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Waste to Energy: Prospects and Applications

Springer

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Editors

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Part I

Introductory Chapters

Emerging Frontiers of Microbes as Agro-Waste Recycler

1

Shalini Rai, Manoj Kumar Solanki, Ajit Kumar Dubedi Anal, Alka Sagar, Anjali Chandrol Solanki, Brijendra Kumar Kashyap, and Akhilesh Kumar Pandey

Abstract

Sustainable agriculture and environmental protection have the foremost importance in the welfare of human being. Annually, agro-wastes are generated at the millions of tonnes scale worldwide that must be degraded in terms of valuable products as well as the concept of sustainable agriculture can also be implemented through the bioconversion of agro residue into other resources without harming and depleting the natural ecosystem. Microbes are a crucial player for the conversion of agro-waste into valuable products, extraction of minerals, enhancement of agriculture, and agro-waste management. So the use of microorganisms with different biotechnological approaches is the most effective method to treat different wastes, in addition to being eco-friendly, cost-effective, and

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environmentally sustainable method. Ultimately, in this way to a meaningful and significant extent, the present chapter can bridge the gap between the adoption of microbial bioconversion technologies for valuable product formation and recycling of agro-waste into wealth, considering innovative and potentially economical approach towards sustainable agriculture as well as the eco-friendly environment.

Keywords

Agro-waste · Microbes · Sustainable agriculture · Bioconversion

1.1 Introduction

Sustainable agriculture has emerged to be the central theme for researchers, scientist, and farmers due to excessive demand for food commodities to the fulfillment world population. In this regards, agricultural production has pressurized to the use of high-yield varieties which were contributed for the vast amount production of agricultural-based residues every year. A report of Belewu and Babalola (2009) were estimated the production of wheat straw and rice straws residues approximately 709.2 and 673.3 million metric tons, respectively. Recently, Sadh et al. (2018) reported that the total production of agro-waste fiber sources is approximately found 147.2 million metric tons in all over the world, which comprises crop residues and processed agricultural wastes. Thus, the accumulation of these agro-wastes causes a severe disposal problem (Sud et al. 2008; Leow et al. 2018). Improper disposal of these agricultural residues has raised several problems concerned with the soil fertility, soil agroecology, environmental pollution, and harmful effect on plant and animal health (Rodríguez-Couto 2008). Several researchers reported that the disposal of untreated agro-waste are treated by dumping, burning or unplanned landfilling (Bhuvaneshwari et al. 2019). Untreated agro-waste has led to a severe deleterious polluting impact on soil fertility, shifting of beneficial microbial communities, emission of greenhouse gases, air pollution, subsequent soil erosion, and climate change (Singh and Nain 2014; Bos and Hamelinck 2014). Indiscriminate and untreated agro-waste disposal have altered agricultural land and created physical, chemical, and biological deterioration of cultivable soil.

The composition of agro-wastes classified into two principal constituents, insoluble chemical constituents (e.g., cellulose, hemicellulose and lignin) and soluble constituents (e.g., sugar, amino acids, and organic acids). Some other reported constituents are fats, oil waxes, resins, pigment, protein, and mineral. However, due to high nutritional composition, these agro-wastes are considered as the primary source for other valuable product formation and developments. Therefore, agro-wastes are the cheapest source that can be used by microbes for growth and produce valuable products through bioconversion and fermentation process. Thus, researchers have been searching for naturally occurring technologies for

enhancement of agriculture and management of agro-waste into valuable products through natural microbial conversion. Hence the recycling of these wastes is not only an ecological necessity but also an economic compulsion in the welfare of humanity.

Microorganisms are widely distributed in the biosphere because of their metabolic ability and nutritional versatility to utilize different substrates to grow in a wide range of environmental conditions. The metabolic activity and biosynthetic capability of certain microorganisms to modify, convert, and utilize agro-waste in order to obtain energy and biomass production give new insight towards microbes based natural bioconversion. In this order, wide ranges of microbial communities are bacteria, archaea, and fungi reported as prime natural bio-converter. The unique nature of microorganisms has been used to advance food processing and safety, food quality improvement, ecological restoration, environmental protection, high-yield crop production, and biotechnology-based bioconversion into valuable products. The application of agro-waste bioconversion through biotechnological process shows immense attention towards involving microorganisms for solving the dangers of many pollutants and advancing for the production of valuable products (Nguyen et al. 2010). In concern of development of effective, low-cost technologies for efficient bioconversion of agro-waste, the biotechnological process is the new thrust of research in concern of soil health, ecological, and environmental restoration and improvising plant nutrition through recycling of residues.

In order of successive bioconversion of agro-waste through different microbes mediated biotechnological processes utilized these as raw material for the production of value-added products such as amino acids, enzymes, organic acids, biofuel, single-cell protein (SCP) animal feed, edible mushroom, bioactive secondary metabolites, nanomaterial and biofertilizers. With the advent of biotechnological innovation, many new opportunities have opened for utilization of agro-waste with minimizing the threats of environmental pollution and animal hazards. This chapter covered the review on the significance of microbe-mediated agro-waste bioconversion and biotechnological approaches for valuable product formation and ecological recycling. The aim of this chapter is to express the current trend the application/role of microorganisms on agro-waste bioconversion or/fermentation into valuable and harmless products. The usefulness of microbe based biotechnological process in producing valuable products has also been summarized with specific examples.

Microorganisms are inhabiting the soil and the surfaces of all living things inside and outside which have the potentiality in biodegradation, bioleaching, bio-composting, nitrogen fixation, improving soil fertility and as well in the production of plant growth hormones. Bioconversion, more specifically composting of agricultural residues refers to step-wise bio-decomposition procedures carried out due to the intervention of different microbial communities under aerobic conditions (Pan et al. 2012). The end product of the aerobic composting yields stabilized organic product, which is beneficial for plant growth and development. Efforts on microbial intervention for better decomposition gained strength from the identification and characterization of such microbial communities from the agricultural soils, composts, vermicompost and humus-rich sites, that prominently catalyzed biodegradation and decomposition (Eida et al. 2012). Scaling-up of bioconversion

processes and large-scale production technologies using microbial inoculants have resulted in producing mass-scale composted material that may be bio-augmented with beneficial microorganisms or fortified with organic inputs, bio-inoculants, and vermicompost (Singh and Sharma 2002; Nair and Okamitsu 2012; Malusá et al. 2012). Composted products were reported to act as soil conditioners in low-cost crop production practices for resource-poor farming communities (Gajalakshmi and Abbasi 2008).

1.2 Agro-Waste

Agro-waste is a considerable term that comprises complex materials such as straws and stems of cereal grains (rice, wheat, barley, and corn), legume waste, bagasse, husks, cobs, fruit peels, and any part of a processed plant source (Yazid et al. 2017). Due to extensive agricultural activities, the global production of agriculture residues in a year is approximately 998 million tons, while 500 million tons, alone reported in India (Loow et al. 2015; Bhuvaneshwari et al. 2019). Agro-waste is produced from various post-harvest agriculture activities. The chemical composition of agriculture residues is comprised of lignocellulosic materials and polyphenolic compounds that required complex processes for bioconversion (Sannik et al. 2013). Agro-waste is broadly classified into two different types of wastes, i.e., agriculture residues and processed agriculture residues (Fig. 1.1).

1.2.1 Agricultural Residues

Agriculture residues are usually produced through farming activities and post-crop harvesting. These residues consist of leaves, stems, plant stalks, hulls, seedpods, vegetable matter, mushroom bedding, molasses, husks, bagasse, seeds, straw, shell, pulp, stubble, peel, roots, that is often useless and will be discarded without proper disposal. In addition to this, various other crops like rice, lentils, maize, chickpeas, fruits, and vegetables are also produced all over the world. A tremendous amount of agriculture residues can be utilized as animal feed, soil improvement, fertilizers, manufacturing, and various other processes.

1.2.2 Processed Agricultural Residues

Processed agricultural residues can be defined as the generation of residues after the crop is processed into a valuable alternate resource. India is the second-largest cereals, fruits, and vegetables producer, while approximately 20% of the production is going waste every year (Rudra et al. 2015). A huge amount of processed agriculture residues are produced every year through the processing industries like juice, beverages, chips, confectionery, fruit, and oil industries. These residues can be utilized for different energy sources. The compositions of process agriculture wastes

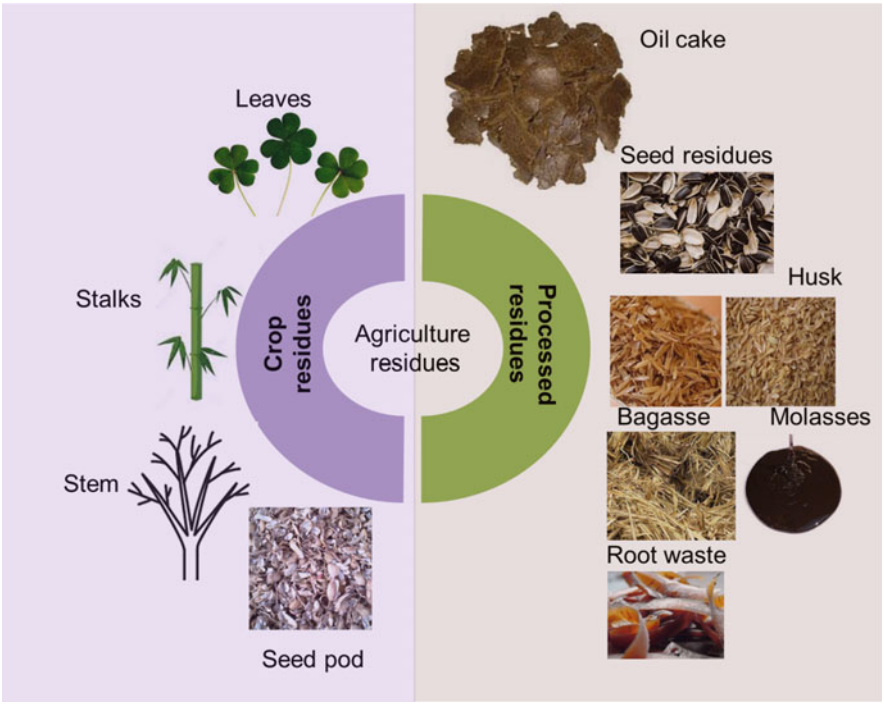

Fig. 1.1 Different kinds of agriculture residues

comprise cellulose, hemicellulose, lignin, moisture, ash, carbon, nitrogen, while bioconversion of these constituents has potential to produce useful products like biogas, bioethanol, biofertilizers, biodiesels, enzymes, and other commercially useful products that reduce the cost of production. One of the potential sources of processed residues is oil industries, produces through the process of oil extraction from seeds (known as oil cakes). The presence of substrate defines the types of oil cakes like canola oil cake, coconut oil cake, cottonseed cake, groundnut oil cake, mustard oil cake, palm kernel cake, sesame oil cake, sunflower oil cake, soybean cake, olive oil cake, and rapeseed cake (Ramachandran et al. 2007). These processed residues are relatively cheap, containing a high amount of constituents that have an unlimited prospective to be consumed as alternative substrates for fermentation.

