuels and decrease the climatic concerns. The use of various microbes to produce renewable sources of energy from biomass and the biological wastes may decrease this threatening concern to more extents (Santoro et al. 2017). Few study have been reported in Table 26.1 in fuel generation using various microbes that were recently increases particularly due

Table 26.1 List of microorganisms producing biofuels or the precursors for biofuel production

Microorganism	Biofuel	Biofuel yield ($g \cdot L^{-1}$)	References
Clostridium acetobutylicum	Butanol	3	Lutke-Eversloh and Bahl (2011)
Clostridium thermocellum	Isobutanol	5.4	Lin et al. (2015)
Escherichia coli	Butanol	30	Shen et al. (2011a)
Escherichia coli	Ethanol	25	Romero-Garcia et al. (2016)
Saccharomyces cerevisiae	Fatty acids	0.38	Yu et al. (2016)
Saccharomyces cerevisiae	Isoprenoid based-biofuel	40	Westfall et al. (2012)
Pseudomonas putida	Butanol	0.05	Nielsen et al. (2009)
Cryptococcus vishniaccii	Lipids	7.8	Deeba et al. (2016)
Zymomonas mobilis	2, 3-butanediol	10	Yang et al. (2016)
Zymomonas mobilis	Ethanol	–	Kremer et al. (2015)
Caldicellulosiruptor bescii	Ethanol	0.70	Chung et al. (2014)
Trichoderma reesei	Ethanol	10	Huang et al. (2014)
Yarrowia lipolytica	Fatty acids	55	Beopoulos et al. (2009)
Synechococcus sp.	Limonene	0.04	Davies et al. (2014a)
Synechococcus Elongates	1,3-propanediol	0.28	Hirokawa et al. (2016)

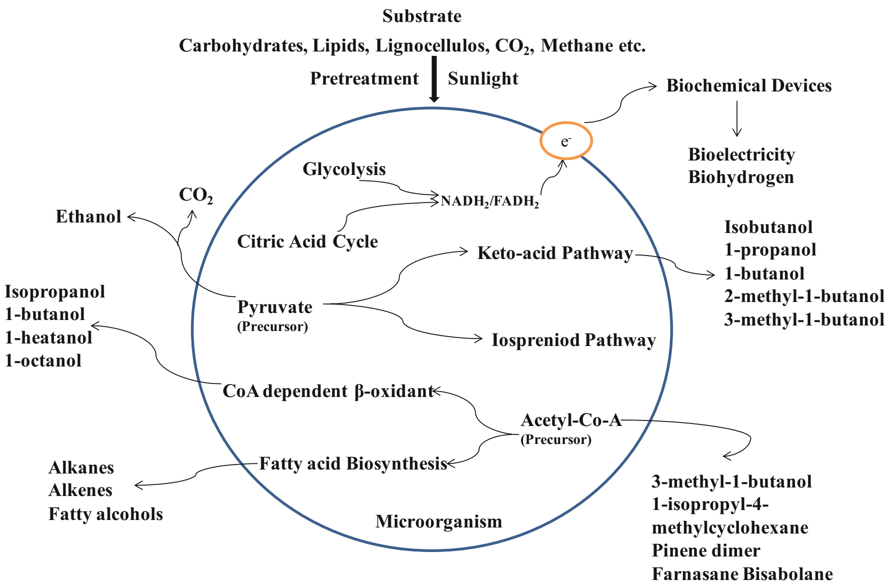


Fig. 26.1 An overview of microbial metabolic pathways for biofuel production (Kumar and Kumar 2017)

to the metabolic diversity of various microbes which enabling the biofuel productions from different substances, viz., in presence of zymase, monosaccharide ($C_6H_{12}O_6$) gets converted into alcohols and cellulolytic microbes may be utilized as plant-driven substances. Microalgae and cyanobacteria possess strength to decrease the atmospheric carbon dioxide into biofuels and methane are used in controlled oxidation process to converts it into methanol in the presence of catalysts (Lutke-Eversloh and Bahl 2011; Lin et al. 2015; Shen et al. 2011a; Romero-Garcia et al. 2016; Yu et al. 2016; Westfall et al. 2012; Nielsen et al. 2009; Deeba et al. 2016; Yang et al. 2016; Kremer et al. 2015; Chung et al. 2014; Huang et al. 2014; Beopoulos et al. 2009; Davies et al. 2014a; Hirokawa et al. 2016). Researchers have also been discussed the action of few bacteria like Geobacter sulfurreducens and Shewanella oneidensis which show peculiar property as "molecular machinery" in the production of biofuels with the help of electron transfer mechanism from microbial outer membrane to conductive surfaces, later on, this property may generalize in bioelectrochemical systems in the production of bioelectricity and biohydrogen (Bond and Lovley 2003; Gorby et al. 2006; Chaudhuri and Lovley 2003; Kracke et al. 2015). Figure 26.1 has been outlined to show the generation of various biofuels using microbial pathways in contrast to select the suitable substrates, microbes and the methods which can easily produce fuels. The ingestion of organic substances by a microbes and its later use in the metabolic methods produces beneficial product which may utilize as a fuel in the production of various forms of energy. The generation of microbial fuels such as alcohols from cereals, also requires more input of energy in the form of fossil fuel in comparison with those processes that involved sugarcane as substrates (Kumar and Kumar 2017; Pal and Sharma 2018; Chaijak et al. 2018).

MFCs, newly designated process for the generation of energy and the degradation of organic materials. In MFCs, microbes are the vital components. Recently reported by Sharma et al. in their study in which Pichia fermentans, a potential microbe has been used in fuel generation in MFCs. The exoelectrogenic quality of this non-pathogenic microbes have been evaluated already to make it an appropriate candidate to be used in anodic chamber while as a cathodic materials, a hybrid, chemical or biological may be used to facilitate the reactions. Few studies have been applied on bio-cathode as to form the system as self sustainable for desire utilization. Laccase based biocathode has already exhibited few promising results in MFCs (Sharma and Arora 2010; Pandey et al. 2016; He et al. 2015; Hou et al. 2011). Thus, the constant production of such types of enzymes in catholyte can furnished a long term solution. Among various microbes, white rot fungi is one of them holds the capacity to generate the lignocellulolytic enzyme. The utilization of such efficiency for the continuous generation of enzymes such as ligninolytic oxidoreductase in cathode chamber to improves the performance of fuel cell. MFCs can be utilized for the production of bioelectricity from different carbohydrates, such as monosaccharide, oligosaccharide and complex carbohydrates such as polysaccharide (starch, molasses) and wastewater from food (cereal) processing plants. MFCs are enchanting biological fuel cells (BFCs) which conveniently exhibit two chambers, i.e. the cathodic and the anodic and using a biological catalyst (oftenly bacteria) to generate electrical energy from naturally occurred organic materials in the environment or from wastes. It has been found that the release of electrons-protons when electrochemically active microbes oxidized the organic materials at the anodic surface (Li et al. 2010; Ghrabi et al. 2011; Doherty et al. 2015). In contrast with Fig. 26.1, Fig. 26.2 represents the fundamental principle of MFCs. The microbes that play a role of biocatalysts to oxidized the inorganic-organic substrate to CO_2 and produce electrons at anode. The transfer of electrons from anode to the surface of cathode via an external circuit, while protons move to the cathode directly via solution took place and seen that other chemicals can accept electrons at the surface of cathode inside the MFCs (Kumar et al. 2015). Every year, as instantaneous increase in demand of energy on large scale, overconsumption and diminishing the natural and nonrenewable energy sources, the generation of microbial sources of energy may lead an important type of bioenergy as MFCs play an effective role in current extraction from biodegradable plant extract materials on large scale and renewable biomasses from easy molecules carbohydrates and proteins of the kind to the complex materials of organic complexes that have been found in nature, i.e., present in living being and food processing wastewaters (Liu et al. 2014). Various sources have been reported by authors revealed that the availability and proficiency of various microbes to use on large scale of organic materials make MFCs a quirky and typical technology for renewable sources of bioelectricity generation. The MFCs technology is an old technology, but their current use and applications are in the limelight from few past decades in research for bioelectricity generation. Not only few classes of

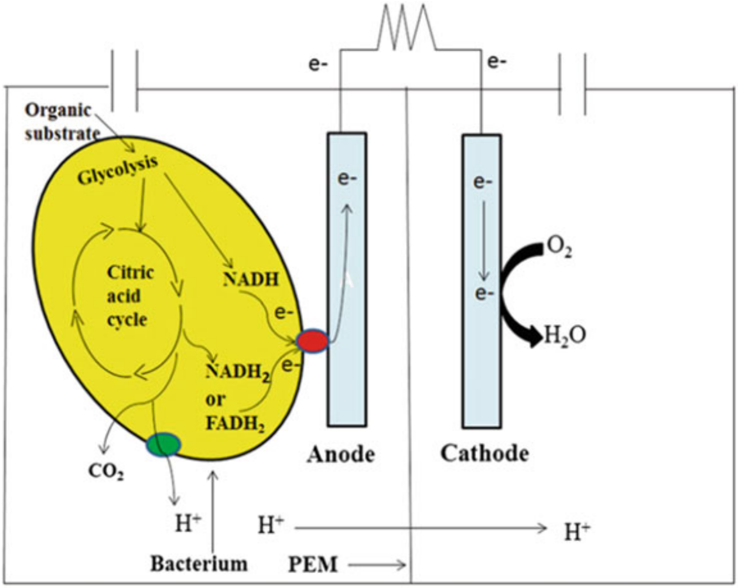


Fig. 26.2 General principle of a microbial fuel cell (Kumar et al. 2015)

microbes such as Firmicutes, Proteobacteria, Acidobacteria phyla etc. shown their ability in the production of bioelectricity, and few fungi, yeast, microalgae etc. have been studied in MFCs fields that were used as substrate or attend the cathode or anode. On the other hand, the ability of reduction of the substrates on the basis of electron transfer, the microbes (bacteria) are so named reducing bacteria in MFCs such as sulphate reducers, iron reducers, etc. (Bond and Lovley 2003; Gorby et al. 2006; Chaudhuri and Lovley 2003). During transfer of electrons, i.e., donating-accepting tendency in the MFCs can be referred as electrode oxidizers-reducers in which one of them is called cathode reducers and anode oxidizers, respectively. Most studied iron-reducing microbes those were used in the production of bioelectricity are as Rhodoferax ferrireducens, Geobacter spp., Aeromonas hydrophila, Shewanella sp., Enterococcus gallinarum, Clostridium butyricum and Pseudomonas aeruginosa, respectively. The conversion of energy is based on Coulombic efficiency (CE) or the percent of electrons recovered from the organic materials varies widely and as functions of the wastewater and type of MFCs that have been discussed by a lot of researchers. By the use of singly-chambered MFCs that contains an air-cathode, the CEs were 40–55%, when a proton-exchange membrane (PEM) has been used, but as the PEM chamber has been removed, the CEs were found to be only 9–12% because of the diffusion of oxygen into anode chamber. In favour of, the CEs of 83% and 89% have been attained by the use of two-chambered aqueous-cathode systems that contains PEM. Low values of CEs have been found even with two-chambered MFCs those contains PEM with their values of 8.1% for prepared sucrose solution and 27% for a cereal-processing wastewater (Chaudhuri and Lovley

2003; He et al. 2005; Min and Logan 2004; Niessen et al. 2004; Oh and Logan 2005).