1.3 Microbes and Agro-Waste Bioconversion/Role of Microorganism in Bioconversion of Agro-Waste

Microorganisms are the key player in the recycling of agricultural wastes (Fig. 1.2). The exceptionality of microorganisms and their biodegrading quality have made them potential candidates for decomposing agricultural residues into valuable products (Kumar and Sai Gopal 2015). Recent reports also indicate multifarious

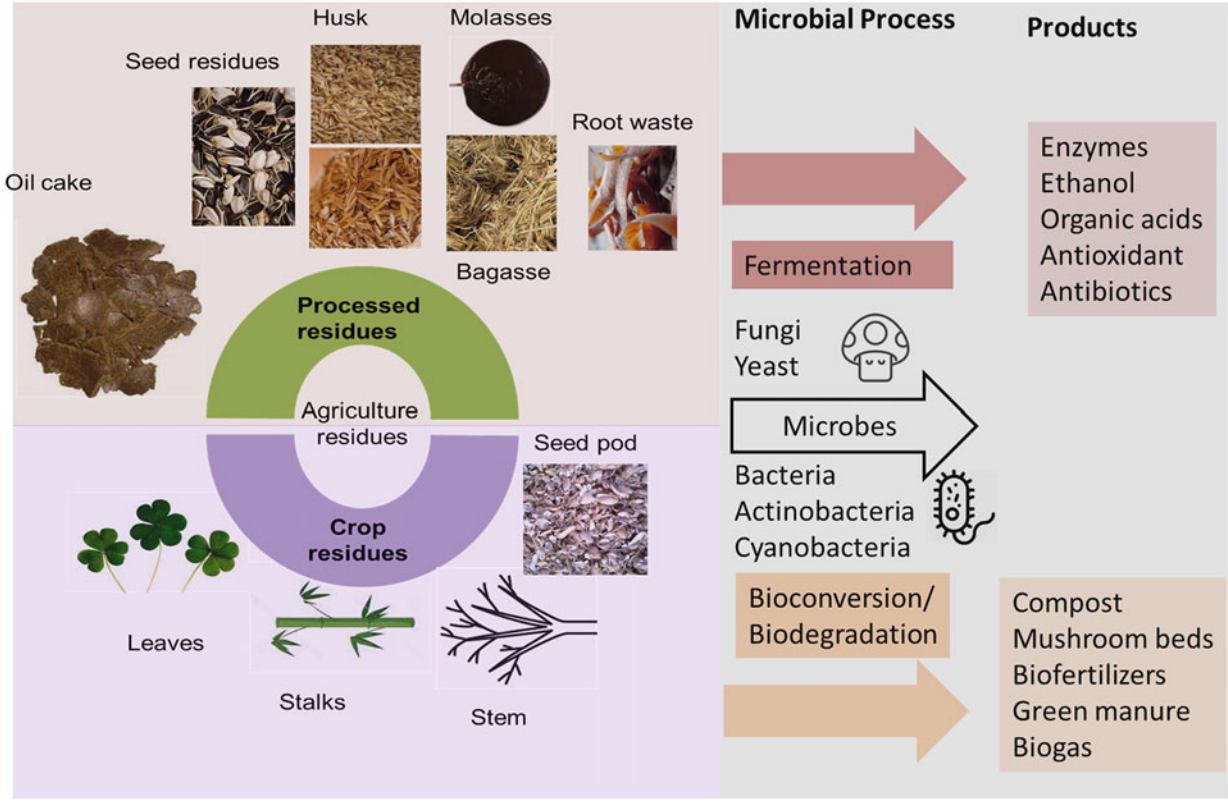

Fig. 1.2 Role of microbes in waste recycling

uses of microorganisms as a modern technique to provide an efficient way to advance human and animal health, food processing, food safety and quality, environmental protection, crop production and production of value-added products. There is a definite need to intensify research on effective microorganisms that convert agriculture waste into high-quality, valuable products in a relatively shorter duration and agricultural biotechnology has made alternatives for large-scale production.

Second most abundant plant material is lignocellulose that is composed of polysaccharides like cellulose, hemicellulose, and lignin which represents the major structural component of agricultural crop residues (Pothiraj et al. 2006; Singh and Nain 2014). Various agricultural residues that contain up to 20–30% lignin–hemicellulose–have potential biotechnological values, and their bioconversion and/or fermentation to yield industrially important constituents including biofuels, biofertilizers, biogas, enzymes, and organic acids (Sorek et al. 2014). The synergistic action microorganisms, viz. bacteria, fungi, and mycorrhiza, are immensely involved in bioconversion of complex lignocellulosic wastes into smaller molecules through the action of microbial enzymes such as cellulases, glucanases, hemicellulases, glycosidase hydrolases, polysaccharide lyases, and carbohydrate esterases (Himmel et al. 2010) which is utilized in the production of value-added products such as chemicals, fuel, textile, paper, and agricultural inputs (Pothiraj et al. 2006). There have been several reports on the isolation and characterization of potential microbial communities (bacteria, actinomycetes, yeast, fungi, and mycorrhizal fungi) that can significantly convert agro-waste and perform functionally better in combination with other organisms for the production of valuable products through different biotechnological approaches (Chandra et al. 2012; Yildirim et al. 2015; Vishan et al. 2017; Ribeiro et al. 2017).

Microbial communities have emerged to decompose the discarded agro-waste and maintained the nutrient pool in the soils, which mobilized into the plants and microbial biomass (Miki et al. 2010; Sadh et al. 2018). It further regulates the cycling of nutrients into the soils. To maintain the nutrient pool in the soil, composting is another way of biological degradation and stabilization of organic agro residues with several benefits such as enhanced soil fertility and soil health which can lead to increased agricultural productivity, improved soil biodiversity, reduced ecological risks, and a healthier environment. These advantages make composting an ideal option for processing of the enormous quantities of agro residues through a natural succession of microflora. Several fungi like *Trichoderma harzianum, Pleurotus ostreatus*, *Polyporus ostriformis,* and *Phanerochaete chrysosporium* are known to play an essential role in composting of lignocellulosic materials. A series of microorganisms and their metabolic actions that help in fast decomposition, biodegradation, and bioconversion of agro residues into valuable products are listed in Table 1.1.

Table 1.1 List of major studies illustrating effect of microorganisms in bioconversion activity of agro-waste

Microorganisms	Agro-waste nature	Mode of bioconversion	Impact	Reference
Bacteria and actinomycetes				
Pseudomonas putida	Agro-waste	Manganese peroxydases and laccase	High potential for degradation of xenobiotic compounds	Ahmad et al. (2010)
Geobacillus strains	Vegetable waste	Ligninolytic enzymes	Boost the total bacterial count to enhance bioconversion process	Pal et al. (2010)
Pseudomonas aeruginosa	Agro-waste	Manganese peroxidases, lipid peroxidase, and laccase	Enhance agro-waste bioconversion and the synthesis of monomer for other product formation	Bholay et al. (2012)
Serratia marcescens	Agro-waste	Manganese peroxidases, lipid peroxidase, and laccase	Degradation of agro-waste into organic material rich compost	Chandra et al. (2012)
Mono and co-cultures of *Bacillus subtilis* and *P. ostreatus*	Apple and plum wastes mixed with cereal wastes	Cellulase	Effective degradation of agro-waste and minimize pollutant effect	Petre et al. (2014)
Citrobacter freundii	Combination of agro-waste and saw dust	Manganese peroxidases, lignin degradation	Degradation of lignocellulytic waste and enhance rate of bioremediation	Ali et al. (2017)
B. cereus, B. megaterium	Organic substrate	Cellulase	Breakdown of cellulose and hemicelluloses in simplest sugar	Ribeiro et al. (2017)
Pseudomonas fragi, P. simiae, Clostridium vincentii, P. jessenii, and Iodobacter fluviatilis	Food waste and maize straw	Cellulase, manganese peroxidases, laccase, and xylanase	Contributed to enhanced composting process with mixed culture at low temperature	
Enterobacter spp	Sugarcane trash, grass powder, sorghum husk, wheat straw,	Cellulase and xylanase	Contributed to enhanced conversion of biomass into enzyme production	Waghmare et al. (2018)

(continued)

Table 1.1 (continued)

Microorganisms	Agro-waste nature	Mode of bioconversion	Impact	Reference
	and water hyacinth			
Actinomycetes strain	Domestic agro-waste	Cellulase, manganese peroxidases, laccase, and xylanase	Contributed to enhanced conversion of biomass into compost formation	Limaye et al. (2017)
Streptomycetes sp	Saw dust and coffee residue	Cellulase	Contributed to enhanced composting process	Eida et al. (2012)
Fungi				
Aspergillus awamori	Agro-wastes	Action of Cellulases	Degradation of cellulose and hemicellulose-containing biomass	Pleissner et al. (2013)
Phanerochaete chrysosporium	Wood shavings, agro-wastes	Lignin peroxydases, glyoxal oxidase, manganese peroxydases enzyme activity	Increased degradation of lignin and phenolics, minimizes risk of composting of lead contaminated agricultural waste	Zhang et al. (2013)
Pleurotus eryngii	Agricultural wastes	Lignocellulose degradation through laccase enzyme activity	Bioconversion and degradation of phenolics	Yildirim et al. (2015)
Pestalotiopsis sp	Forest litter mixed with agro-waste	Cellulases and laccases	Degradation of cellulose and lignocellulosic biomass and enzyme production for bioremediation	
Trichoderma harzianum, T. Koningii	Oil palm empty fruit bunches	Hemicellulose degradation	Pre-decomposition of organic matter for production of compost	Saud et al. (2013)
Marasmius sp	Agricultural wastes	Laccase	Bioconversion of lignocellulosic biomass and enzyme production for bioremediation of azo dyes	Vantamuri and Kaliwal (2016)

1.3.1 Bacterial Bioconversion

The role of bacterial community as bioconversion agents is essential due to their fast ability to convert cellulosic and lignocellulosic wastes into organic materials. Cellulose-degrading bacterial community is ubiquitous that hasten the biodegradation of crop residues such as straw, leaves, trash, etc., that ultimately solubilize and modify into the nonhazardous and valuable products in human welfare. Successful bioconversion of organic matter by the addition of bacteria had been reported earlier for many agro residues, including rice bran, wheat bran, maize straw, paddy straw, black gram husk, vegetable waste, apple, plum wastes mixed with cereal wastes, and sawdust (Faisal et al. 2014; Kaur et al. 2015; Oliveira et al. 2017; Singh et al. 2019). Recent findings concerning cellulose-degrading bacteria include the *Bacillus cereus, B. megaterium*, *Amycolatopsis mediterranean, Xanthomonas campestris, Pseudomonas* spp., and *Serratia marcescens* able to degrade lignocellulosic material with the action of bacterial enzymes, such as cellulase, xylanase, laccase, manganese peroxidases, and lipid peroxidase (Vastrad and Neelagund 2011a, b; Vidhyalakshmi et al. 2012; Chandra et al. 2012; Faisal et al. 2014; Sadh et al. 2018).

1.3.2 Fungal and Mycorrhizal Bioconversion

Fungi are eukaryotic, saprophytic, aerobic microorganisms which include unicellular (yeasts) to mycelial (molds). Fungal communities have emerged to influence agro-waste decomposability and maintain the nutrient pool in the soils. Fungi are considered as the most efficient bio-degrader of natural polymeric compounds of agro-waste with the help of extracellular multienzyme complexes and eliminate the hazardous wastes from the environment. Similarly, mycorrhiza is an association between a fungus and roots of a vascular plant that can degrade complicated organic matter of agro-waste, induce nutrient mineralization and maintain the nutrient pool in the soils.

Various fungal communities were reported as fast decomposers, bio-degraders, and bio-converters of non-useful products (Gautam et al. 2012). Fungal communities are saprophytic and develop fast in the straw residue due to the presence of well-equipped enzymatic machinery and metabolic pathways that help to degrade agro residues (Ma et al. 2013). Their hyphal system provides a mechanical assistant to colonize and penetrate substrates rapidly that helps in transporting and redistribution of nutrients within their biomass. Several fungi like *Aspergillus niger*, *A. awamori*, *Trichoderma harzianum, T. reesei, Penicillium brasilianum, Pleurotus ostreatus, P. eryngii, Polyporus ostriformis,* and *Phanerochaete chrysosporium* are known to play an important role in biodegradation/bioconversion of lignocellulosic materials through production of several enzymes, viz., cellulases, xylanases, lignin peroxidases, glyoxal oxidase, manganese peroxidases, laccase, glucosidase, and esterase (Jorgensen et al. 2003; Romero et al. 2007; Pleissner et al. 2013; Zhang et al. 2013; Yildirim et al. 2015; Mahalakshmi and Jayalakshmi 2016). Several

researchers reported various fungal genera, namely, *Pleurotus fabellatus*, *Trametes versicolor,* and *Phanerochaete chrysosporium* were proved to be the potential organisms for enhanced decomposition and degradation when applied on a different combination of agricultural residues (Rice straw, sisal leaves, sugarcane bagasse, and woody shavings) (Cabuk et al. 2006; Mshandete and Cuf 2008; Huang et al. 2009). Potential microorganisms with impressive enzymatic capabilities for fast degradation/bioconversion/fermentation of rich lignocellulosic material and their impact on the environment are discussed (Table 1.1).

1.4 Factor Affecting Microbial Agro-Waste Conversion

Bioconversion process is the sequential degradation, immobilization, and/or detoxification of various agro-wastes comprising high lignocellulosic material from the environment through the action of bacteria, fungi, invertebrates, and plants. The efficiency of bioconversion depends on many factors; including, the biochemical nature and concentration of organic content in agro-waste, physicochemical characteristics of the environment, and their availability to microorganisms (Abatenh et al. 2017; Singh et al. 2019). The bioconversion processes is a complex system due to many factors, such as a microbial population capable of degrading the agro-waste, the availability of nutrient of agro-waste to the microbial population and environment factors (types of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients). The metabolic characteristics of the microorganisms and physicochemical properties of the different agro-wastes determine possible interaction during the bioconversion process. Microorganism growth, activity and kinetics of degradation are affected by soil structure, solubility in water, and availability of nutrients, pH, temperature, moisture, redox potential, and oxygen content.

To survive and continue their microbial activities microorganisms need a number of nutrients such as carbon, nitrogen, and phosphorous that channelize the nutrient balance for microbial growth and reproduction as well as increasing the biodegradation rate and effectiveness of agro-waste. Other most important determining physical factors for the survival of microorganisms and degradation of constituents of the agro-waste are temperature. The microbial physiological properties fluctuate due to change in temperature. As a result, temperature influence the bioconversion process either speed up or slow down. Microbial enzymes have participated in the degradation pathway that is maximum at optimum temperature and will not have the same metabolic turnover for every temperature.