Besides in the generation of bioelectricity, microbes have also been used in the production of biodiesel which are an alternative and research to increase the efficiency of production utilize the Jatropha curcas L. Aim is to write this chapter to provide the knowledge about the basic properties of microbes and their effects on activity, stability and selectivity of as an enzyme and their useful applications in various fields that will be helpful in academia as well as in industrial biotechnology in the near future.

26.2 Development of Biofuels

Biofuel generation is an excellent area of bio-sustainability research in recent years. In the production of these fuels, enzymes play a vital role in the conversion of biomass to liquid fuels utilizing the different feed stocks including carbohydrates, municipal waste or woody biomass. The production of biofuels is classified into four generations. In the first generation, enzymes are used to decompose the starch rich biomass into simple saccharides to produce bioethanol followed by fermentation process (Uhlen and Svahn 2011). Proceeding of second generation based on the production of bioethanol from cellulose based biomass like waste materials after manufacturing of food. The third generation basically based on metabolic engineering to generate more energy-efficient biofuels as compared to bioethanol, isobutanol and different forms of alkanes; the second generation biofuels. Hence, new microbes fermenting "biodiesel" at the place of bioethanol can be visualized, although most efforts so far are devoted to the production of more valuable chemicals. Biofuels production in the fourth generation was basically without the need of beginning of biomass. This can be gained by photo-synthesizing microbes, like algae or cyanobacteria, which have been engineered to generate the biofuels with the help of sunlight, CO_2 and water as shown in Fig. 26.3. Researchers and the industrialist have needed to develop and improve the production and obtain commercially feasible processes. Following are the various applications of microbes for the production of various forms of energy (Uhlen and Svahn 2011).

26.3 Applications

26.3.1 Biofuel Production from Brown Macroalgae

In the production of biofuels, brown macroalgae show few salient features of an ideal feedstock and renewable facile chemical compounds. Expecting none of the resources of fresh water, arable land or fertilizers, cultivation of the crops overreach the economic concerns associated with five times higher than those with the ethanol generation from cereals. Examples including mannitol, alginate and glucan are the most abundant carbohydrates in brown macroalgae (Somerville et al. 2010; Huang

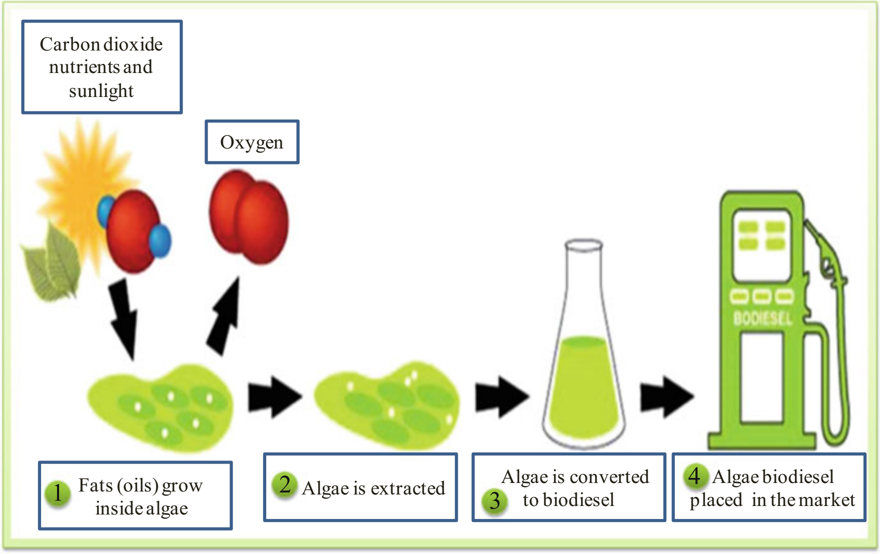


Fig. 26.3 Schematic of biofuel production using algae (Uhlen and Svahn 2011)

et al. 2011a, 2012). The production of bioethanol from these sugars (glucan and mannitol) approx 0.08–0.12 w/w to ethanol/dry macroalgae is reported (Roesijadi et al. 2010). Nevertheless, the full potential of production of bioethanol from macroalgae cannot presently be recognized due to the lack of ability of industrial microbes to metabolic the alginate compounds. As for example, the production of bioethanol via fermentation of glucan using *Saccharomyces cerevisiae* was found to be ~0.45% v/v in *Saccharina latissima*. The compared study with glucose, the catabolism of mannitol produces excess reducing equivalents, causing an unbalanced redox atmosphere under fermentation processes. Therefore, the production of bioethanol from mannitol is spontaneous only in micro-aerobic conditions which are also known as electron shunts. Semi-fermentative criteria enabled for the production of bioethanol from mannitol using *Zymobacter palmae* with a yield of 0.38 w/w ethanol/mannitol (Adams et al. 2008; Horn et al. 2000a, b).

Many of the metabolic engineering exploits to utilize a pair of aboriginal and non-analogous genes, viz., the generation of 1,3-propanediol, a precursor of anti-malarial drug artemisinic acid and more recent, production of isobutanol in *E. coli*. As for example, the over expression of the heterologous enzymes may hold stress via consuming the pool of available amino acids, chiefly various codon usage organisms where the production of the heterologous genes as compared with the generation of strains (Nakamura and Whited 2003; Ro et al. 2006; Atsumi et al. 2008). Accompanying with more heterologous proteins have been now codon-optimized to mobilize preponderantly on the greater availability of the transfer RNA (t-RNA) pools and to facilitate potential problems with the structure of messenger RNA (m-RNA). Additionally, simultaneous explanation of both the native and

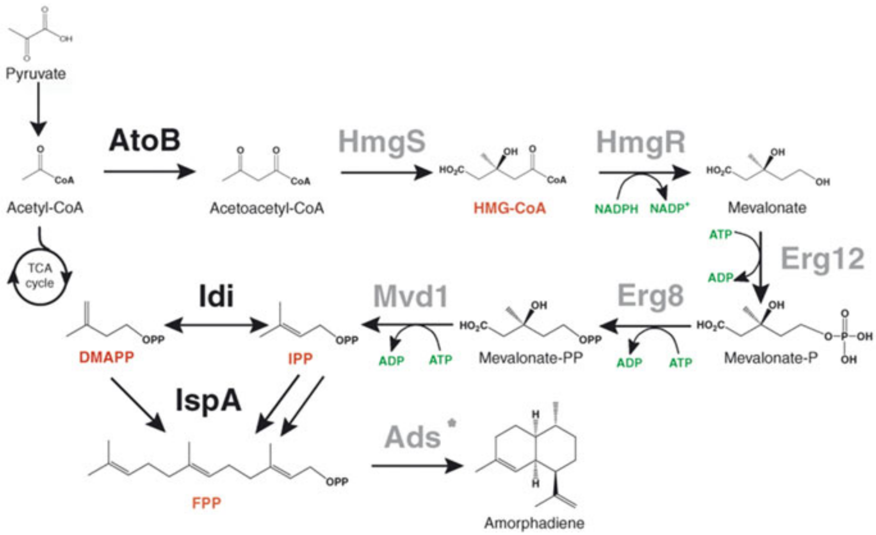


Fig. 26.4 An example of an engineered pathway for the production of the anti-malarial drug precursor, amorphadiene. This pathway demonstrates several key points that are pertinent to any metabolic engineering project. Namely, there are a large number of genes that must be co-expressed; all enzymes in grey were derived from S. cerevisiae, with the exception of ads, which was obtained from Artemisia annua, while enzymes in black were over expressed but were native to E. coli. Toxic intermediates are shown in red. The toxicity of these intermediates was overcome by balancing the expression levels of the corresponding enzymes (Mukhopadhyay et al. 2008)

non-native routes may the reason of unbalancing in the redox cellular reactions by modifying the ratios of NAD^+/NADH and $NADP^+$/NADPH that may be result to the overflow of metabolism. Unbalancing in the enzymatic activities may also results in the assemblage of inhibitory or toxic route intermediate that may drastically decrease simultaneously in the cellular development and the generation level of the microbial fuels. For example, the accruement of *3-hydroxy-3-methyl-glutaryl*-coenzyme A has been found under investigation by researchers as a bottleneck in the generation of isoprenoids biofuels in E. coli and was corrected over expression of t-HMG. The different exemptions of metabolic engineering for the generation of amorphadiene in *E. coli* are as they have been summarized in Fig. 26.4 (Gustafsson et al. 2004; Vemuri et al. 2006; Kizer et al. 2008; Pitera et al. 2007; Pfleger et al. 2006; Mukhopadhyay et al. 2008). The microbial production of 1,3-propandiol using Du Pont-Genen core are an excellent example in the successful reported by researchers in systems biology. In the beginning of 2000, Chotani et al. have been reported the importance of associating bottlenecks by the use of functional genomics emphases. To achieve a viable titre, various barriers had to be overwhelmed. The main route in which glycerol was used has been replaced by a pathway capable of using the cheap substance like glucose. The route and the host optimization eventually results in the level of production of 135 g/L are reported. This engineered system continues to be the focus of cell-wide studies to characterize its phenotype (Chotani et al. 2000).