Moreover, the degradation process for the specific composition of agro-waste needs specific temperature. Among the physical factors, pH is the most important one to determining the survival of microorganisms and nature of the agro-waste which may be acidic, basic, and alkaline that can be converted into the valuable product through microbial metabolic. Higher or lower pH values showed inferior results, while metabolic processes are optimum at the correct pH. Microorganisms require adequate water to accomplish their growth, so moisture content has a significant effect on the bioconversion of agro-waste. The concentration of oxygen

is another decisive factor for microbial growth as well as bioconversion of agro-waste. The requirement of oxygen is different for different organisms (aerobic, anaerobic, facultative, and obligate aerobic or anaerobic) which facilitate the bioconversion rate in a better way. Microbial degradation is carried out in aerobic and anaerobic condition through degradation, bioconversion, and fermentation process because oxygen is a gaseous requirement for most living organisms.

1.5 Biotechnological Approaches of Microbial Bioconversion of Agro-Waste

The application of biotechnological approaches in the production of different bio-products has been widely reported including enzymes, organic acids, biofertilizers, biopesticides, biosurfactants, bioethanol, aroma compounds, animal feed, pigments, vitamins, and antibiotics (Tsouko et al. 2017). A variety of microorganisms are used for the production of these valuable products through bioconversion/fermentation processes. These biotechnological approaches have opened a new model of bioconversion of agro-wastes through the production of biologically active metabolites both at the lab and industrial scale. Therefore, biotechnological approaches and their technologies for the formation of value-added products by bioconversion/fermentation process are reviewed and listed in Table 1.2.

The production of different valuable products depends upon the basic composition of agro-waste. The variety of processed agro-waste such as coconut husks, corn cobs, candelilla stalks, oil cakes, fruit peel waste, Rice bran, wheat bran, black gram bran, soybean, and sugarcane bagasse were used by several researchers for the production valuable enzymes (Buenrostro et al. 2013; Mehta and Duhan 2014; Saharan et al. 2017). The variable composition of agro-waste supports the growth of microorganisms and through fermentation different valuable enzymes such as amylase (Duhan et al. 2013; Kumar et al. 2013), glucoamylase (Suganthi et al. 2011), invertase (Mehta and Duhan 2014), cellulase, lipase (Oliveira et al. 2017), xylanase, Pectin methylesterase (Gayen and Ghosh 2011), and β-glucosidase (Sadh et al. 2017). Several researchers reported enormous bacterial, viz., *Bacillus* sp. (Sodhi et al. 2005), *Pseudomonas aeruginosa* (Dharmendra 2012), and fungal, viz., *Aspergillus niger* (Sharanappa et al. 2011; Sindiri et al. 2013), *Penicillium notatum* (Gayen and Ghosh 2011), *Candida rugosa* (Rekha et al. 2012) species for different enzymes production. Similarly, antioxidants are produced through different agro-waste (pineapple waste, orange peel, pomegranate, and lemon peel) with the use of microorganisms (*A. awamori* and *A. oryzae*) (Hegazy and Ibrahium 2012; Singh and Genitha 2014; Rashad et al. 2015; Sadh et al. 2017). The beneficial properties of natural antioxidants such as antiviral, anti-inflammatory, anti-cancer, anti-tumor, and hepatoprotective activity tend it to be safer use for human beings (Nigam et al. 2009). Recent studies have demonstrated that antibiotics production through different agro-waste including coconut oil cake, ground nutshell, corn cobs, sawdust, and rice hulls, are another promising valuable product for the production of different.

Table 1.2 Recent studies of biotechnological approaches using different microorganisms and agro-wastes for bioconversion and degradation of agricultural residues into valuable products

Agro-waste nature	Microorganisms	Biotechnological approaches	Valuable products	Reference
Potato peel	*Xanthomonas campestris*	Solid state fermentation	Xanthan	Vidhyalakshmi et al. (2012)
Wheat bran, rice husk, black gram husk, wheat straw, sugarcane bagasse, maize straw, and paddy straw	*Bacillus licheniformis, B. amyloliquefaciens*	Solid state fermentation	Amylase	Rai and Solanki (2014), Kaur et al. (2015)
BUP6 groundnut oil cake, coconut oil cake, SOC, and CSC	*Pseudomonas* spp.	Solid state fermentation	Lipase	Faisal et al. (2014)
Rice bran, wheat bran, black gram bran, soybean, groundnut oil cake, and coconut oil cake	*Aspergillus niger, Achromobacter, xylosoxidans*	Solid state fermentation	Amylase, cellulase and xylanase	Kumar and Duhan (2011), Suganthi et al. (2011), Mahalakshmi and Jayalakshmi (2016)
Soybean meal waste	*A. oryzae*	Solid state fermentation	Protease enzyme	Thakur et al. (2015)
Corn cob cassava peel, soybeans, wheat bran, and citrus pulp	*Rhizopus arrhizus* and *Mucor subtillissimus*	Solid state fermentation	Protease	Nascimento et al. (2015)
Banana stem	*A. ellipticus* and *A. fumigatus*	Fermentation technique	Bioethanol	Ingale et al. (2014)
Starch containing agriculture waste	*Clostridium beijerinckii*	Fermentation technique	Butanol	Maiti et al. (2016)
Vegetable's waste-potato peel, carrot peel,	*Saccharomyces cerevisiae*	Fermentation technique	Bioethanol	Mushimiyimana and Tallapragada (2016)

(continued)

Table 1.2 (continued)

Agro-waste nature	Microorganisms	Biotechnological approaches	Valuable products	Reference
and onion peel				
Pineapple wastes	–	Fermentation technique	Antioxidant	Rashad et al. (2015)
Fruits peel	–	Fermentation technique	Antioxidant	Singh and Genitha (2014)
Paddy and pulses waste	*A. awamori* and *A. oryzae*	Solid state fermentation	Antioxidant	Saharan and Duhan (2013), Sadh et al. (2017)
Coconut oil cake and ground nut shell	*Amycolatopsis mediterranean*	Solid state fermentation	Antibiotics	Vastrad and Neelagund (2011a, b)
Fruits peel waste	*A. niger*	Fermentation technique	Invertase enzyme	Mehta and Duhan (2014)
Papaya waste and orange peel	*A. niger*	Fermentation technique	α-Amylase enzyme	Sharanappa et al. (2011), Sindiri et al. (2013)
Apple pomace	*A. niger*	Solid state fermentation	β-Mannanase	Yin et al. 2013
Groundnut oil and linseed oil waste	*P. aeruginosa, C. rugosa*	Fermentation technique	Lipase	Dharmendra (2012), Rekha et al. (2012)
Rice bran	*Pediococcal* sp.	Biotransformation	Ferulic acid	Kaur et al. (2013)
Paddy straw	Bacteria (*Eupenicillium crustaceum, Paceliomyces* sp., *Bacillus atropheus* and *Bacillus* sp.) and commercial fungal consortia (*Aspergillus awamori, Aspergillus nidulans, Trichoderma viride* and *Phanerochaete chrysosporium*)	Aerobic and anaerobic bioconversion	Biofertilizer	Shukla et al. (2016)
Rice straw, maize stover, and mixed weed biomass	Combined inoculation of earthworm (*Eisenia fetida*) and cellulose-degrading	Bioconversion	Compost	Rajkhowa et al. (2019)

(continued)

Table 1.2 (continued)

Agro-waste nature	Microorganisms	Biotechnological approaches	Valuable products	Reference
	microorganism (*Pseudomonas* sp.)			
Agro-waste	Recombinant *Pediococcus acidilactici BD16*	Bioconversion	Vanillin	Chakraborty et al. (2017)
Wheat bran and orange peel	*Penicillium notatum*	Fermentation technique	Pectin methyl esterase	Gayen and Ghosh (2011)
Castor oil, sunflower oil, barley bran, peanut cake, and rice bran	*Pseudomonas aeruginosa*	Solid state fermentation	Biosurfactant	Saravanan and Vijayakumar (2014)
Paddy straw, Banana stalks, Bahia grass	*Pleurotus tuber-regium, P. sajor-caju, P. eryngii*	Bio-conservation of lingo-cellulosic wastes	Edible mushroom	Siqueira et al. (2011), Kumhomkul and Panich (2013), Lakshmi and Sornaraj (2014), Yildirim et al. (2015)
Cucumber and orange peels	*S. cerevisiae*	Submerged fermentation	Single-cell protein	Mondal et al. (2012)
Agro-waste	*Methylomicrobium alcaliphilum, Methylosinus trichosporium,* and *Methylococcus capsulatus*	Bio-conservation of lingo-cellulosic wastes	Biogas	Henard et al. (2018)

Oxytetracycline, rifamycin B, L-Asparagine, Penicillin, and other important antibiotics, were also described as a potential product for inhibiting the growth or kill pathogenic microorganisms (Tripathi 2008; Vastrad and Neelagund 2011b). At present, most of the microorganisms are mainly reported to have production of antibiotics such as *Streptomyces rimosus, Amycolatopsis mediterranean*, *Penicillin chrysogenum,* and *Pseudomonas plecoglossicida*. Recently, there is an increasing interest in developing the potential biotechnological applications of high yield producing microorganism/or genetically modified organism for enzyme production, purification and quantification of end products during downstream processing of fermentation technology. Biosurfactant is another beneficial product for humankind that can be produced by using agro-waste such as castor oil, sunflower oil, barley

bran, peanut cake, and rice bran, through the action of microbes (*Pseudomonas aeruginosa*) (Saravanan and Vijayakumar 2014).

The fast-growing population and rapid development of industrialization cause the high input demand for fuels. The production of the low-priced energy source as biofuel from agricultural waste residues becomes attractive substitute of fossil fuels. Several studies revealed the production of biofuels from different agro residues containing high lignocellulosic composition like corn stalks, rice straw, potato waste, sweet potato waste, sawdust, sugarcane bagasse, sugar beet, and vegetable waste like potato peel, carrot peel, and onion peel (Duhan et al. 2013; Saini et al. 2014; Kumar et al. 2014, 2016). The most promising microorganisms that reported for the making of ethanol were described by researchers as, *Saccharomyces cerevisiae* (Mushimiyimana and Tallapragada 2016), *Aspergillus ellipticus,* and *Aspergillus fumigatus* (Ingale et al. 2014), *Clostridium beijerinckii* (Maiti et al. 2016). Biogas production from agro-waste is another significant approach as a substitute of fuels. Paepatung et al. (2009) reported the production of biogas from various agriculture residues as well as two weeds, i.e., *Typha angustifolia* L. and *Eichornia crassipessolms*. Another researcher reported the production of biogas by sequential bioconversion of various agriculture residues, and slurries of animal residues were carried out by a series of microorganism (Paepatung et al. 2009). The lignocellulosic-derived biofuels production through biotechnological approaches is cost-effective as well as eco-friendly and alternative source of energy for the upcoming future.

Production of mushroom worked as a noticeable method of biotechnology for the ecological as well as economic points of view by the transformation of agro-based residues into protein-rich food using various microorganisms (Randive 2012). Mushroom used either as a protein-rich food or bioremediation tool for degradation of lignocellulosic material rich agro-waste (wheat bran, rice bran, paddy straw, banana stalks, and bahiagrass) through the action of dominating fruiting bodies as *Pleurotus tuber-regium, Pleurotus sajor-caju, Pleurotus eous, and Pleurotus platypus* (Babu and Subhasree 2010; Siqueira et al. 2011; Jonathan and Babalola 2013; Lakshmi and Sornaraj 2014). Similarly, a single-cell protein obtained from the bioconversion of agro-wastes (cucumber and orange peels) using microbes, viz., *S. cerevisiae* is economical and nutritionally contained a high content of protein (Mondal et al. 2012).

1.6 Bioconversion of Agro-Waste in Bio-Compost for Sustainable Agriculture

Composting is a sequential bioconversion of agricultural waste into a useful resource. The agricultural residues degraded through the action of lignocellulolytic microorganisms to manage and recycle this waste into a high economic valuable product and efficient compost (Sánchez 2009; Lauwers et al. 2013). The application of compost in the soil improves physical, chemical, and biological properties of soil, restore nutrient pools, enhance soil fertility and health (Huang et al. 2010; Clara et al.

2017; Han et al. 2017). The composting is achieved by a natural succession of microflora that includes bacteria, actinomycetes, and several fungi (Vargas Garcia et al. 2010; Bohacz 2017). Most of the researchers reported significant degradation and bioconversion of agro-waste through bacterial and actinomycetes actions, named as, *Bacillus. Subtilis, B. polymyxa, B. licheniformis, B. pumilus, B. brevis, B. firmus, B. circulans, B. megaterium, B. cereus, Cellulomonas, Cytophaga*, *Pseudomonas* spp., *Clostridium vincentii*, *Sporocytophaga*, *Streptomyces, Micromonospora,* and *Thermoactinomyces* (Awasthi et al. 2016; Bohacz 2017). Several fungi like *Trichoderma harzianum, T. viride, Pleurotus ostreatus, Polyporus ostriformis,* and *Phanerochaete chrysosporium* are known to play an essential role in composting of lignocellulosic materials (Schuster and Schmoll 2010; Awasthi et al. 2016; Varma et al. 2015). The co-inoculation practices are applied to improve crop productivity through diverse mechanisms through nutrient acquisition, mineralization, carbon addition, and phytohormone production (Rashid et al. 2016; Meena et al. 2017). Several beneficial bacterial and fungal species of *Rhizobium, Azotobacter, Azospirillum, Pseudomonas, Bacillus, Burkholderia cepacia, Candida oleophila, Coniothyrium minitans, C. sclerotiorum, Aspergillus niger, Fusarium oxysporum* (nonpathogenic), *Gliocladium* spp., *Phlebia gigantean, Pythium oligandrum, Streptomyces griseoviridis,* and *Trichoderma* spp. that are currently being used with organic matter-rich compost can add to the soil health, when added in combination with the compost can also provide significant support to agriculture (Reddy and Saravanan 2013; Sharma et al. 2013; Rai et al. 2016). The process of decomposition of crop residues involves differentially variable conditions (pH, temperature, moisture, nutrient availability) for the microbial communities involved during the period of degradation.

In the context of sustainable agriculture, compost is an unavoidable natural resource for the management of agro-waste and high-yield production in the farmers' fields. In this order, controlled composting conducted by potential microbial communities to decompose agricultural residues properly and provide high-value low-cost bioorganic compost for farmers (Ahmad et al. 2007; Singh and Nain 2014; Sudharmaidevi et al. 2017). The way of composting processes can help farmers to attract towards organic compost rather than chemical fertilizers, and simultaneously it enhanced the production of high-value commercial crops like vegetables, fruits, flowers, and organic crops (Hoornweg et al. 2000; Seyedbagheri 2010). The application of biofortified compost with bioagents, controlled the soil-, seed- or seedling-borne fungal pathogens in the field that reduces the application of biopesticide (Siddiqui et al. 2008; Ng et al. 2016). Similarly, farmers also applied consortium of microorganisms that are capable for fixing nitrogen, solubilizing phosphorus, zinc, and mobilizing potassium that can be fortified with compost (Baig et al. 2012; Pallavi Chandra and Sharma 2017). These scientific approaches provide knowledge and progression in sustainable agriculture and awareness of the farmer's regarding their need, expertise, indigenous resource availability, local conditions, and existing human resources.

1.7 Develop Eco-Innovative Strategies to Agro-Waste Conversion to Farmers

The campaigning and adoption of these microbial technologies as eco-innovative strategies to farmers provide information about benefits of microbe-mediated composting processes, biotechnological aspects of agro-waste bioconversion, the involvement of microorganisms invaluable product formation, benefits of microbial fortified, and enriched compost in crop yield production. These eco-innovative strategies are simple and easily adaptable by the farming communities. The application of these technologies helps reintroduce organic matter to the soils along with the beneficial microorganisms that help soils to improve nutrient status for plant growth and development. The develop link between farmers, and eco-innovative technologies are a significant problem for sustainable agriculture.

The scientific approaches that targeted farmer-friendly microbe-mediated agro-waste bioconversion for composting among the grass-root stakeholders are a matter of perception and preference. Several factors that hamper the awareness of technologies among the farmers are lack of knowledge about soil and plant characters, less awareness about the effect of chemicals over agricultural foods with human health, a dilemma to adopt new technologies, and short-sightedness towards long-term benefits of organic and fortified compost in agriculture. To overcome these problems, awareness programs using ICT tools or by videos, learning materials or by technical demonstration kits, new government programs regarding sustainable agriculture are connect farmers to adopt these technologies (Karubanga et al. 2017). These efforts can yield desirable impacts on crop yield production, minimizes the application of high-cost chemical fertilizers, integrated farm management practices, limiting the risk of pollutants due to residual effects of pesticides, lowering the production cost of the crops, converting agriculture residue into useful compost and enhancing soil fertility level that lost due to countable changes among farming communities (Muller 2009; Aktar et al. 2009; Settle et al. 2012; Yadav et al. 2013). Therefore, the Indian government has shown keen interest in promoting the adaptation of such environment- and agriculture-friendly practices in farmers through various developmental schemes and funding projects.

1.8 Conclusion and Future Perspectives

Agro residues are rich in nutrient composition and bioactive compounds such as sugars, minerals, and proteins; that is why it considered as “raw material” for several industrial processes. The occurrence of such nutrients in these residues offers suitable productive conditions for the growth of microorganisms that can produce several value-added products through bioconversion/fermentation processes. With the help of microbial interventions and developing biotechnological approaches, the raw residues can be transformed firstly into demanding valuable products and subsequently, the spent waste can further be converted into microbe-enriched biofertilizers/bio-formulations/compost having the specific functional trait. One of

the major benefits of using biotechnological approaches for agro-waste bioconversion is to making feasible the availability of the ready-to-use valuable product in the welfare of human beings. Secondly, this can also help to advent genetic engineering approaches to add desired microbial genes with specific functions, which perform fastest bioconversion/fermentation into the valuable product as well as also involved in other biological bioconversion/bioremediation process. Thirdly, proper composting of agro-waste through microbe-mediated process provides organic materials in the soil that enhances the mineralization of nutrient and ensures proper availability of micronutrients for a longer time duration in the soil. The presence of microbial communities in the soils as emerging frontiers in agro-waste recycling not only produced valuable product but also reduces the environmental risk. In an integrated way, these microbe-mediated processes help improve ecological services and awareness about the eco-innovative strategies of agro-waste recycling drag attention of significant farming communities of India for valuable product formation as well as sustainable agriculture.

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Microbes: The Next-Generation Bioenergy Producers

2

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Abstract

An amalgamation of eternal-increasing energy outlay and global warming concerns has created an international imperative to seek alternative energy that is renewable and can be produced sustainably. Methodical studies have consistently shown that liquid fuels through microbial conversion derived from plant biomass are one of the excellent alternatives if it is lucrative means of commercial production. Yield, titre, and competent reconstruction of feedstock into fuel are the three most imperative factors for engineering microbes that can produce biofuels on an industrial scale. The role of microbial population is indispensable not only in the conversion of plant biomass into liquid fuels but also gaining momentum in the conversion of organic material into other forms of renewable energy sources: bioethanol, biodiesel, biohydrogen, and bioelectricity. Hence, contemporary research demands to understand the metabolomics of these microbial populations and ways and means to transform them to utilize organic waste into renewable energy source effectively. Recombinant technology combined with genomics and proteomics helps to understand and modulate the microbial communities to a better yielding strain. This review will discuss the role of different microbes in bioenergy production and highlight the techniques involved in their transformation, pros and cons of these microbial bioenergy producers in fulfilling the future energy demand.

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2.1 Introduction

The energy demand and non-sustainable nature of existing energy systems demands an alternative renewable and sustainable energy resource development. The United Nationals Development programme has initiated a global agenda for sustainable energy. Conventional energy relies on fossils and is supply focussed whereas bioenergy when converted to modern energy carriers like liquid and gaseous fuels besides electricity can be a potential alternative for sustainable energy production. Energy sources are classified into fossil fuel, renewable source, and nuclear sources. The bioenergy can be made an alternate sustainable energy system if appropriately designed and implemented. The bioenergy system can address the problems relating to the environment, improved livelihood of humankind and productivity. The conversion of biomass into electricity and biofuels can results in a cleaner and efficient use of biomass resources than the traditional direct combustion, leading to the sustainable production of clean, reliable, cost-effective energy. However, the design and implementation required to consider environmental and socioeconomic impacts for humankind.

2.2 Bioenergy Sources

The sources of bioenergy include residues and wastes from agriculture, industry, and domestic, energy crops, and natural vegetation. Crop residues and agricultural processing residues can be a reliable source for generating electricity and biogas. Growing crops for energy production would result in significant energy production but at the same time competition for land use between biomass energy plantation and agriculture should be considered and the biomass energy plantation should be targeted on barren and degraded lands. Methods need to be developed to address the challenges in growing energy crops in barren and degraded lands. Production and cultivation of energy crops can be encouraged in exclusive areas or as co-production with non-energy crops which may address environmental and socioeconomic issues on land usage and labour involvement. While choosing the biomass feedstock's for bioenergy production, the following criteria are considered critical, such as high yield, low cost, low adverse eventual and social impact, and sustainable and renewable use. The various sources of bioenergy are depicted in Fig. 2.1. Production and conversion of biomass into biofuels, bioelectricity and/or co-generation of multiple energy products (heat and power) should involve low investment and high thermodynamic efficiencies for which many research developments are warranted.

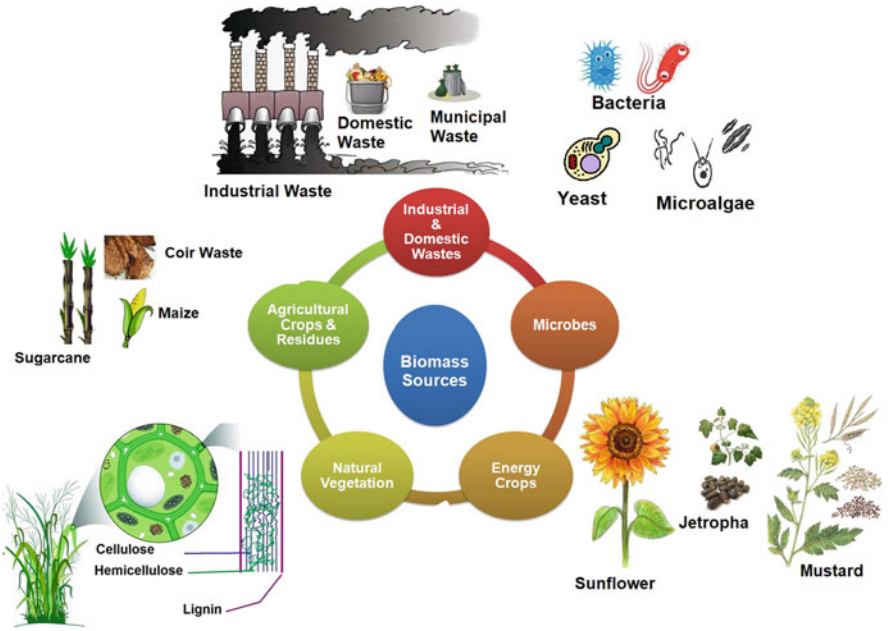

Fig. 2.1 Various sources of sustainable bioenergy

2.3 Microbes as a Source of Biofuel

Microbes not only involved in the conversion of biomass into biofuels but can also be a direct source of bioenergy. The microalgae and oleaginous microorganisms accumulate intracellular oil which serves as a source for biofuel production. The microbes also secrete certain valuable products which may serve as an indirect source of biofuels. Microbial fuel cells can also use bacteria to convert chemical compounds to bioelectricity and biohydrogen. Microbes which can store oil in their cells with ~20% of biomass are generally referred to as oleaginous microbes (Meng et al. 2009). Some species of bacteria, yeast, fungi, and microalgae produce such microbial oil (Borowitzka 2006). The prokaryotic bacteria synthesis specific lipids, while the eukaryotic fungi, yeast, and microalgae produce triacylglycerols (TAG) which can be converted into biodiesel through transesterification. The enzyme is converted from one form to other and microbes play an essential role in transforming chemical energy from biomass into energy bearing fuels. The ability to consume organic substrate and further utilization in metabolic processing by microbes generates useful products that can be utilized for biofuel production. The selection of microbes, substrate, and production process is considered critical for the production of biofuels. For commercialization, a biofuel with more positive net balance

energy is considered advantageous. Metabolic engineering has been a vital breakthrough technology for upscaling of biofuel production. Microbes exhibit specific metabolic pathway and different enzymes for biofuel production. Metabolic engineering of such pathways will result in a fruitful increase of yield in microbial biofuel production. Engineering of microbes for various substrate utilization for biofuel production is an essential strategy. Besides this, bioelectrical cells and microbial fuel cells have gained significant impetus in the generation of bioenergy from wastewater and organic biomass (Logan et al. 2015; Dai et al. 2016).

2.3.1 Oleaginous Bacteria

Bacteria can also accumulate oil under particular conditions. However, only a few bacteria can produce oil (TAG) that can be converted as biodiesel, since the lipid composition produced by bacteria is different (complex lipoid) from other microbial oils (Yi and Zheng 2006). Some of the bacteria reported accumulating oil include *Mycobacterium*, *Nocardia, Rhodococcus, Streptomyces, Acinetobacter,* and *Gordonia*. It is relatively easy to modify the bacteria through genetic engineering and metabolic engineering to improve its oil accumulation as many gene regulation mechanisms in fatty acid synthesis is already known (Wentzel et al. 2007; Alvarez and Steinbuchel 2002).

2.3.2 Yeast and Mould

Many yeast species were found to accumulate lipid under some cultivation conditions. Some of the oleaginous yeast species include *Cryptococcus* sp., *Lipomyces* sp., *Rhodosporidium* sp., *Rhodotorula* sp., and *Trichosporon* sp. (Tsouko et al. 2016). Similarly, fungi species also accumulate lipids, and most of them produce specific lipids like Docosahexaenoic Acid (DHA), Gamma-linolenic acid (GLA), Eicosapentaenoic Acid (EPA), and Arachidonic acid (ARA). Although many mould species can produce and accumulate lipids, only a few reports are available on the utilization of fungal oils for biodiesel production.