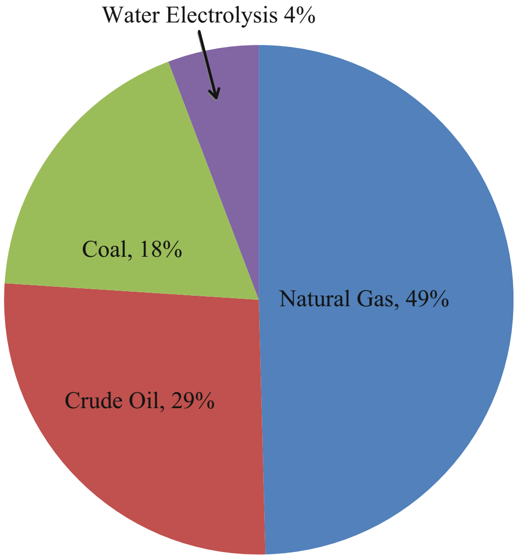
26.3.2 Metabolic Engineering to Upscale Biofuel Production

The microbes show various types of catalytic enzymes and the different production routes of microbial fuels. A microorganism *Saccharomyces cerevisiae* leads to the production of bioethanol to direct decarboxylation of pyruvates while in E. coli, coenzymes activate the acyl group the decarboxylation of pyruvates and converted it into ethanol, respectively. Such types of conversion in the metabolic engineering can be productive in the production of microbial fuels. This method could be applied in various paths to enhance the production of microbial fuels. It has been reported by a number of researchers as the pathways for the production of ethanol in yeast and in *E. coli*, respectively. The production of ethanol in the absence of coenzymes is examined as an efficient path as discussed by Liao et al. (Liao et al. 2016). Hence, this route may be conveyed in various microorganisms via genetic engineering technique for the generation of bioethanol. As like as the microbes lack the metabolic routes for individual microbial fuels can be injected with insistent genes are isolated from an efficient microbial fuels generating organism, transforming those microbes which are the non-biofuel producer into the biofuel producing microbes. This method can be more advantageous in microbial engineering for employing different substrates for the production of biofuels. The competing routes which may drains the microbial fuels or the precursors like acetyl coenzymes, pyruvates etc. or the enzymes that interfere with the synthetic routes of microbial fuels which can be severe with the use of metabolic engineering pathway. E.g., an inhibition process occurs by acyl carrier protein for in *E. coli* for the biosynthetic route of fatty acids. The over expression of the enzyme thioesterase can reduce the inhibition process and allow to synthesize the free fatty acids that subsequent resulting in the production of precursor (acyl-coenzyme, for the synthesis of fatty alcohol). Nevertheless, the substrate-specific enzymes and their catalytic activity and the number of turnovers may enhance by influencing the genetic materials of the enzyme by the use of more advanced techniques. With regard of the study based on computation proteins may be adapted to artificial structured amino acid to generate unnatural enzymes of desired functions which can be later used in the generation of biofuels. Hence, it can be seen that these above mentioned pathways have been extensively utilized for the production of biofuels in MFCs (Davies et al. 2014b).

26.3.3 Bioelectricity and Biohydrogen Production

Recently, bioelectrochemical cells (BECs) have achieved a considerable interest in the production of bioenergy from wastewater. Particularly, MFCs and electrolytic cells based on microbes (MECs) have been usually employed for the production of bioelectricity and biohydrogen. From a biological view, both MFCs and MECs work

Fig. 26.5 Hydrogen productions from different sources (Raj et al. 2012)



on the same principle and hence, the common microbes may be positioned in both cells for the generation of bioenergy. The peculiar characteristics of these microbes in BECs are the accumulation of unique "molecular machinery" which can be in the transfer of electrons from the outer membrane of microbes to the surfaces of the conductive film. Subsequently, these electrons were used to produce bioelectricity and biohydrogen. Nevertheless, the results from both of the fuel cells described above are insufficient for the real-world applications and presently not spontaneous for commercialization. Logan et al. have been theoretically reported the maximum voltage generation by MFCs is 1.2 V and the production of by MECs was found to be 3.4 mol hydrogen to mol-acetate ratio (Kracke et al. 2015; Logan et al. 2015; Dai et al. 2016). Focus on the implementation in BECs on wide scale that might be the cost effective altered per unit of the production of energy in the systems. The BECs having many scopes for the expedition of its future concerns towards to improve the production of bioenergy and biohydrogen. Good assumption to understand the routes of the microbial action which were important in the performance of BECs like electron transfer mechanisms, formation of the electroactive bio-membrane and later handling may help to increase in the output of energy from these sources. Nevertheless, this method would be highly effective in terms to reduce the initial time and towards amending the BEC'S performance (Kracke et al. 2015).

Hydrogen is examined as the purest fuel to the present energy assumption because of their non-polluting nature and have a high combustion yield than that of hydrocarbon, i.e., 122 kJ/g of the energy production has been reported by researcher. Moreover, in light of liquid biofuels, H_2 can be transformed into electrical energy using MFCs. Currently the global demands of hydrogen stand at approx 45 million tons per year (Cabrol et al. 2017). Figure 26.5 shows the production of hydrogen from various sources describe the 96% of the total hydrogen supply from steam regenerating of the traditional fossil fuel while 4% of the total is derives from the water electrolysis has been reported by researchers. Numerous works focused on the improvement of the promising technologies for the microbial transformation of different waste materials into biohydrogen (Raj et al. 2012). All of the different biotechnologies utilized in the production of biohydrogen, dark fermentation method with interracial hydrogen association to generating microbes and focused more attention because of the following assumptions: (a) they can used a broad range of organic substance, mainly waste streams, (b) large production ratio evinced as the rate of evolution of hydrogen (in mmol·L^{-1}·h^{-1}), and (c) the negligible production of CO_2 as major of the produced CO_2 during the metabolism of carbon is limited and used for the growth of biomass and the production of energy. Enzymes such as glyceraldehyde-3-phosphate dehydrogenase and pyruvate ferredoxin oxidoreductase are take part in producing decreased cofactors for potential production of H_2 have been reported by researchers (Bibra et al. 2015; Mahmod et al. 2017; Haiza et al. 2013; Liu and Ren 2008; Reischl and Rittmann 2018). Verhaart et al. have been examined the various mechanistic pathways by which the extremely thermophilic and hyperthermophilic bacteria and archaea like *Caldicellulosiruptor saccharolyticus, Thermotoga maritima*, *Pyrococcus furiosus* and *Thermoanaerobacter tengcongensis* have been involved in the hydrogen production via reductant disposal (Verhaart et al. 2010). One of an important melalloenzyme hydrogenase is produced by microbe takes part in the consumption and production of hydrogen biofuels. They possess various active sites and are distinguished on the active site metal consumption such as [Fe], [Fe-Fe] and [Ni-Fe] hydrogenase. In the production of biohydrogen, NADH and ferredoxin have also been participated which is also known as an electron-bifurcating hydrogenase enzymes (Kim and Kim 2011). A number of studied reveals that the microbial ecology in the production of biohydrogen after the success of the techniques based on molecular biology and has reported as a broad phylogenetic ecology by the researchers that also play a major role in the production of biohydrogen from complex substances. *Clostridium sp*. is one of the predominate and the most efficient hydrogen producing bacteria in the H_2 reactors shown the producing capacity of hydrogen ranges from 1.5 to 3 mol/mol of H_2/hexose ratio. *Clostridium acetobutyricum*, *Clostridium beijerinckii*, *Clostridium butyricum* and *Clostridium pasteurianum* are reported those were produced hydrogen from the diverse organic waste materials like condensed molasses, brewery yeast waste, starch-containing waste by few authors. Another activity known as cellulolytic activity were shown by few bacteria such as *Clostridium tyrobutyricum* and *Clostridium celerecrescens* which being apart from the production of biohydrogen were also reported. *Klebsiella sp.* was reported as the prevalent member in the production of biohydrogen community which coproduced hydrogen and ethanol from glycerol. *Pseudomonas stutzeri* and *Shewanella oneidensis* are belonging as facultative anaerobic Gammaproteo bacteria are identified as hydrogen producing bacteria. Limited investigations have been conducted to characterize in the production of hydrogen in the extreme environments (Buckel and Thauer 2013; Yang and Wang 2018; Masset et al. 2012; Patel et al. 2014).

26.3.4 Microbial Bioenergy Production

A lot of microbes have been extensively experimented in MFCs in the production of electricity, bioremediation and various manifold applications. Figure 26.6 is shown the Bioenergy conversion processes from natural sources (Uhlen and Svahn 2011). Other than these, few nutrients such as carbohydrates, proteins, etc. and wastewaters such as swine, paper recycling, chocolate industry and protein-excesses wastewaters, etc. from different sources were extensively used in the growth of microbes as substrate in MFCs technology. Study based on the production of electricity, a lot of available microbes and substrates but only specific microbes are well known in

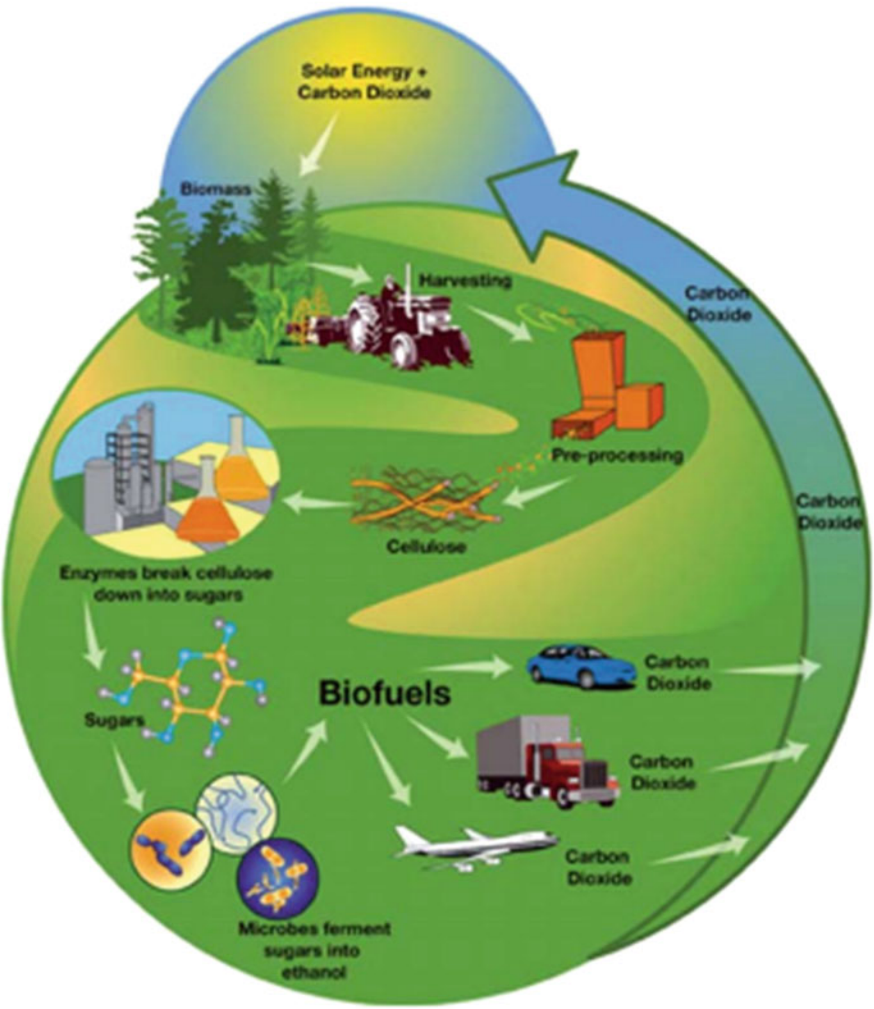


Fig. 26.6 Bioenergy conversion processes (Uhlen and Svahn 2011)