2.3.3 Microalgae

Microalgae are generally rich in lipid content which varies between 1 and 70% and under certain conditions can reach up to 90% of dry weight (Metting 1996; Spolaore et al. 2006). Both prokaryotic (cyanobacteria) and eukaryotic (diatoms, green algae, red algae, and golden algae) microalgae can be used to obtain biodiesel (from microalgal oil), biomethane (anaerobic digestion of microalgal biomass), bioethanol (fermentation), and biohydrogen (dark fermentation). The built-in chlorophyll in microalgae renders them to perform photosynthesis and grow abundantly. They can also adapt to various environmental changes. Because of their ability to utilize

various available nutrients to produce valuable metabolites like sterols, fatty acids, pigments, lipids, and oil, they could be used for the production of various kinds of biofuels. The algal biomass can be converted into biofuels either by biochemical or thermochemical processes to yield biogas, liquid biofuels, like ethanol, butanol, and acetone, biohydrogen, bio-oil, and bioelectricity.

The production of bioenergy from microalgae involves many steps, viz. species selection, cultivation, harvesting, biomass concentration, pretreatment and conversion (Matuszewska 2016; Peng et al. 2020). The selection of appropriate species plays a crucial step in algal biomass production which largely depends on the composition of cell/cell walls, oil, lipid, overall productivity, and adaptability to the culture system. The microalgae can be cultivated in photobioreactors or open ponds. The strain improvement and selection/design of appropriate cultivation system can increase the yield. The production cost should also be kept in mind while developing cultivation systems. Since oil/lipid content is a major factor for biodiesel production, the species and growth conditions which favour higher oil yield would be preferred. When compared with energy crops (oil crops), microalgae require less land area. The yield of oil is also higher as much as to 10–28% than the crops (Chisti 2007). The only limitation is that microalgal oil contains polyunsaturated fatty acids as it cannot be directly used for automobile fuels, but can be overcome by partial catalytic hydrogenation of double bonds.

Besides oil, microalgae can also be used as feedstock for anaerobic digestion in biogas production. The production of methane (~350 L/kg Vs) and biogas (~600 L/kg Vs) have been reported in the literature by *Phaeodaetylum tricornutum* and *Chlamydomonas reinhardtii,* respectively (Debowski et al. 2013). The reduced H_2S content in the gaseous product of microalgae is an added advantage. The yield of methane or biogas from microalgae can be increased by pretreatment, which disintegrates the cell wall and increases the availability of raw material for methanogens. The pretreatment methods include mechanical, thermal, chemical or biological process, which depends on the type of microalgae and its characteristics. Carbon/Nitrogen (C/N) ratio plays an important role in anaerobic digestion and microalgae have low C/N ratio, leading to inhibition of methane production, co-digestion with a carbon-rich substrate like silage/sludge is encouraged. Cell wall containing algae are less susceptible to microbial degradation while those algal species devoid of the cell wall (*Dunaliella, Pavtora*) and algal species containing glycoprotein as cell wall (*Euglena, Chlamydomonas*) are made susceptible to microbial degradation leading to higher yields of biogas and methane (Okuda 2002; Gerken et al. 2013). Table 2.1 represents the microbes in use for bioenergy production in the current scenario.

Table 2.1 List of bioenergy producing microorganisms in the current scenario

SNo	Microbes	Source	Technology	Bioenergy	Reference
1	*Clostridia acetobutylicum*	Starch, molasses and lignocellulosic feedstock	Fermentation	Bioethanol Biobutanol	Yoo and Soucaille (2020)
2	*Clostridium thermocellum*	Lignocellulosic feedstock	Fermentation	Bioethanol	Walker et al. (2020)
3	*Clostridium butyricum*	Lignocellulosic feedstock	Fermentation	Bioethanol	Spiller et al. (2020)
4	*Neocallimastigales*	Lignocellulosic feedstock	Fermentation	Bioethanol	Narwal et al. (2020)
5	*Trichoderma reesei*	Glucose, molasses and lignocellulosic feedstock	Fermentation	Bioethanol	Chen et al. (2020b)
6	*Aspergillus niger*	Lignocellulosic feedstock	Fermentation	Bioethanol	Siqueira et al. (2020)
7	*Penicillium capsulatum*	Hemicellulose	Fermentation	Bioethanol	Narwal et al. (2020)
8	*Talaromyces emersonii*	Hemicellulose	Fermentation	Biobutanol	Narwal et al. (2020)
9	*Thermomonospora fusca BD25*	Carboxymethylcellulose and rice straw	Fermentation	Bioethanol	Chen et al. (2020a)
10	*Thermobifida Cellulosilytica TB100T*	Carboxymethylcellulose and rice straw	Fermentation	Bioethanol Biobutanol	Elmahdy et al. (2020)
11	*Zymomonas mobilis*	Glucose	Fermentation	Bioethanol	Wang et al. (2020) and Zhao et al. (2020)
12	*Saccharomyces cerevisiae*	Glucose	Fermentation	Bioethanol	Tran et al. (2020) and Zhao et al. (2020)
14	*Pichia stipites*	Xylose and Quercus aegilops	Fermentation	Bioethanol	Das et al. (2020) and Zhao et al. (2020)
15	*Kluyveromyces marxianus*	Xylose and Quercus aegilops	Fermentation	Bioethanol	Das et al. (2020)
16	*Escherichia coli*	Glucose, xylose lignocellulose	Fermentation	Bioethanol	Shanmugam et al. (2020) and Zhao et al. (2020)
17	*Chlorella vulgaris*	Algal feedstocks or algal waste	Photo synthetic process	Bioethanol Biodiesel	Varaprasad et al. (2020) Khan and Fu (2020)
18	*Phaeodactylum tricornutum*	Algal feedstocks	Photo synthetic process	Biodiesel	Khan and Fu (2020)

19	*Coccomyxa sp. strain obi*	Algal feedstocks	Mutagenesis	Biodiesel	Khan and Fu (2020)
20	*Aspergillus nidulans A773*	Spent coffee grounds oils	Transesterification	Biodiesel	Goncalves et al. (2020)
21	*Klebsiella sp. WL1316*	Cotton stalk hydrolysate	Fermentation	Biohydrogen	Li et al. (2020)
22	*Thermotoga maritime*	Biomass	Fermentation	Biohydrogen	Shao et al. (2020)
23	*Chlamydomonas reinhardtii*	Algal feedstocks	Photo synthetic process	Biohydrogen	Khan and Fu (2020)
24	*Chlorococcum minutum*	Algal feedstocks	Photo synthetic process	Biohydrogen	Khan and Fu (2020)

2.4 Role of Microbes in Biomass Conversion and Bioenergy Products

Apart from energy crops and residual biomass, the microbial population also play a critical role as a source of bioenergy. Biofuels can make a substantial contribution to the future energy needs and is projected to be even more environmentally friendly than first-generation biofuels, with the potential to reduce greenhouse gas emissions by an estimated 85% compared to gasoline (Fulton et al. 2004). Different strategies for enzymatic hydrolysis and fermentation have been developed to address specific engineering process issues. Separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and Simultaneous saccharification and co-fermentation (SSCF). All these strategies require different levels of additional fermentation technology development. Microorganisms are a vital component of the technology used in different fermentation regimes. The potential of several microorganisms has been studied, including yeasts such as the naturally glucose-fermenting yeast *S. cerevisiae*, mesophilic bacteria such as *Klebsiella oxytoca* (Ingram et al. 1999), and cellulolytic thermophilic anaerobes such as *Clostridium thermocellum* (Lu et al. 2006). *Saccharomyces cerevisiae* remains the gold standard in industrial ethanol production, attaining a production rate of 170 g ethanol per litre per hour on glucose under optimal laboratory conditions (Cheryan and Mehaia 1984). The ability of *S. cerevisiae* to tolerate low pH and rapidly produce ethanol helps to prevent contamination, and it has good tolerance to ethanol and other inhibitors, making it a strong candidate for further development and commercialization in cellulosic ethanol production from biomass (Almeida et al. 2007).

An alternative fermentation microorganism is the mesophilic Gram-negative bacterium *Zymomonas mobilis*. *Z. mobilis* produces ethanol at a high yield and a high specific rate, and it also has a very high ethanol tolerance (Rogers et al. 1982; Gunasekaran et al. 1990). Besides, the organism has been proved to be robust to other inhibitors, and hence it is suitable for fermentation of lignocellulosic materials. Although not as hardy as industrial *S. cerevisiae* strains, the ethanol yield of *Z. mobilis* per unit of fermented glucose is 5–10% higher than that of *S. cerevisiae* due to its unique glucose metabolism (Lin and Tanaka 2006). Several groups have reported success in the cloning of bacterial endoglucanases gene for biofuel production. Use of a cellulase mixture from different microorganisms or a mixture of cellulases and other enzymes for the hydrolysis of cellulosic materials has been extensively studied. In addition to that synergism between Endo-Endo, Exo-Exo, and Endo-Exo cellulases may result in improved cellulase activity than the single cellulase enzyme activity (Henrissat et al. 1985). The simultaneous combined action of two cellulases is not intrinsically necessary to observe synergistic action, and therefore a sequential attack seems to be possible. The addition of β-glucosidases into the bacterial cellulases system achieved better saccharification than the system without β-glucosidases. The challenge here is the identification of microorganisms that can carry out this fermentation efficiently, such as the engineered *Z. mobilis* strain (Aden et al. 2002; Aden 2008). One potential problem is that most commonly

used fermentation organisms strongly prefer glucose as a carbon source, leading to inefficient or underutilization of xylose and other C5 sugars (Wyman et al. 2005).

Genetic improvement of organisms and processes for the bioconversion of lignocellulose to ethanol has the potential to increase efficiency and reduce the costs of fuel ethanol to that or less than petroleum. The cost of the cellulase enzymes to be a critical factor is considered in ethanol production. Consolidated bioprocessing (CBP) also known as a direct microbial conversion is a technology in which one or more microorganisms carry out enzymatic hydrolysis and ethanol fermentation simultaneously in a single vessel (Taherzadeh and Karimi 2007). Despite the potential for significant cost reductions, the technology faces challenging technical hurdles. CBP requires strains that can efficiently (in terms of yield and rates) convert glucose and C5 sugars to ethanol, adequately express multiple cellulases, exhibit robustness under industrial conditions, and for which the requisite genetic and metabolic pathway background knowledge exists (Chang et al. 2007).

Fungi are well-known agents of decomposition of organic matter in general and of cellulosic substrates in particular. Cellulolytic enzyme systems can be produced by several different fungi such as white-rot fungi, soft rot fungi, and anaerobic fungi. All fungal cellulases studied so far have been shown to contain a multiplicity of enzyme components. The actual number of components depends on the source of the fungus and the manner in which it has been cultured. *Trichoderma* sp. cellulases have been most extensively studied. They have been shown to contain four to eight endoglucanases, two cellobiohydrolases, and one to two β-glucosidases. *Penicillium funiculosum, Penicillium pinophilum* cellulase contains two cellobiohydrolases, five to eight endoglucanases and two β-glucosidases. Other cellulases are equally heterogeneous. These cellulase genes were cloned and expressed into various hosts such as *E. coli, Z. mobilis,* and *S. cerevisia* for bioethanol production.

Filamentous fungi are the primary source of commercial cellulases. Cellulolytic fungi belonging to the genus *Trichoderma* (*T. viride*, *T. longibrachiatum*, *T. reesei*) have long been considered the most productive and powerful destroyers of crystalline cellulose. Commercial cellulase preparations based on mutant strains of *T. reesei* (also known as *Hypocrea jecorina*) are produced on an industrial scale by many companies worldwide (Gusakov 2011). Thus, it is not surprising that most R&D projects on bioethanol production from lignocelluloses focus on using *T. reesei* cellulases. As a result, many involved in bioethanol production projects believe that *T. reesei* is the only and indispensable choice for enzymatic cellulose saccharification. However, recent publications have increasingly demonstrated that alternatives to *T. reesei* enzymes in the production of second-generation biofuels exist and results were reported successfully in the cloning of fungal endoglucanases gene into an ethanologenic host and producing cellulosic ethanol production (Nigam and Singh 2011).