MFCs. Exoelectrogens of the different types like algae, yeast, Gram-(+) and Gram-(−) bacteria, cyanobacteria and even fungi can be utilized in various forms of MFCs as reported by the researchers (Liu et al. 2004; Rabaey et al. 2005; Reguera et al. 2005). Those microbes are significantly essential in the production of electric current which can be fully breakdown the complex organic substances into their corresponding components at the anode. While a specific exoelectrogen can oxidize a particular substrate for their development and generation of electrical energy. Furthermore, based on the nature of substrate, each exoelectrogens have various routes and genes, proteins or enzymes for the oxidation or degradation of bacteria (Logan 2004). That is why the selection of appropriate bacterial association and preferred substrate give the product of MFCs. An early study has been found that describe when an MFCs was operated for three months and it fed with anaerobic-aerobic sludge substances (glucose and inoculums) in the conversion of electricity rates 7 folds. Starch, cellulose, proteins and lipids are the organic substrates which play as an electron donor for the redox chemical reactions in MFCs at the anode in the production of bioenergy. These molecules further undergo in glycolysis to produce acetyl coenzyme which further undergo to precede citric acid cycles (CACs). In (CACs), 3 reduced NADH equivalents are produced from 3 NAD^+, one FAD reduced to $FADH_2$ and carbon dioxide as a by-product in single turn of cycle (Logan 2009). The well known metabolic routes such as glycolysis and Krebs cycle processed in both the bacteria and yeast. An electron carrier agents like NADH and $FADH_2$ which may transfer the electrons to the electron transport chain to generate a molecule to carry energy known as adenosine triphosphate. In prokaryotes, respiratory reactions occur in cell membrane, the assembly constituting all the enzymes or proteins required to transfer of electrons while in eukaryotes, the electron transfer chain resides on the inner membrane of mitochondria. The electron transfer chain in eukaryotes typically consists of four types of protein intermediate such as ubiquinone, cytochromes, coenzyme Q and NADH dehydrogenase, respectively. Earlier to the prominence, bacteria may facilitate the transfer of electron; a chemical intermediate was utilized to catalyse the transfer of electrons interior to the bacterial cell to the interface of anode. These intermediate combine with electron transfer chain and free from the bacterial cell and electrons transfer towards the anode is found (Singh et al. 2013). Furthermore, bacterial metabolism may switch from oxidative phosphorylation to fermentative metabolism depends on the potential of the anode. The low potential at anode, prokaryotes adapts an oxidative metabolism in the presence of an electron acceptor and deposition of electrons occurs on electron acceptors. In the absence of electrons acceptor, the deposition of electrons on electron acceptors is due the metabolic activity by bacteria. As for example, during the fermentation of glucose, 1/3rd of the electrons are utilized in the electricity generation while remaining of electron resides in fermentation products, which may later oxidized by anaerobia bacteria like *Geobacter sp.* in the MFCs in the production of current. Beyond the production of electricity, a lot of bacteria, e.g., *Clostridium sp., Enterococcus sp.* have been communicated by anaerobic method in MFCs to generate fermentation product. *Geobacter sp.* and *Clostridium sp.* are most efficient exoelectrogens towards the production of hydrogen in MFCs (Singh and Wahid 2015; Huang et al. 2011b).

Logan et al. have been investigated whether MFCs could be used for the production of electricity production using corn stover hydrolysates. Power density has been evaluated using a single-chamber, air-cathode MFCs lacking a PEM which are produced 494 mW/m^2 (CE = 9–12%) with glucose and 146 mW/m^2 (CE = 20%) with domestic wastewater. This same system with a PEM produced maximum power densities of only 262 mW/m^2 (CE = 40–55%) with glucose and 28 mW/m^2 (CE = 28%) with domestic wastewater (Zuo et al. 2006).

26.3.5 Solar-to-Chemical and Fuel Production

Cyano- and/or photoautotrophic bacteria are the prognosticating microbial platform for continuous generation of biofuels and biochemicals CO_2 and light due to applied maximum attribution to convert CO_2 directly using cyanobacteria (Robertson et al. 2011). Moreover, cyanobacterial metabolic engineering was focused for direct generation, release of product and also the optimization of method. Various works have been focused in the production of biochemical and biofuels in improvement of the native routes and by involving heterologous pathways. Currently, these bacteria have been applied to metabolic engineering pathway has etiolated to increase the total production of butane-2,3-diol later inserting with simple mono-, disaccharides under diurnal conditions. By doing so, heterologous illustration of the supplier of various kinds such as xylose, galactose, xylulokinase and xylose isomerase from *E. coli* to a butane-2,3-diol generating forms of cyanobacteria was essential and resulting in the continuous production of butane-2,3-diol in dark. In such condition, 70% of carbon was obtained from glucose consumption based on the analysis of 13C-U-labled glucose (Case and Atsumi 2016). The work has been reported by authors for the production of D-lactic acid and ethylene using the photomixotrophic methods under constant sunlight in engineered cyanobacteria by increasing coil 1.9 fold for D-lactic acid and 1.6 fold for ethylene, respectively, which has been compared with D-lactic acid and ethylene autotrophic productions. In accordance with bicarbonate of 13C-labled and xylose of 12C-U-labled, half amount (approx 50%) of the carbon has been obtained from the utilized xylose, tested in the photomixotrophic conditions. The percentage compositions and productivity have been increased as the sources of sugar carbon were added, while the life cycle possession of the mixotrophic increment of cyanobacteria and also their generation could be examined in order of the net decrease in the CO_2 emission rates due to the cellular activities in phototrophic cyanobacteria (Savakis and Hellingwerf 2015; Gudmundsson and Nogales 2015; McEwen et al. 2016). The facile formation of the chemical products are affected strongly in absence and presence of sunlight, the aspect of genetically regulation and the metabolic activity of the cyanobacteria under the diurnal condition, those have also been discussed by the authors. Significantly, various observations on metabolic activity of cyanobacterial carbon have been clarified to reveal their plasticity and complexity, but more information regarding the functions of gene remains unknown. The presence of activities of the enzymes such as 2-oxoglutarate decarboxylase and succinate semialdehyde dehydrogenase were also demonstrated for the completion the tricarboxylic acid cycles and while in light of the glyoxylate cycle, the Entner–Doudoroff pathway and γ-aminobutyric acid passage have been validated in cyanobacteria (Varman et al. 2013). Study on the scalable bioelectrochemical system based on hydrolysis of water into their constituents has been recently reported as a catalytic system coordinated with a microbial engineered system to fascinate solar energy and convert carbon dioxide into desired alcohols or a number of substrates. It has been found that decomposition of various cathodic-anodic substances (cobalt phosphate, Ni, W, Zn and/or stainless steel) were found that allows to the growth of bacterial cell at the electrode potential of the cell was set on 2.3–3.0 V in a single cell set up by oxidizing hydrogen and fixing carbon dioxide in a chloride free source at neutral pH with the phosphate concentration 36 mM (Lee et al. 2015; Cohen and Golden 2015; Saha et al. 2016). Later on, biochemical cell in stainless steel is assumed as a cathode with engineered chemo litho-autotrophic *Ralstonia eutropha* which was genetically arranged to express genes for enzymes ketothiolase, acetoacetate decarboxylase, acetoacetyl-coenzyme transferase and alcohol dehydrogenase were used and found to be the production of isopropanol about 216 mg·L^{-1} in five days from carbon dioxide. In combination with metabolic engineering of *R. eutropha*, obtained hybrid system gained an energy efficiency of carbon dioxide of over 10% for biomass, bioplastic and fuel alcohols which were determined to increase the production of natural photo-systems. The hybrid system obtained from cobalt phosphate- R. eutropha has been shown to act as artificial cyanobacteria which was intrinsically sociable with diurnal situations which depends on the production of hydrogen in the day time. This R. eutropha has engineered in metabolic processes which have developed in the production of methyl ketone and also β-hydroxyisobutyric acid from carbon dioxide and hydrogen can be interrelated to hybrid hydrolytic bacterial systems. Bacteria of the type of *Chemolithoautotrophic* are nonphototrophic, carbon dioxide utilizing microbes, redox process occurs in which hydrogen oxidized by microbes and the reduction due to metabolically accept electrons. Carbon dioxide acts as fixation agent via numerous metabolic paths in chemolithoautotrophs which may be aggregated into the dark phase of photoautotrophs. Bioelectricity from various renewable sources is directly used an electron-utilized autotrophic acetogen strain as a source of energy production (Nichols et al. 2015; Torella et al. 2015; Liu et al. 2016). A hybrid setup which has been developed to produce acetate from carbon dioxide through the Wood–Ljungdahl route was based on a self-photosensitizes and nonphotosynthetically forms of the *Moorella thermoacetica* bacterium with equivalent to the reduction method. Whatever with the reduction equivalent, nascent hydrogen i.e., [H] has been produced using electron via carbon dioxide utilized hosts in studied hybrid systems were introduced for the production of value added chemicals through synthetic metabolic paths (Nichols et al. 2015). Light absorbed silicon nano-wire array interacts with *Sporomusa ovate*, a type of anaerobic bacterium which was generated acetate at a high reaction rate of carbon dioxide and with selective control of the transport of mass inside the nano-wire under desirable conditions. Interesting work focused for the production of bioenergy from the electrosynthetic acetate which has been commonly used as biosynthetic feedstocks for *E. coli* engineering which produced biobutanol, polyhydroxybutyrate polymer and the isoprenoids, respectively (Liu et al. 2016). Moreover, solar-operative photochemically evaluated hydrogen can produce hydrogen in an uninterrupted process of reduction to a microbe for carbon dioxide fixation and conversions. In the absence of an external electrical bias and/or sacrificial chemical quenchers, a hybrid bioinorganic system that consists a biocompatible evolution reaction for hydrogen electro-catalyst and the conversion of carbon dioxide by *Methanosarcina barkeri*, which allow the production of methane with 86% overall Faradaic efficiency for over seven days (Muller et al. 2013; Przybylski et al. 2015; Kang et al. 2015; Li et al. 2012).