One approach to increase biofuel production is to clone and express of cellulase genes that encode the enzymes of the cellulase enzyme complex. The cloned genes may also be of use in introducing cellulolytic properties into non-cellulolytic organisms which have commercial or ecological importance. Cloning of the cellulase genes from these organisms would be useful not only for increasing the enzyme

productivity but also for obtaining information on the structure and function relationship. A thermotolerant yeast strain, *Kluyveromyces fragilis* (NCIM 3358) that can undergo fermentation at temperatures above 40 °C gives better yields of ethanol than *S. cerevisiae* from lignocellulosic wastes (sugar cane leaves and *Antigonum leptopus* leaves). They are found to be suitable alternatives for developing the simultaneous saccharification and fermentation (SSF) process to overcome the limitations of SHF. Increased ethanol yields were obtained when the cellulase was supplemented with β-glucosidase (Hari Krishna et al. 2001) in a short time. Glucose analogue 2-deoxy-d-glucose resistant thermotolerant fermenting yeast strains showed improved fermentation ability at elevated temperatures (Rincon et al. 2001) and also increase its fermentation efficiency. Similarly, a *Candida molischiana* mutant, resistant to 2-deoxy-d-glucose was capable of producing ethanol at 45 °C, unlike its wild-type counterparts (Geiger et al. 2014). Using site-directed mutagenesis (SDM) a methylotrophic yeast of *Hansenula polymorpha* was subjected for a mutation to produce ethanol from cellobiose, glucose, and xylose at high temperatures (Gírio et al. 2010). Xylose-utilizing ability by *H. polymorpha* strain was improved through site-specific mutagenesis of the endogenous xylose reductase gene resulting in a sevenfold increase in ethanol production (Dmytruk et al. 2008).

2.4.1 Bioethanol Production

Traditionally clean-burning fuel, ethanol is produced from biomass crops rich in starch (corn), sugar (sugarcane) or lignocelluloses material (wood and grasses). Brazil is the leading producer of ethanol from sugarcane, and most of the countries produce (about 80 developing countries) ethanol from sugarcane, bagasse, and molasses (Larson and Kartha 2000; Mohanty and Swain 2019; Kumar et al. 2020). Ethanol production depends on the raw materials used and commonly involves three steps: (1) Pretreatment of raw materials (Biomass) to obtain fermentable sugars, (2) Fermentation of sugars into ethanol, and (3) Separation and purification of ethanol by distillation. *E. coli* forms an interesting host for biofuel production from lignocellulosic biomass (Feldmann et al. 1992) as it can utilize hexoses and pentoses. The first genetically modified *E. coli* has the potential to produce bioethanol harbouring pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) under the control of *lac* operon (Ingram et al. 1987, 1998) in pET.

Homoethanolic fermentation was achieved by expressing PDC and ADH genes of *Z. mobilis* into *E. coli,* the first successful applicant of metabolic engineering for biofuel production. The transformation of lignocellulosic feedstocks into next-generation biofuel is well appreciated for its social and economic benefits. Efficient conversion of cellulose, hemicellulose, and lignin into liquid fuel by specialized microbes harvesting an array of enzymes through genetically engineering *E. coli* would be a healthy option for biofuel production. Through engineering *E. coli,* the biofuels like ethanol, isopropanol, butanol, and other short-chain alcohols are produced. Apart from these pathways, for isoprenoid biosynthesis has also been

successfully engineered in *E. coli* (Alper et al. 2005; Pitera et al. 2007). The application of genetic engineering in *E. coli* for biofuel production is well analysed and reviewed (Liu and Khosla 2010).

In the fermentation process, the hydrolytic products, including monomeric hexoses (glucose, mannose, and galactose) and pentoses (xylose and arabinose) will be fermented to valuable products such as ethanol. Among these hydrolytic products, glucose is usually the most abundant, followed by xylose or mannose and other lower concentration sugars. *S. cerevisiae* and *Z. mobilis* are the most frequently and traditionally used microorganism for fermenting ethanol from starch-based residues at industrial scales (Hahn-Hagerdal et al. 2006). *S. cerevisiae* has a few advantages, such as its wide public acceptance, high fermentation rate, and high ethanol tolerance that make it the best candidate for fermentation processes (Chu and Lee 2007).

An ethanol plant conventionally uses baker's yeast as first course cultures, because of its low cost and accessibility at the necessary amount. The indigenous (wild) yeast isolated from the distillery was efficient for ethanol production compared to other strains as they could survive the recycling process (Basso et al. 1993). Silva-Filho et al. (2005) and co-workers reported that PCR-fingerprinting based analysis of yeast samples from few distilleries, that indigenous strains could be more personalized to the industrial process than commercial ones, also identifying foremost strains. The rationale for using starter yeast strains was incapable of enduring could be due to the distressing situation imposed by industrial fermentation. High ethanol concentration, high temperature, osmotic stress due to sugar and salts, acidity, sulphite and bacterial contamination are recognized stress conditions faced by yeast during the industrial processes (Alves 1994) some of them acting synergistically (Dorta et al. 2006) and particularly with cell recycling.

2.4.2 Biogas Production

Anaerobic digestion is a low-temperature biological process involving microbial conversion of biomass into combustible biogas. Animal waste, sewage sludge, crop residues, industrial by-products, and landfill materials can be used as feedstocks for anaerobic digestion. The role of microbes in anaerobic digestion is critical to the production of bioenergy. Fermentative bacteria, acetogens, and methanogens help in the conversion of biomass into bioenergy. The striking balance between these microbial populations at various stages of digestion decides the quantity and quality of biogas production. The successive degradation of organic biomass into biogas (methane and CO_2) is a result of the action of complex microbial consortia. The microbes are involved in the decomposition of organic substances consisting of successive biochemical processes, viz. hydrolysis/ cellulolysis, acidogenesis, acetogenesis, and methanogenesis (Zabranska and Pokorna 2018; Farghali et al. 2020). The first three reactions were carried out by fermentative bacteria, while the methanogenesis is carried out by methanogenic archaea (Hassa et al. 2018). Anaerobic digestion is a sustainable option for reduction of environmental concern over

wastewater (vinasse) generation during ethanol fermentation from sugarcane bagasse, as vinasse can be used for generation of biogas such as hydrogen and methane. Reports on the potential microbial conversion of sugarcane vinasse into hydrogen and methane were documented (Santos et al. 2014; Souza et al. 1992; Mota et al. 2013; Ferraz Júnior et al. 2016; Lazaro et al. 2014). However, information on the biogas producing microbes needs to be probed for improving the technologies necessary for increasing the sustainable use of ethanol plants for the production of biofuels. The occurrence of thermophilic and mesophilic hydrogen produces are reported. Some of the bacteria reported producing hydrogen include *Ethanoligenens*, *Clostridium*, *Pectinatus*, *Megasphaera*, *Propionispora*, *Thermoanaerobacterium,* and *Thermohydrogenium* (Ferraz Júnior et al. 2015a, b).

2.4.3 Biohydrogen Production

Hydrogen is considered as an ideal clean energy source for the future as it can be converted into electrical energy in fuel cells or burnt and converted into mechanical energy (Malhotra and Gosnell 2007). Biological hydrogen production is an energy-saving, cost-effective, environmentally friendly approach (Das and Veziroğlu 2001; Esper et al. 2006). Algal and cyanobacterial biophotolysis of water or photo fermentation of organic substances by photosynthetic bacteria results in biohydrogen production. Two main approaches are available for generation of biohydrogen, viz. Direct (Biophotolysis) and indirect (photo and dark fermentation). The indirect approach utilizes the potential of photosynthesis to build biomass and converts the accumulated biomass into biohydrogen through photo/dark fermentation (Benemann 1996; Sarangi and Nanda 2020; Seibert et al. 2008). The direct approach depends on the photolysis of water into hydrogen and oxygen in the presence of sunlight for biohydrogen generation. Microalgae and cyanobacteria are involved in biophotolysis while purple bacteria convert organic substrate like acetate, lactate, butyrate into hydrogen and CO_2 in the presence of sunlight. Anaerobes (*Clostridia*), facultative Anaerobes (*Enterobacteriaceae*), methanotrophs (*Methanogens*, *Ruminococcus*), and photosynthetic bacteria (*Rhodospirillum*) are involved in biohydrogen production. Cyanobacteria (*Anabaena, Synechococcus, Oscillatoria*) have also been reported to produce hydrogen (Nandi and Sengupta 1998). The approaches for improvement of biohydrogen production by fermentation are reviewed in detail (Nath and Das 2004). The biohydrogen and methane production and the microbial community dynamics in industrial wastewater treatment plants (Elreedy et al. 2019), saline industrial wastewater treatment plants (Manal Ali et al. 2019), and anaerobic bacteria consortium from brewery wastewater were also reported to have salt-tolerant bacterial (*Desulfovibrio*, *Oscillibacter*) archeae (*Methanosarcina*, *Methanocorpusculum,* and *Methanobacterium*) populations (Pachiega et al. 2019).

2.4.4 Biodiesel Production

Transesterification of fatty acids from vegetable oil, microbial oil, and algal oil leads to the production of biodiesel, an alternative for diesel from fossil fuel. The biodiesel produced by bacteria is referred to as micro-diesel (Kalscheuer et al. 2006). Engineered *E. coli* is used for the production of biodiesel. The use of photosynthetic organism for biofuel production is cheap and eco-friendly, and the biomass serves as a source of synthesis of biohydrogen, bioethanol, biodiesel, and bioelectricity. Microalgae is considered as an attractive feedstock for biodiesel production (Slade and Bauen 2013; Chisti 2007; Poudyal et al. 2015). Several species of algae are reported to be useful in the production of biofuels, and attempts are made for identification of desired strains (Razaghifard 2013; Singh et al. 2011).

2.4.5 Bioelectricity Production and Microbial Fuel Cell (MFC)

Microbial fuel cell and bioelectrochemical systems (BES) use microbial derivatives for bio electrocatalytic conversion of chemical and electrical inputs. Microbes actively catabolize substrate and generate bioelectricity, and hence they can be used in MFC for power generation. In MFC, the active microorganism is used as a biocatalyst in an anaerobic anode compartment for production of bioelectricity. The ability of the bacteria to produce electric current was observed by Potter (1911) but gained momentum only after 2000. MFC consists of anode and cathode chambers physically separated by a proton exchange membrane (PEM). The organic substrates are oxidized by active microbes in the anode and produce electrons and protons. The electrons move to the external circuit while protons are transported to the cathode chamber through PEM. Since O_2 can inhibit the production of electricity, bacteria in the anodic chamber are maintained in anaerobic condition. When organic waste is used as the source of microbes to be used in MFC to generate electrons, it leads to not only bioelectricity production but also organic waste removal.

The central part of the MFC is anaerobic anode compartment. The anode chamber is filled with substrate, mediator (optimal), microbes, and the anode electrode. The factors which influence the performance of MFC include electrode material, equipment, concentration, and species of electron acceptor, catalyst performance, and separators. The ideal electrode material should have the following salient features such as good electrical conductivity, low resistance (internal), chemical stability, anti-corrosive, large surface area, relevant mechanical strength and toughness. The frequently used anode materials include carbon, graphite, nanostructures (CNT) of carbon, metal-based anodes with conductive polymers like PANI (polyaniline) or titanium dioxide composite, poly tetra fluoro ethylene (PTFE). The electron travels to the cathode chamber and transmits into oxygen.

2.5 Approach for Characterizing and Improving Microbial Communities

Biotechnological methods in bioenergy production include fermentation techniques, nanotechnology, omics approaches, biorefinery concept, system biological, and metabolic engineering. The metagenome, metatranscriptome, metaproteome, and metabolome provide information on community function and activity. Next-generation sequencing (NGS) is used for characterizing community composition. Several 16S rRNA based methods have developed like denaturing gradient gel electrophoresis (DGGE), automated ribosomal intergenic spacer analysis (ARISA), terminal restriction fragment length polymorphism ((T-RFLP), and 16S rRNA tagged pyrosequencing. All these strategies help in characterization of

- Community biomass-quantity and distribution of microbes.
- Community taxonomy-types and relative abundance of microbes.
- Community function-the nature of the reaction and its regulation.

Metabolic engineering, genetic engineering, and synthetic biology help in improvisation of microbes for bioenergy production.

2.5.1 Metagenomics and Next-Generation Sequencing

Metagenomics gained importance due to the advent of NGS to understand the composition of microbial consortia involved in bioenergy conversion and insights into their functionality. The roles of uncultivable microbes were established because of the availability of high throughput sequencing of environmental whole community DNA/RNA. The understanding of the microbial community's composition and function in bioconversion provides opportunities for their management and engineering (Koch et al. 2014; Carballa et al. 2015). The compositional makeup of microbial consortia and phylogenetic relationship between the community members were studied by PCR amplification of specific marker genes from whole metagenomic DNA. 16S rRNA amplicon sequencing is widely accepted and used approach for profiling of microbial community without the need for cultivation of microbes. About nine hypervariable regions separated by conserved regions are present in 16S rRNA gene, which provides organism-specific sequences for identification of taxa. These specific regions or the whole of the 16S rRNA gene can be considered as a taxonomic marker gene (Yang et al. 2016). The 16S rRNA gene nucleotide sequence identity is based on the taxonomic thresholds for species and genus level of 98.65% and 94.5%, respectively (Kim et al. 2014; Yarza et al. 2014).

Several fingerprinting techniques were developed for targeting 16s rRNA gene amplicons like DGGE analysis, T-RLFP analysis, and Amplified Ribosomal DNA Restriction (ARDRA) Analysis (Muyzer et al. 1993; Moyer et al. 1994; Liu et al. 1997). DGGE and ARDRA were the low-cost technologies employed to get rapid insights into community structure and function (Wang et al. 2016; Yamei et al.

2017). On the other hand, T-RFLP analysis provides valuable data on the structural fluctuations and influence of different parameters on the microbial community (Goux et al. 2015; Krakat et al. 2010; Theuerl et al. 2015; Witarsa et al. 2016). Presently, fingerprinting tools coupled with NGS are used for monitoring microbial community structures (Akyol et al. 2016; Sun et al. 2016; Ozbayram et al. 2017). Pre-screening of samples for NGS using fingerprinting techniques helps to focus on particular functional groups of microbes (Sun et al. 2016; Ozbayram et al. 2017). NGS platforms help to predict the accurate taxonomic classification of microbial communities (Liu et al. 2008).

NGS is a high throughput technology for identification of the entire population in a community since it has comprehensive coverage of sequences without compromising the quality of sequencing. It can be used to explore the diversity of microbes in a community by sequencing the whole genome and comparative analysis or sequencing their ribosomal RNA sequence alone (Kircher and Kelso 2010; Shokralla et al. 2012). NGS is used to identify the underrepresented bacterial communities in a population while the traditional approaches concentrate on dominant members. Some of the NGS platforms include Illumina (Solex) sequencing, Ion torrent sequencing, Roche 454 pyrosequencing. NGS has provided a great platform for comprehensive characterization of complex microbial consortia in a cost-effective manner. The best and most used NGS platform is sequencing by synthesis (SBS) through Illumina, which provides information on genome, transcriptome, and epigenome with precision. The microbial diversity of fermentation reactions were uncovered by sequencing the hypervariable regions of the 16S rRNA gene through Illumina Miseq sequencing (Gupta 2014; Jeon et al. 2015). The Illumina Miseq approach is also widely accepted as a promising platform to study microbial community analysis, especially for uncultivable organisms. Pyrosequencing provides essential data on bacterial diversity of mixed cultures and completely identifies all microbial populations. Pyrosequencing involves DNA synthesis and real-time monitoring of pyrophosphate production during DNA polymerization through light emitted by enzyme action of luciferase upon addition of each base (Nyren et al. 1993). High throughput sequence-based metagenomic characterization of microbial communities in biomethanation process (Pore et al. 2016) and biohydrogen production (Ratti et al. 2015) were achieved by 454 pyrosequencing and SOLiD NGS methods. After the launch of ion torrent PGM technique, it is widely used for microbial composition analysis with better and highest throughput compared to 454 pyrosequencing (Wang et al. 2013; Luo and Angelidaki 2014). Most of the microbes are unculturable; hence reference datasets are essential. For 16S rRNA gene sequence analysis reference databases like Greengenes, NCBI, SILVA, and RDP were consulted (Cole and Tiedje 2014; Balvociute and Huson 2017). The completeness and correctness of corresponding reference databases for sequence classification is critical for 16S rRNA gene amplicon analysis (Ranjan et al. 2016). The taxonomic composition of bacterial and archaeal communities of biogas plants was reviewed extensively (Hassa et al. 2018). High-resolution taxonomic profiling is made feasible by NGS 16S rRNA gene amplicon sequencing. Environmental gene tags (EGTs), metagenome assembly, and binning strategies

provide valuable information on genome sequence and functional properties of uncultivable microbes (Krause et al. 2008; Campanaro et al. 2016). A comprehensive NGS and metagenomic approaches for identification of bioenergy producers were shown in Fig. 2.2.

2.5.2 Functional Genomics/Metabolomics

Functional genomics includes metatranscriptomics and metaproteomics, which aids in metabolic profiling of active enzymes and metabolites. The metatranscriptome analysis is done by total RNA preparations from the microbial consortium and metatranscriptome profiling of transcripts encoding key enzymes responsible for bioenergy production (Zakrzewski et al. 2012; Gullert et al. 2016). Mapping of transcriptome sequences to metagenome assembled genomes (MAG) in a genome enabled metatranscriptomics approach allows detection of metabolically active enzymes (Xia et al. 2014; Gullert et al. 2016; Maus et al. 2016; Stolze et al. 2016). The influence of process temperature (Lin et al. 2016), long-chain fatty acid addition (Treu et al. 2016) to lab-scale bioreactors for biogas production were also analysed successfully by metatranscriptomic analysis.

Metaproteome analysis was conducted by extracting the protein followed by tryptic digestion and peptide analysis through mass spectrometry. The different proteins were identified by database search. Metaproteome analysis was done for the study of the hydrolytic pathway (Hanreich et al. 2013), and the differential profile of mesophilic and thermophilic processes during biogas process (Heyer et al. 2013). The correlation between process parameters (ammonia concentration, temperature, retention time, etc.) and protein expression were analysed using metaproteomic analysis (Heyer et al. 2016). Both the metagenome datasets and metaproteome were used to predict the function of the proteins. The metagenome assemblies provide the information regarding the genetic context of proteins identified in metaproteome analysis. Also, the metagenomic contigs encoding proteins of the metaproteome provides information on gene available near the target genome. The compilation of a protein database for different communities would improve protein identification in metaproteome analysis (Heyer et al. 2016; Ortseifen et al. 2016). Quantitative metaproteomics employing LC-MS helped in the elucidation of a variety of enzymes involved in the degradation of lignocellulosic biomass (Zhu et al. 2016). In another study involving induction of enzyme expression in response to cellulase addition, several enzymes, viz. cellulases, xylanases, cellobiose, phosphorylases, cellobiosidases, and glycol hydrolases were identified in the extracellular metaproteome of the community bacteria associated with cellulose metabolism (Speda et al. 2017). The protein profiling of mesophilic vs thermophilic conditions in biogas plant was also studied through the metaproteome of community microbiomes (Abendroth et al. 2017).

Elaborate metabolome profiling has been carried out in lab-scale biogas production units, and it could be extended to other community analysis involved in alternate bioenergy production. Metatranscriptomics, metaproteomics complemented by

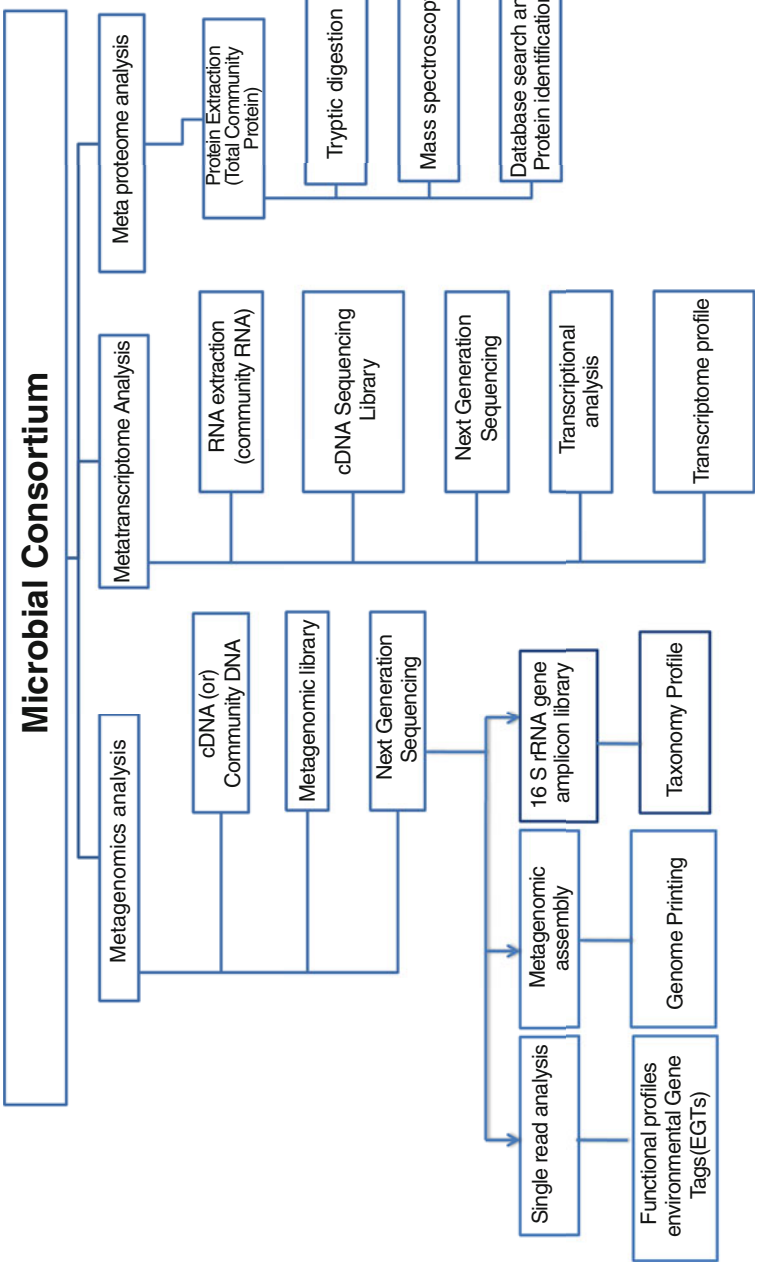

Fig. 2.2 Analysis of microbial communities through Metagenomics and Next-Generation Sequencing (Caporaso et al. 2010; Hassa et al. 2018)

metabolome analysis can be correlated with gene expression (metagenomics) to metabolite profiling, and these approaches would provide deep insides into composition, performance, and inter-relations of the community microbiome and also the effect of Fed substrate and process parameters on the community bacteria. Carbohydrate active enzyme databases (CAZy) and fungal oxidative lignin enzymes (FOLy) databases are developed for the enumeration and classification of enzymes involved in the breakdown of carbohydrates and lignin, respectively. CAZy database currently classifies CAZy enzymes into six family, viz. glycoside hydrolases, Glycosyltransferase, polysaccharide lyases, auxillary activity enzymes, carbohydrate esterases, and carbohydrate-binding molecules (Lombard et al. 2013). Similarly, FOLy database classifies the lignin-degrading enzymes into two main classes: lignin oxidases (laccases, lignin peroxidases, manganese peroxidases, chloro peroxidases, cellobiose dehydrogenase) and lignin-degrading auxillary enzymes (aryl alcohol oxidase, vanillyl alcohol oxidase, glyoxal oxidase, pyranose oxidase, galactose oxidase, glucose oxidase, and benzoquinone reductase) (Levasseur et al. 2008). Recent developments employing advanced sequencing technologies for genomic studies of cellulase and lignin-degrading microbes were reviewed extensively (Kameshwar and Qin 2017). The importance of metagenomic tool in the biofuel sector, especially for the unculturable microorganisms was dealt in discussing the novel biocatalytic agents and its pivotal role in enhancing the efficiency (Bilal et al. 2018).

2.5.3 Metabolic Engineering and Genetic Engineering

Genetic engineering attempts have been made to enhance biofuel production, especially ethanol fermentation, through transferring genes from one species to another. Gene cloning and expression of various enzymes, including enzymes for creating a new metabolic pathway, are also known. However, none of these discoveries has successfully broadened the fermentable substrate range of a microorganism which could not previously ferment pentose sugars to ethanol. For a long time, research is being done to enhance the digestibility of lignocellulosic biomass mainly for the efficient conversion of (hemi-) cellulose to ethanol. It is, however, not clear which characteristics of the lignocellulosic biomass are essential, to determine a successful pretreatment. Different pretreatment methods were used to enhance the digestibility of lignocellulosic material for efficient cellulosic ethanol production. In the function-driven approach, the metagenomic library is screened for the clones that express a desirable metabolic function (Schloss and Handelsman 2003). While the sequence driven approach involve identification of gene of interest directly from metagenomic DNA or metagenomic library through PCR amplification and hybridization probes based on conserved regions (Simon and Daniel 2011). Whole-genome sequencing of the microbial community enables the reconstruction of genomes and helps to establish the relationships of metabolic pathway among species for energy generation (Tyson et al. 2004). The analysis of data generated by pyrosequencing of a fosmid library constructed from the microbial community for sugarcane bagasse

enabled the taxonomic profiling of the microbial community and helped to derive information on the protein-coding genes involved in biogas conversion (Mhuantong et al. 2015). The mining of environmental genomes resulted in the identification of several novel genes of CAZymes (Revised in Ferraz Júnior et al. 2017).