26.3.6 Microorganisms in Bioethanol and Biobutanol Production

Production of ethanol by the activity of microbes commonly called bioethanol is one of the most renewable materials used in transportation fuel. The worldwide used of this microbial fuel basically in internal combustion engines in the beginning of the nineteenth century. In the recent year Brazil and US become the highest bioethanol producers and lead to approx 85% of the worldwide production of ethanol. The utilization of ethanol as in the pure form of ethanol or conflated with gasoline to inhibit the emission of exhaust gases. This has also increase the fuel quality over gasoline viz., large octane number, border flammability limits and increased heat of vapourization (Azhar et al. 2017). In comparison to petroleum, ethanol is found to be biodegradable and low toxic and possessing very low polluting tendency. An interesting research work has been recently focused in the second generation production as the cellulosic bioethanol emerges as the primary feedstocks and these fuels are abundantly and low cost effective. Figure 26.7 (Pejin et al. 2009) describes the various paths in the generation of cellulose based bioethanol as reported by researcher. Microbes show their activity in the production of bioethanol from cellulose, especially hydrolysis and fermentation. Fungi in the form of filament like structures have been broadly utilized in the generation of cellulolytic enzyme by submerged fermentation pathways. Recent work has been focused on *Trichoderma reesei* having the properties of mutagenesis in the development of secreting mutants. Nevertheless, as exhibited as secretome and genome analyses, *Trichoderma reesei* generates very low amounts of β-glucosidase which result in the ineffective cellobiose hydrolysis and resultant product inhibition of *Trichoderma reesei* enzymatic systems as discussed by researchers (Pejin et al. 2009; Balat and Balat 2009; Bardhan et al. 2019; Zhang et al. 2017). Various filamentous fungi such as Aspergillus, Myceliophthora, Penicillium, Humicola, etc., have tendency to produce cellulase are under the limitation of the intensified work on hydrolysis of cellobiose. In favour of this work, Penicillium cellulase has been upgraded that containing good efficiency meanwhile the sachharification of biomass as it is dense in β-glucosidase. This substantially decreases the requirement of cellulase, thereby

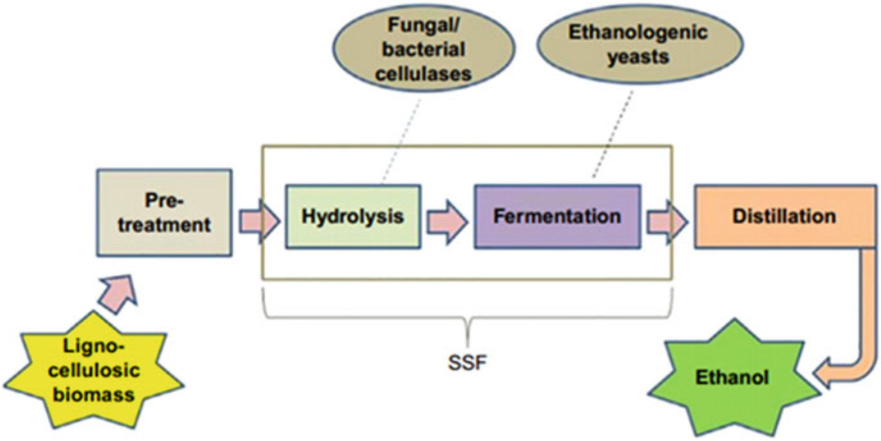


Fig. 26.7 The steps involved in the production of bioethanol from lignocellulosic biomass (Pejin et al. 2009)

reducing the cost of cellulase. Yeasts show vital role in the production of bioethanol using fermentation of the wide range of saccharides to ethanol. The strains like yeast such as S. cerevisiae, Kluyveromyces fragilis and Pichia stipitis have extensively studied by the researcher that were used in the production of bioethanol from various types of sugars, respectively. These have been used for the commercial bioethanol production because of their yields higher than 90%, bioethanol tolerance greater than 40 $g{\cdot}L^{-1}$, productivity of ethanol greater than 1 $g{\cdot}L^{-1}\ h^{-1}$, suppress the growth of contaminating microbes (Mussatto et al. 2012). One the known yeast S. cerevisiae (also known as Baker's yeast) is simply used in the production of bioethanol on industrial scale. However, study revealed that during the industrial production, stressful atmosphere were occurred such as osmotic stress, temperature, concentration of ethanol increases, inhibitory compounds generation and the contamination with those types of yeasts and bacteria alter the nonviable yeast strain production. Bioethanol when obtained from the fermentation of sugars, it has seen a problem arises due to the inability of S. Cerevisiae to co-ferment into pento−/hexoses. Very few literatures are reported on yeast as Candida, Pachylosen, Pichia and Schizosaccharomyces are the common genera are able to ferment pentose sugar to bioethanol (Gottumukkala et al. 2013).

Biobutanol is also considered as an alternate conventional liquid fuels have attracted attention because of its production from renewable source by microbial fermentation methods. It is a drop-in or finished fuel having an excellent fuel property in comparison with bioethanol due to their high energy density and similar property with gasoline. Biobutanol synthesis was put forward by Chaim Weizmann in 1912 on industrial scale from starch using a strain of C. acetobutylicum. Figure 26.8 (Gottumukkala et al. 2013) described the fermentation process of ethanol, butanol and acetone in C. acetobutylicum and they can switch from acidogenesis solventogenesis metabolism, i.e., the production of butanol and acetone.

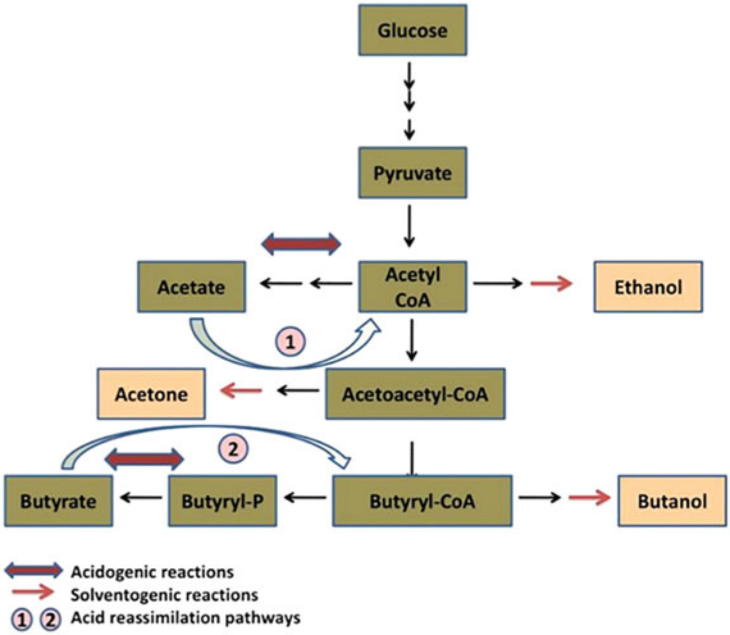


Fig. 26.8 ABE pathways in Clostridium acetobutylicum. Trends and advances in conversion of lignocellulosic biomass to biobutanol: microbes, bioprocesses and industrial viability (Gottumukkala et al. 2013)

Solventogenesis processes in C. acetobutylicum have been reported in favoured under low pH and low growth rate and the high carbohydrate concentrations. Few of the Clostridium strain are saccharolytic and may generate biobutanol from cellulosic substances (Azhar et al. 2017; Pejin et al. 2009; Balat and Balat 2009; Bardhan et al. 2019; Zhang et al. 2017). Higher production of ethanol, acetone and butanol were obtained with C. beijerinckii by the use of hydrolyses-based medium from green seaweed Ulva lactuca in absence of any nutrient supplement. Other interesting bacteria like C. ljungdahlii is able to utilize CO and H_2 from synthetic gas as carbon and energy source in acetone and butanol production. Few other Clostridial species like *C. pasteurinum*, *C. saccharoperbutylacetonicum*, *C. saccahrobutylicum* and *C. sporogenes* have been studied by the authors for the production of biobutanol (Adsul et al. 2007; Singhania et al. 2014; Thang et al. 2010; Sabra et al. 2014). Jang et al. have been studied on the metabolic engineering and select the *C. acetobutylicum* that enable biobutanol tolerance of enhance the production up to 19 $g{\cdot}L^{-1}$ and the production of biobutanol 0.71 mol/mol butanol/glucose which was found to be 160% and 245% higher than of the wild-type yeast strains. Cuenca et al. investigated the key genes in P. putida which can be involved in tolerance of butanol and their relationship (Jang et al. 2012). Transcriptomic and proteomic studies justified that P. Putida initiate biobutanol relationship through alcohol-aldehyde dehydrogenase. Metabolic strategies in the production of butanol in non-clostridial microbes like *E. coli* involve in the transfer of acetone, butanol and ethanol from *C. acetobutylicum* yeast in the generation of larger alcohol, viz., 1-butanol and 1-propanol have been reported by Shen et al. (Shen et al. 2011b).

26.4 Future Outlook

The more challenging task in the production of microbial fuels by the use of "microbial factory" is to produce a high amount of biofuel on a comparatively low cost, easiest way and more efficiency in comparison with nonrenewable sources. In other words, the replacement of petrol with ethanol, the latter should be an efficient and low cost additives, which might be a most challenging hurdles in convenience with the daily needed. E.g., in USA, approx 19 million barrels of petrol are consumed in a day that is to produce this large amount to use on the large scale may be a challenge. Therefore, to increase the adequacy of microbial fuels, its production process should be prioritized in near future. Progress in the areas of metabolic engineering, the synthetic and system biology have further enhanced characteristics to successful implementation and to analyse numerous schemes to engineer *E. coli* in the production of highly efficient microbial fuels using the different metabolic routes. The continuous progress of synthetic and systems biological techniques that slow the time required to make genetic constructs as well as increase the predictability and reliability of the systems should highly improve the metabolic engineering techniques for an effective production of a wide variety of biofuels and bio-chemicals. Furthermore, utilizing the system biological tools, viz., genomics, proteomics, transcriptomics, fluxomics and metabolomics will be helpful to facilitate the design, characterization and integration of recent metabolic routes for the production of biofuel. The continuous use and progress in the conspiracy and the system tools and techniques in biological fields can only functionalization to later increase the concentrations and the production of enormous varieties of biofuels from numerous feedstocks that represents an excellent route for the viable production on industrial scale of renewable fuels critical to drop-off our dependence on fossil fuels.

26.5 Conclusions

A number of technology have been discussed for the production of biofuels such as biodiesel, biohydrogen, bioenergy, etc. are the renewable sources in recent time. These sources of energy have been demonstrated from microbes in MFCs and MECs. MFCs are a novel bioelectrochemical device that integrates treatment of organic wastes and wastewater and applied in the production of bioelectricity. The bioconversion methods that are based on the microbes are an excellent approach in the production of these biofuels from large renewable sources. A number of well-known processes such as synthetic biology and metabolic engineering, *E. coli* acts as a biocatalyst to yield a large variety of potential biofuels from a number of biomass constituents. Besides in the generation of bioelectricity, microbes have also been used in the production of biodiesel which are an alternative and research to improve the production efficiency. Therefore, microbes can become an ideal platform for the production of various bio-products in the near future.

References

Adams JM, Gallagher JI, Donnison SJ (2008) Fermentation study on Saccharina latissima for bioethanol production considering variable pre-treatments. Appl Phycol 21:569. https://doi.org/10.1007/s10811-008-9384-7

Adsul MG, Bastawde KB, Varma AJ, Gokhale DV (2007) Strain improvement of Penicillium janthinellum NCIM 1171 for increased cellulase production. Bioresour Technol 98:1467–1473. https://doi.org/10.1016/j.biortech.2006.02.036

Atsumi S, Hanai T, Liao JC (2008) Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 451:86–89. https://doi.org/10.1038/nature06450

Azhar SHM, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Faik AAM, Rodrigues KF (2017) Yeasts in sustainable bioethanol production: a review. Biochem Biophys Rep 10:52–61. https://doi.org/10.1016/j.bbrep.2017.03.003

Balat M, Balat H (2009) Recent trends in global production and utilization of bio-ethanol fuel. Appl Energy 86:2273–2228. https://doi.org/10.1016/j.apenergy.2009.03.015

Bardhan P, Gupta K, Mandal M (2019) Microbes as bio-resource for sustainable production of biofuels and other bioenergy products, Ch. 15. https://doi.org/10.1016/B978-0-444-64191-5.00015-8

Beopoulos A, Cescut J, Haddouche R, Uribelarrea JL, Molina-Jouve C, Nicaud JM (2009) Yarrowia lipolytica as a model for bio-oil production. Prog Lipid Res 48:375–387. https://doi.org/10.1016/j.plipres.2009.08.00

Bibra M, Wang J, Pinkelman RJ, Papendick S, Schneiderman S, Wood V, Amar V, Kumar S, Salem D, Sani RK (2015) Biofuels and value-added products from extremophiles, Ch. 2. Springer

Bond DR, Lovley DR (2003) Electricity production by Geobacter sulfurreducensattached to electrodes. Appl Environ Microbiol 69:1548–1555. https://doi.org/10.1128/AEM.69.3.1548-1555.2003

Buckel W, Thauer RK (2013) Energy conservation via electron bifurcating ferredoxin reduction and proton/Na + translocating ferredoxin oxidation. Biochim Biophys Acta Bioenerg 1827:94–113. https://doi.org/10.1016/j.bbabio.2012.07.002

Cabrol L, Marone A, Tapia-venegas E, Steyer J, Ruiz-filippi G, Trably E (2017) Microbial ecology of fermentative hydrogen producing bio-processes: useful insights for driving the ecosystem function. FEMS Microbiol Rev 41:158–181. https://doi.org/10.1093/femsre/fuw043

Case AE, Atsumi S (2016) Cyanobacterial chemical production. J Biotechnol 231:106–114. https://doi.org/10.1016/j.jbiotec.2016.05.023

Chaijak P, Sukkasem C, Lertworapreecha M, Boonsawang P, Wijasika S, Sato C (2018) Enhancing electricity generation using a laccase-based microbial fuel cell with yeast Galactomyces reessii on the cathodes. J Microbiol Biotechnol 28:1360–1366. https://doi.org/10.4014/jmb.1803.03015

Chaudhuri SK, Lovley DR (2003) Electricity generation by direct oxidation of glucose in mediatorless micro-bial fuel cells. Nat Biotechnol 21:1229–1232. https://doi.org/10.1038/nbt867

Chotani G, Dodge T, Hsu A, Kumar M, LaDuca R, Trimbur D, Weyler W, Sanford K (2000) The commercial production of chemicals using pathway engineering. Biochim Biophys Acta-Protein Struct Mol Enzymol 1543:434–455. https://doi.org/10.1016/s0167-4838(00)00234-x

Chung D, Cha M, Guss AM, Westpheling J (2014) Direct conversion of plant biomass to ethanol by engineered Caldicellulosiruptor bescii. Proc Natl Acad Sci U S A 111:8931–8936. https://doi.org/10.1073/pnas.1402210111

Cohen SE, Golden SS (2015) Circadian rhythms in cyanobacteria. Microbiol Mol Biol Rev 79:373–385. https://doi.org/10.1128/MMBR.00036-15

Dai H, Yang H, Liu X, Jian X, Liang Z (2016) Electrochemical evaluation of nano-Mg(OH)2/graphene as a catalyst for hydrogen evolution in microbial electrolysis cell. Fuel 174:251–256. https://doi.org/10.1016/j.fuel.2016.02.013

Davies FK, Work VH, Beliaev AS, Posewitz MC (2014a) Engineering limonene and bisabolene production in wild type and a glycogen-deficient mutant of Synechococcus sp. PCC 7002. Front Bioeng Biotechnol 2:21. https://doi.org/10.3389/fbioe.2014.00021

Davies FK, Work VH, Beliaev AS, Posewitz MC (2014b) Engineering limonene and bisabolene production in wild type and a glycogen-deficient mutant of Synechococcus sp. PCC 7002. Front Bioeng Biotechnol 2:22. https://doi.org/10.3389/fbioe.2014.00021

Deeba F, Pruthi V, Negi YS (2016) Converting paper mill sludge into neutral lipids by oleaginous yeast Cryptococcus vishniaccii for biodiesel production. Bioresour Technol 213:96–102. https://doi.org/10.1016/j.biortech.2016.02.105

Doherty L, Zhao Y, Zhao X, Wang W (2015) Nutrient and organics removal from swine slurry with simultaneous electricity generation in an alum sludge-based constructed wetland incorporating microbial fuel cell technology. Chem Eng J 266:74–81. https://doi.org/10.1016/j.cej.2014.12.063

Ghrabi A, Bousselmi L, Masi F, Regelsberger M (2011) Constructed wetland as a low cost and sustainable solution for wastewater treatment adapted to rural settlements: the Chorfech wastewater treatment pilot plant. Water Sci Technol 63:3006–3012. https://doi.org/10.2166/wst.2011.563

Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealson KH, Fredrickson JK (2006) Electrically conductive bacterial nanowires produced by Shewanella oneidensisstrain MR-1 and other microorganisms. Proc Natl Acad Sci U S A 103:11358–11363. https://doi.org/10.1073/pnas.0604517103

Gottumukkala LD, Parameswaran B, Valappil SK, Mathiyazhakan K, Pandey A, Sukumaran RK (2013) Biobutanol production from rice straw by a non acetone producing Clostridium sporogenes BE01. Bioresour Technol 145:182–187. https://doi.org/10.1016/j.biortech.2013.01.046

Gudmundsson S, Nogales J (2015) Cyanobacteria as photosynthetic biocatalysts: a systems biology perspective. Mol BioSyst 11:60–70. https://doi.org/10.1039/C4MB00335G

Gustafsson C, Govindarajan S, Minshull J (2004) Codon bias and heterologous protein expression. Trends Biotechnol 22:346–3532. https://doi.org/10.1016/j.tibtech.2004.04.006

Haiza N, Yasin M, Mumtaz T, Ali M, Aini N, Rahman A (2013) Food waste and food processingwaste for biohydrogen production: a review. J Environ Manag 130:375–385. https://doi.org/10.1016/j.jenvman.2013.09.009

He Z, Minteer SD, Angenent LT (2005) Electricity generation from artificial wastewater using an upflow microbial fuel cell. Environ Sci Technol 39:5262–5267. https://doi.org/10.1021/es0502876

He CS, Mu ZX, Yang HY, Wang YZ, Mu Y, Yu HQ (2015) Electron acceptors for energy generation in microbial fuel cells fed with wastewaters: a mini-review. Chemosphere 140:12–17. https://doi.org/10.1016/j.chemosphere.2015.03.059

Hirokawa Y, Maki Y, Tatsuke T, Hanai T (2016) Cyanobacterial production of 1,3-propanediol directly from carbon dioxide using a synthetic metabolic pathway. Metab Eng 34:97–103. https://doi.org/10.1016/j.ymben.2015.12.008

Horn SJ, Aasen IM, Ostgaard K (2000a) Production of ethanol from mannitol by Zymobacter palmae. J Ind Microbiol Biotechnol 24:51. https://doi.org/10.1038/sj.jim.2900771

Horn SJ, Aasen IM, Ostgaard K (2000b) Ethanol production from seaweed extract. J Ind Microbiol Biotechnol 25:249. https://doi.org/10.1038/sj.jim.7000065

Hou B, Sun J, Hu YY (2011) Simultaneous Congo red decolorization and electricity generation in air-cathode single-chamber microbial fuel cell with different microfiltration, ultrafiltration and proton exchange membranes. Bioresour Technol 102:4433–4438. https://doi.org/10.1016/j.biortech.2010.12.092

Huang L, Chai X, Chen G, Logan BE (2011a) Effect of set potential on hexavalent chromium reduction and electricity generation from biocathode microbial fuel cells. Environ Sci Technol 45:5025–5031. https://doi.org/10.1021/es103875d

Huang L, Chai X, Chen G, Logan BE (2011b) Effect of set potential on hexavalent chromium reduction and electricity generation from biocathode microbial fuel cells. Environ Sci Technol 45:5025–5503. https://doi.org/10.1021/es103875d

Huang L, Chai X, Quan X, Logan BE, Chen G (2012) Reductive dechlorination and mineralization of penta-chlorophenol in biocathode microbial fuel cells. Bioresour Technol 111:167–174. https://doi.org/10.1016/j.biortech.2012.01.171

Huang J, Chen D, Wei Y, Wang Q, Li Z, Chen Y et al (2014) Direct ethanol production from lignocellulosic sugars and sugarcane bagasse by a recombinant Trichoderma reesei strain HJ48. Sci World J 798683. https://doi.org/10.1155/2014/798683

Jang Y, Lee Y, Lee J, Park H, Im A, Eom M, Lee J, Lee SH, Song H, Cho JH, Lee SY (2012) Enhanced butanol production obtained by reinforcing the direct butanol-forming route in Clostridium acetobutylicum. MBio 3:1–9. https://doi.org/10.1128/mBio.00314-12

Kang U, Choi SK, Ham DJ, Ji SM, Choi W, Han DS, Abdel-Wahabe A, Park H (2015) Photosynthesis of formate from CO_2 and water at 1% energy efficiency via copper iron oxide catalysis. Energy Environ Sci 8:2638–2643. https://doi.org/10.1039/c5ee01410g

Kim D, Kim M (2011) Hydrogenases for biological hydrogen production. Bioresour Technol 102:8423–8431. https://doi.org/10.1016/j.biortech.2011.02.113

Kizer L, Pitera DJ, Pfleger BF, Keasling JD (2008) Application of functional genomics to pathway optimization for increased isoprenoid production. Appl Environ Microbiol 74:3229–3241. https://doi.org/10.1128/aem.02750-07

Kracke F, Vassilev I, Kromer JO (2015) Microbial electron transport and energy conservation–the foundation for optimizing bioelectrochemical systems. Front Microbiol 6:575. https://doi.org/10.3389/fmicb.2015.00575

Kremer TA, LaSarre B, Posto AL, McKinlay JB (2015) N2 gas is an effective fertilizer for bioethanol production by Zymomonas mobilis. Proc Natl Acad Sci U S A 112:2222–2226. https://doi.org/10.1073/pnas.1420663112

Kumar R, Kumar P (2017) Future microbial applications for bioenergy production: a perspective. Front Microbiol 8:1–4. https://doi.org/10.3389/fmicb.2017.00450

Kumar R, Singh L, Wahid ZA (2015) Chapter 9. Role of microorganisms in microbial fuel cells for bioelectricity production. In: Kalia VC (ed) Microbial factories, pp 136–154. https://doi.org/10.1007/978-81-322-2598-0_9

Lee TC, Xiong W, Paddock T, Carrieri D, Chang IF, Chiu HF, Ungerer J, Juo SH, Maness PC, Yu J (2015) Engineered xylose utilization enhances bio-products productivity in the cyanobacterium Synechocystis sp. PCC 6803. Metab Eng 30:179–189. https://doi.org/10.1016/j.ymben.2015.06.002

Li Z, Zhang X, Lin J, Han S, Lei L (2010) Azo dye treatment with simultaneous electricity production in an anaerobic–aerobic sequential reactor and microbial fuel cell coupled system. Bioresour Technol 101:4440–4445. https://doi.org/10.1016/j.biortech.2010.01.114

Li H, Opgenorth PH, Wernick DG, Rogers S, Wu TY, Higashide W, Malati P, Huo YX, Cho KM, Liao JC (2012) Integrated electromicrobial conversion of CO_2 to higher alcohols. Science 335:1596. https://doi.org/10.1126/science.1217643

Liao JC, Mi L, Pontrelli S, Luo S (2016) Fuelling the future: microbial engineering for the production of sustainable biofuels. Nat Rev Microbiol 14:288–304. https://doi.org/10.1038/nrmicro.2016.32

Lin PP, Mi L, Morioka AH, Yoshino KM, Konishi S, Xu SC et al (2015) Consolidated bioprocessing of cellulose to isobutanol using Clostridium thermocellum. Metab Eng 31:44–52. https://doi.org/10.1016/j.ymben.2015.07.001

Liu X, Ren N (2008) Recent advances in fermentative biohydrogen production. Prog Nat Sci 18:253–258. https://doi.org/10.1016/j.pnsc.2007.10.002

Liu H, Ramnarayanan R, Logan BE (2004) Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ Sci Technol 38:2281–2285. https://doi.org/10.1021/es034923g

Liu S, Song H, Wei S, Yang F, Li X (2014) Bio-cathode materials evaluation and configuration optimization for power output of vertical subsurface flow constructed wetland—microbial fuel cell systems. Bioresour Technol 166:575–583. https://doi.org/10.1016/j.biortech.2014.05.104

Liu C, Colon BC, Ziesack M, Silver PA, Nocera DG (2016) Water splitting-biosynthetic system with CO_2 reduction efficiencies exceeding photosynthesis. Science 352:1210–1213. https://doi.org/10.1126/science.aaf5039

Logan BE (2004) Extracting hydrogen electricity from renewable resources. Environ Sci Technol 38:160–167. https://doi.org/10.1021/es040468

Logan BE (2009) Exoelectrogenic bacteria that power microbial fuel cells. Nat Rev Microbiol 7:375–381. https://doi.org/10.1038/nrmicro2113

Logan BE, Wallack MJ, Kim KY, He W, Feng Y, Saikaly PE (2015) Assessment of microbial fuel cell configurations and power densities. Environ Sci Technol Lett 2:206–214. https://doi.org/10.1021/acs.estlett.5b00180

Lutke-Eversloh T, Bahl H (2011) Metabolic engineering of Clostridium acetobutylicum: recent advances to improve butanol production. Curr Opin Biotechnol 22:634–647. https://doi.org/10.1016/j.copbio.2011.01.011

Mahmod SS, Jahim JM, Abdul PM (2017) Pretreatment conditions of palm oil mill effluent (POME) for thermophilic biohydrogen production by mixed culture. Int J Hydrog Energy 42:27512–27522. https://doi.org/10.1016/j.ijhydene.2017.07.178

Marousek J, Itoh S, Higa O, Kondo Y, Ueno M, Suwa R, Komiya Y, Tominaga J, Kawamitsu Y (2012) The use of underwater high-voltage discharges to improve the efficiency of *Jatropha curcas* L. biodiesel production. Biotechnol Appl Biochem 59:451–456. https://doi.org/10.1002/bab.1045

Marousek J, Hasckova S, Zeman R, Vachal J, Vaníckova R (2014) Nutrient management in processing of steam-exploded lignocellulose phytomass. Chem Eng Technol 37:1945–1948. https://doi.org/10.1002/ceat.201400341

Masset J, Calusinska M, Hamilton C, Hiligsmann S, Joris B, Wilmotte A, Thonart P (2012) Fermentative hydrogen production from glucose and starch using pure strains and artificial co-cultures of Clostridium spp. Biotechnol Biofuels 5:35. https://doi.org/10.1186/1754-6834-5-35

McEwen JT, Kanno M, Atsumi S (2016) 2,3 Butanediol production in an obligate photoautotrophic cyanobacterium in dark conditions via diverse sugar consumption. Metab Eng 36:28–36. https://doi.org/10.1016/j.ymben.2016.03.004

Min B, Logan BE (2004) Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. Environ Sci Technol 38:5809–5814. https://doi.org/10.1021/es0491026

Mukhopadhyay A, Redding AM, Rutherford BJ, Keasling JD (2008) Importance of systems biology in engineering microbes for biofuel production. Curr Opn Biotechnol 19:228–234. https://doi.org/10.1016/j.copbio.2008.05.003

Muller J, MacEachran D, Burd H, Sathitsuksanoh N, Bi C, Yeh YC, Lee TS, Hillson NJ, Chhabra SR, Singer SW, Beller HR (2013) Engineering of Ralstonia eutropha H16 for autotrophic and heterotrophic production of methyl ketones. Appl Environ Microbiol 79:4433–4439. https://doi.org/10.1128/aem.00973-13

Mussatto SI, Machado EMS, Carneiro LM, Teixeira JA (2012) Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. Appl Energy 92:763–768. https://doi.org/10.1016/j.apenergy.2011.08.020

Nakamura C, Whited GM (2003) Metabolic engineering for the microbial production of 1,3-propanediol. Curr Opin Biotechnol 14:454–459. https://doi.org/10.1016/j.copbio.2003.08.005

Nichols EM, Gallagher JJ, Liu C, Su Y, Resasco J, Yu Y, Sun Y, Yang P, Chang MC, Chang CJ (2015) Hybrid bioinorganic approach to solar-to-chemical conversion. Proc Natl Acad Sci U S A 112:11461–11466. https://doi.org/10.1073/pnas.1508075112

Nielsen DR, Leonard E, Yoon SH, Tseng HC, Yuan C, Prather KLJ (2009) Engineering alternative butanol production platforms in heterologous bacteria. Metab Eng 11:262–273. https://doi.org/10.1016/j.ymben.2009.05.003

Niessen J, Schroder U, Scholz F (2004) Exploiting complex carbohy-drates for microbial electricity generations – a bacteria fuel cell operating on starch. Electrochem Commun 6:955–958. https://doi.org/10.1016/j.elecom.2004.07.010

Oh S, Logan BE (2005) Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. Water Res 39:4673–4682. https://doi.org/10.1016/j.watres.2005.09.019

Pal M, Sharma RK (2018) Exoelectrogenic response of Pichia fermentans influenced by mediator and reactor design. J Biosci Bioeng. https://doi.org/10.1016/j.jbiosc.2018.11.004

Pandey P, Shinde VN, Deopurkar RL, Kale SP, Patil SA, Pant D (2016) Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery. Appl Energy 168:706–723. https://doi.org/10.1016/J.APENERGY.2016.01.056

Panwar NL, Kaushik SC, Kothari S (2011) Role of renewable energy sources in environmental protection: a review. Renew Sust Energ Rev 15:1513–1524. https://doi.org/10.1016/J.RSER.2010.11.037

Patel SKS, Kumar P, Mehariya S, Purohit HJ, Lee J, Kalia VC (2014) Enhancement in hydrogen production by co-cultures of Bacillus and Enterobacter. Int J Hydrogen Energ 39:14663–14668. https://doi.org/10.1016/j.ijhydene.2014.07.084

Pejin J, Mojovic LJ, Vučurovic V, Pejin J, Denčic S, Rakin M (2009) Fermentation of wheat and triticale hydrolysates: a comparative study. Fuel 88:1625–1628. https://doi.org/10.1016/j.fuel.2009.01.011

Pfleger B, Pitera DJ, Smolke CD, Keasling JD (2006) Combinatorial engineering of intergenic regions in operons tunes expression of multiple genes. Nat Biotechnol 8:1027–1032. https://doi.org/10.1038/nbt1226

Pitera DJ, Paddon CJ, Newman JD, Keasling JD (2007) Balancing a heterologous mevalonate pathway for improved isoprenoid production in Escherichia coli. Metab Eng 9:193–207. https://doi.org/10.1016/j.ymben.2006.11.002

Przybylski D, Rohwerder T, Dilssner C, Maskow T, Harms H, Muller RH (2015) Exploiting mixtures of H_2, CO_2, and O_2 for improved production of methacrylate precursor 2-hydroxyisobutyric acid by engineered Cupriavidus necator strains. Appl Microbiol Biotechnol 99:2131–2145. https://doi.org/10.1007/s00253-014-6266-6

Rabaey K, Hofte M, Vesrtraete W, Boon N (2005) Microbial phenazine production enhances electron transfer in biofuel cells. Environ Sci Technol 39:3401–3340. https://doi.org/10.1021/es048563o

Raj SM, Talluri S, Christopher LP (2012) Thermophilic hydrogen production from renewable resources: current status and future perspectives. Bioenerg Res 5:515–531. https://doi.org/10.1007/s12155-012-9184-4

Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. Nature 435:1098–1101. https://doi.org/10.1038/nature03661

Reischl B, Rittmann SKR (2018) Biohydrogen production characteristics of Desulfurococcus amylolyticus DSM 16532. Int J Hydrog Energy 43:8747–8753. https://doi.org/10.1016/j.ijhydene.2018.03.121

Ro DK, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, Ho KA, Eachus RA, Ham TS, Kirby J, Chang MCY, Withers ST, Shiba Y, Sarpong R, Keasling JD (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440:940–943. https://doi.org/10.1038/nature04640

Robertson DE, Jacobson SA, Morgan F, Berry D, Church GM, Afeyan NB (2011) A new dawn for industrial photosynthesis. Photosynth Res 107:269–277. https://doi.org/10.1007/s11120-011-9631-7

Roesijadi G, Jones SB, Snowden-Swan LJ, Zhu Y (2010) Macroalgae as a biomass feedstock: a preliminary analysis, prepared for the U.S. Department of Energy under contract DE-AC05-76RL01830 by Pacific Northwest National Laboratory

Romero-Garcia JM, Martínez-Patio C, Ruiz E, Romero I, Castro E (2016) Ethanol production from olive stone hydrolysates by xylose fermenting microorganisms. Bioethanol 2:51–65. https://doi.org/10.1515/bioeth-2016-0002

Sabra W, Groeger C, Sharma PN, Zeng A (2014) Improved n-butanol production by a non-acetone producing Clostridium pasteurianum DSMZ 525 in mixed substrate fermentation. Appl Microbiol Biotechnol 98:4267–4276. https://doi.org/10.1007/s00253-014-5588-8

Saha R, Liu D, Hoynes-Oconnor A, Liberton M, Yu J, Bhattacharyya-Pakrasi M, Balassy A, Zhang F, Moon TS, Maranas CD, Pakrasi HB (2016) Diurnal regulation of cellular processes in the Cyanobacterium Synechocystis sp. strain PCC 6803: insights from transcriptomic, fluxomic, and physiological analyses. MBio 7. https://doi.org/10.1128/mBio.00464-16

Santoro C, Arbizzani C, Erable B, Ieropoulos I (2017) Microbial fuel cells: from fundamentals to applications, a review. J Power Sources 356:225–244. https://doi.org/10.1016/J.JPOWSOUR.2017.03.109

Savakis P, Hellingwerf KJ (2015) Engineering cyanobacteria for direct biofuel production from CO_2. Curr Opin Biotechnol 33:8–14. https://doi.org/10.1016/j.copbio.2014.09.007

Sharma RK, Arora DS (2010) Production of lignocellulolytic enzymes and enhancement of in vitro digestibility during solid state fermentation of wheat straw by Phlebia floridensis. Bioresour Technol 101:9248–9253. https://doi.org/10.1016/j.biortech.2010.07.042

Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM, Liao JC (2011a) Driving forces enable high-titer anaerobic 1-butanol synthesis in Escherichia coli. Appl Environ Microbiol 77:2905–2915. https://doi.org/10.1128/AEM.03034-10

Shen CR, Lan EI, Dekishima Y, Baez A, Cho KM, Liao JC (2011b) Driving forces enable high-titer anaerobic 1-butanol synthesis in Escherichia coli. Appl Environ Microbiol 77:2905–2915. https://doi.org/10.1128/AEM.03034-10

Singh L, Wahid ZA (2015) Enhancement of hydrogen production from palm oil mill effluent via cell immobilisation technique. Int J Energy Res 3:21522. https://doi.org/10.1002/er.3231

Singh L, Siddiqui MF, Ahmad A, Rahim MH, Sakinah M, Wahid ZA (2013) Biohydrogen production from palm oil mill effluent using immobilized mixed culture. J Ind Eng Chem 19:659–664. https://doi.org/10.1016/j.jiec.2012.10.001

Singhania RR, Saini JK, Saini R, Adsul M, Mathur A, Gupta R, Tuli DK (2014) Bioethanol production fromwheat strawvia enzymatic route employing Penicillium janthinellumcellulases. Bioresour Technol 169:490–495. https://doi.org/10.1016/j.biortech.2014.07.011

Slate AJ, Whitehead KA, Brownson DAC, Banks CE (2019) Microbial fuel cells: an overview of current technology. Renew Sust Energ Rev 101:60–81. https://doi.org/10.1016/J.RSER.2018.09.044

Somerville C, Youngs H, Taylor C, Davis SC, Long SP (2010) Feedstocks for Lignocellulosic biofuels. Science 329:790. https://doi.org/10.1126/science.1189268

Thang VH, Kanda K, Kobayashi G (2010) Production of acetone–butanol–ethanol (ABE) in direct fermentation of cassava by Clostridium sac-charoperbutylacetonicum N1-4. Appl Biochem Biotechnol 161:157–170. https://doi.org/10.1007/s12010-009-8770-1

Torella JP, Gagliardi CJ, Chen JS, Bediako DK, Colon B, Way JC, Silver PA, Nocera DG (2015) Efficient solar-to-fuels production from a hybrid microbial-water-splitting catalyst system. Proc Natl Acad Sci U S A 112:2337–2342. https://doi.org/10.1073/pnas.1424872112

Uhlen M, Svahn HA (2011) Lab on a chip technologies for bioenergy and biosustainability research. Lab Chip 11:3389–3393. https://doi.org/10.1039/c1lc90063c

Varman AM, Yu Y, You L, Tang YJ (2013) Photoautotrophic production of D-lactic acid in an engineered cyanobacterium. Microb Cell Factories 12:117. https://doi.org/10.1186/1475-2859-12-117

Vemuri GN, Altman E, Sangurdekar DP, Khodursky AB, Eiteman MA (2006) Overflow metabolism in *Escherichia coli* during steady-state growth: transcriptional regulation and effect of the redox ratio. Appl Environ Microbiol 72:3653–3661. https://doi.org/10.1128/aem.72.5.3653-3661.2006

Verhaart MRA, Bielen AAM, Van der Oost J, Alfons JM, Kengen SWM, Verhaart MRA, Alfons JM (2010) Hydrogen production by hyperthermophilic and extremely thermophilic bacteria and archaea: mechanisms for reductant disposal. Environ Technol 31:993–1003. https://doi.org/10.1080/09593331003710244

Westfall PJ, Pitera DJ, Lenihan JR, Eng D, Woolard FX, Regentin R et al (2012) Production of amorphadiene in yeast, and its conversion to dihydroartemisinic acid, precursor to the antimalarial agent artemisinin. Proc Natl Acad Sci U S A 109:111–118. https://doi.org/10.1073/pnas.1110740109

Yang G, Wang J (2018) Kinetics and microbial community analysis for hydrogen production using raw grass inoculated with different pretreated mixed culture. Bioresour Technol 247:954–962. https://doi.org/10.1016/j.biortech.2017.09.041

Yang S, Mohagheghi A, Franden MA, Chou YC, Chen X, Dowe N et al (2016) Metabolic engineering of Zymomonas mobilis for 2, 3-butanediol production from lignocellulosic biomass sugars. Biotechnol Biofuels 9:189. https://doi.org/10.1186/s13068-016-0606-y

Yilanci A, Dincer I, Ozturk HK (2009) A review on solar-hydrogen/fuel cell hybrid energy systems for stationary applications. Prog Energy Combust Sci 35:231–244. https://doi.org/10.1016/j.pecs.2008.07.004

Yu AQ, Juwono NKP, Foo JL, Leong SSJ, Chang MW (2016) Metabolic engineering of Saccharomyces cerevisiae for the overproduction of short branched-chain fatty acids. Metab Eng 34:36–43. https://doi.org/10.1016/j.ymben.2015.12.005

Zhang X, Zi L, Ge X, Li Y, Liu C (2017) Development of Trichoderma reeseimutants by combined mutagenesis and induction of cellulase by low-cost corn starch hydrolysate. Proc Biochemist 54:96–101. https://doi.org/10.1016/j.procbio.2016.12.027

Zuo Y, Maness PC, Logan BE (2006) Electricity production from steam-exploded corn stover biomass. Energy Fuel 20:1716–1721. https://doi.org/10.1021/ef060033l