Cellulolytic enzymes have been expressed in bacteria (Wood and Ingram 1992) and yeast (Murai et al. 1998; Van Rensburg et al. 1996) as a way of reducing the cost of cellulase production and other pretreatments in the process of ethanol production from cellulosic materials (Lynd et al. 1999; Zaldivar et al. 2001). Some researchers have developed ethanologenic bacteria (Guedon et al. 2002) and yeast (Cho and Yoo 1999) that can produce ethanol from cellulosic materials. The recombinant *Klebsiella oxytoca* SZ21 was able to directly produce ethanol from amorphous cellulose, although with insufficient ethanol yield (Zhou and Ingram 2001). When using other recombinant ethanologenic bacteria or yeast to ferment cellulose, the addition of commercial cellulase is necessary for ethanol production. The high expression of heterologous genes in *Z. mobilis* is possible when the genes are expressed from their promoters. A CMCase gene of *Cellulomonas uda*, CB4, was cloned on pZA22, a cloning vector for *Z. mobilis* (Misawa et al. 1988). *Z. mobilis* carrying this gene synthesized cellulase immunologically identical with that of *C. uda*. In all these cases, no endoglucanase activity was detected in the culture supernatant, and a poor expression in *Z. mobilis* was obtained when compared to *E. coli*. However, the cellulase gene from *E. chrysanthemi* coding for endoglucanase was subcloned into a broad-host-range vector pGSS33, and was conjugally transferred into *Z. mobilis* with the help of RP4 (Goachet et al. 1989). In this case, most of the endoglucanase accumulated in the periplasmic space, suggesting an efficient export of this foreign protein in *Z. mobilis*.

Production of advanced biofuels by microbes should meet up specific criteria, viz. efficient fermentation, resistance against contamination, high productivity, yield and high tolerance to fermentation inhibitors and products. Engineering of microbes to produce biologically derived replacements for diesel, gasoline, and aviation fuel is an area of current research focus (Ko et al. 2020). The next-generation biofuels like long-chain alcohols, fuels derived from fatty acid or isoprenoid pathway through the engineering of microbes offers promising new biofuels. The next-generation biofuels have many advantages; the toxicity of the microbes is a major problem. Engineering of organisms for both tolerance and biofuel production through the transfer of tolerance mechanism from microbes that survive in hydrocarbon environment to host-microbe would improve yields. The stress and tolerance of microbial community in next-generation biofuel production have been reviewed, and specific mechanism for improving tolerance like activation of stress response gene, expression of efflux pump, membrane modifying proteins and heat shock proteins are discussed in detail (Nicolaou et al. 2010; Dunlop 2011). The solvent tolerance pumps from resistance-nodulation division family in gram-negative bacteria are composed of three protein subunits essential for pump function. These efflux pumps recognize and export toxic compounds outside the cell through proton motive force. The solvent resistant pumps srpABC from *Pseudomonas putida*, acrAB-tolC pump from *E. coli*, mex pumps from *P. aeruginosa* have been shown to export

solvents (Kieboom et al. 1998; Takatsuka et al. 2010). Expression of efflux pumps for longer chain alcohols, alkenes, alkanes, and cyclic hydrocarbons is a promising engineering strategy for improved biofuel production and tolerance. Solvent tolerant microbes can block the entry of solvents by actively changing the membrane composition, especially, the fatty acid. The shift from cis to trans unsaturated fatty acids and an increase in the ratio of trans to cis fatty acids is corroborated with an increase in solvent tolerance due to decrease in membrane fluidity (Holtwick et al. 1997; Junker and Ramos 1999; Kiran et al. 2004). Modifications in the phospholipid headgroups or phospholipid chain length also shown to increase solvent tolerance (Ramos et al. 2002). Besides, membrane protein is also altered in response to solvent stress, and together genomics has identified specific genes involved in stress response and engineering of them may be useful in increasing the production of bioenergy. Integration of multiple strategies will significantly improve biofuel tolerance in the host, which in turn can increase the biofuel yield. Bioengineering of microalgae for biohydrogen and biofuel production by metabolic engineering has been dealt with in detail (Beer et al. 2009; Wargacki et al. 2012; Larkum et al. 2012).

2.5.4 Synthetic Biology

It is an emerging field that blends engineering principles into genetic manipulation of microbes. Biofuels production depends absolutely on the cost reduction process, and it is necessary to involve synthetic biology, which can help in the reduction of time needed to make genetic constructs while increasing reliability and predictability. Creation of novel organisms with minimal genomes and assembling new metabolic pathways in an existing organism are the primary aim of synthetic biologists for biofuel production (Lee et al. 2008; Peralta-Yahya and Keasling 2010). Integrating the functional genomics to monitor thousands of parameters simultaneously and data modelling, one should be able to predict the potential bottlenecks in biofuel production and address it effectively. The microbes can be engineered, and the targeted genomic profiling and metabolic flux analysis can identify the potential constraints in production and toxicity from pathway expression. The system biology can be used to predict the useful model to be incorporated into the microbe for cost-effective and efficient biofuel production. Synthetic biology offers more time-efficient, less resources intensive system for improved biofuel yields. Construction, heterologous expression, and in vivo assembly of hydrolytic enzymes into chimeric scaffolds have been attempted in non-cellulolytic microbes (Cho et al. 2004; Perret et al. 2004; Mingardon et al. 2005). Such synthetic cellulosomes are useful for the production of new generation biofuels by conversion of lignocellulosic biomass (Elkins et al. 2010).

Synthetic biology helps in design or chemically synthesizes genetic sequences that can be incorporated into the host. The construction of genetic control elements, optimization of genes and functional genetic circuits can be used to modulate the biosynthetic pathways involved in biofuel production. Engineering of a synthetic

pathway for construction of hydrogen-producing cells (Yoshida et al. 2007) and using synthetic pathways for isoprenoid based fuel (Lee et al. 2008) are few recent developments of synthetic biology. Synthetic programming of the system for biofuel production and construction of cells with minimum genomes essential for cell function and biofuel yield makes this synthetic biology approach more effective and most warranted alternatives for exploiting the innate microbial capacity and importing biosynthetic potential for biofuel production (Alper and Stephanopoulos 2009).

2.6 Pros and Cons of Advanced Biofuels

Advanced biofuels by re-routing of amino acid biosynthetic pathways for short-chain alcohol have been successful but stumble upon certain setbacks like feedback inhibition, generation of the mixture of alcohols; however, these can be overcome by metabolic engineering. Although *E. coli* and *S. Cerevisiae* were proven to produce a wide array of isoprenoid based biofuels (Hanai et al. 2007), the yield and productivities (needed for economical viable production) are still questionable in terms of economic feasibility (Pitera et al. 2007; Chang et al. 2007). Fatty acid production, on the other hand, is a promising platform where the engineered *E. coli* have shown to overexpress fatty acid which can be converted into small chain alcohols (Lu et al. 2008). Microbial fuel cells and bioelectrochemical cells have been extensively exploited for biohydrogen and bioelectricity production (Logan et al. 2015; Dai et al. 2016). However, the commercial feasibility is questionable as the upscaling process involves more cost; however, proper manipulation of the electron transfer mechanism can help in the improvement of energy output. Table 2.2 discides the pros and cons of modern biofuel production.

Table 2.2 Pros and cons of advanced biofuel production

Merits	Demerits
Renewable	Toxic to microbes
High energy density	Time and resources allocation
Low vapour pressure	Economic viability
Less hydroscopic	Net energy gain
Less corrosive	Carbon neutrality
Reduce dependence on fossil fuels	Different efficiency of light utilization by phototrophs under different solar light industries
Zero-emission of greenhouse gases and environmental pollutants	O_2 interference on hydrogenases and pathways
Used directly for internal combustion of the engine	Rate limiting in carbon assimilation and further conversion to biohydrogen is low
Used to power fuel cells for electricity	Storage systems need to be developed

2.7 Impediments to the Commercialization of Microalgal Fuel and Mitigation

Availability of carbon dioxide is essential for the growth of algae, and 1.83 tons of CO_2 is required for the production of one ton of algal biomass (Chisti 2008). Flue gases from the combustion of coal, cement industry are the main sources of concentrated CO_2 emissions. Insufficiency of concentrated sources of carbon dioxide hampers the use of microalgae for the production of biofuel. The normal atmospheric CO_2, if can be tapped efficiently at low cost, it can be a source for algal culture and methods to accelerate carbon capture via genetic modifications could be a possible solution (Savile and Lalonde 2011). In addition to CO_2, algal growth requires nitrogen and phosphorus as principal nutrients. Supply of these nutrients rely on fertilizers, but the production of fertilizers releases CO_2 and hence recycling and reclamation of nutrients is essential for sustainable production of algal fuels (Chisti 2010). Biological nitrogen fixation by photosynthetic bacteria and cyanobacteria and metabolic engineering can be a possible solution. Wastewater can also be a source of the N and P nutrients for producing algae (Kosaric et al. 1974; Woertz et al. 2009; Kumar et al. 2010; Christenson and Sims 2011; Craggs et al. 2011). The supply of fresh water and brackish water is insufficient to support any substantial scale up production of algal fuels; the realistic options are the use of seawater and marine algae. However, fresh water is necessary for washing biomass before oil extraction, closed production facilities, and water recycling strategies (Pate et al. 2011) may be employed to mitigate this issue. Algal biofuel production requires the input of energy for biomass harvesting, processing, and extraction of biofuel. The energy ratio between the energy contained in microalgae and energy required for the production of biofuel is an important measure to determine the worth of biofuel production from microalgae. Improvements in energy efficiency for photobioreactors (Wongluang et al. 2013), hydrothermal liquefaction (Barreiro et al. 2013) of whole biomass or spent biomass may offer opportune for enhancing the energy ratio in biofuel production. Development of inexpensive and low energy processes for recovering algal biomass and oil extraction is necessary to alleviate the impediments for commercialization. Biomass production that relies on sunlight can achieve high productivity and biomass concentration. Improved light penetration and availability of sunlight spectrum can be broadened through genetic engineering and overexpression of photosystems is a potential strategy for improved light capture (Stephenson et al. 2011). Focussed research on algal biology and engineering of production system could improve the production of bioenergy from microalgae.

2.8 Conclusion

Over 70% of global energy demands growth was met by oil, natural gas, and coal, while renewables accounted for almost all of the rest. Renewable energies are derived from renewable resources, which are constantly replenished by nature and are environment friendly. The potential of the world's renewable energy resources is

much higher than the current global energy demand. To increase energy security, renewable resources will have a significant share in the future energy mix. The recent rise in oil prices, global energy demand, and depletion of fossil resources increased our interest to seek alternatives sources for bioenergy production. A further major consideration with respect to the prospects for an increased role for microbes for an alternative to the fossil fuel industry is the source of development. Various approaches are being examined for the use of microbes in bioenergy production. The novel omics era has thrown light on the efficient use of microbes and ways and means to engineer them for the production of efficient, cost-effective alternatives to fossil fuels. This review discusses major technological advances that could be used to improve the microbial population for bioenergy production.

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Emerging and Eco-friendly Approaches for Waste Management

3

Shuchi Saxena and Anand Kumar Pandey

Abstract

The exponential increase in waste accumulation with the increase in population at a global level is posing a great threat to the environment. Along with this, poor management and disposal systems, in turn, increase the adversity. Management of such enormously growing waste is a need for the scenario. Various organic and inorganic contaminants present in the waste possess the potential to harm the environment and need proper treatment before they encounter an environmental niche, to reduce their adverse effects. Conventional physical, chemical and biological treatments though can break these contaminants but are either less efficient or in turn cause damage to the environment due to the utilization of harsh chemicals, temperature or pressure conditions. High-efficiency eco-friendly treatments like enzymatic degradation, bioremediation, phytoremediation and composting can not only reduce the produced waste to a great extent but can also produce valuable products like biofuels, biofertilizers, etc. Henceforth, the present chapter illustrates the conventional as well as emerging eco-friendly approaches which can be utilized at a global scale to minimize the increasing threat of waste accumulation. Moreover, updated researches and waste management trends have been discussed to present an actual status of the eco-friendly approaches in waste management.

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Waste management · Eco-friendly approaches · Enzymatic degradation · Bioremediation · Composting · Phytoremediation

3.1 Introduction

Globally increasing population and industrialization not only upsurge the requirement of resources but also enhances waste production. According to the World Bank report entitled "What a waste 2.0", the global waste is expected to rise to 3.40 billion tonnes by 2050. Currently, the whole world generates approximately 2 billion of solid waste annually, and out of that 33% of waste is not being treated in an eco-friendly and sustainable manner (Kaza et al. 2018). The most probable associated reasons are population growth, rapid urbanization and higher income. Globally enhanced production of different types of waste including household, municipal waste, agricultural waste, industrial waste, etc. adversely affects the environment. The release of unprocessed and incompletely treated waste from industries and municipality has caused a great threat to various life forms. The waste released from various industries like iron and steel processing, mining, distilleries, textile, leather processing, paper milling, etc. produces harmful and toxic pollutants such as sulphides, halides, phenols, heavy metals, polymers, synthetic dyes, pathogens which pose a serious impact on natural resources as well as are responsible for causing severe health hazards (Table 3.1) (Abdel-Shafy and Mansour 2018; Sharma and Bhattacharya 2017). The huge amount of cellulosic waste from agricultural industries and the contaminated water containing pesticides, insecticides and other chemicals, in turn, cause adverse effects on the environment.

Some of the conventional methods of treating waste are incineration, landfill, adsorption, composting, thermal treatment, etc. but due to their high operational cost

Table 3.1 The various health-related and environmental problems caused by toxic waste

Toxic waste	Problem	Reference
Chemicals such as POPs (persistent organic pollutants), phthalates, hydrocarbons	Neurological disorders Endocrine diseases	Zeliger (2013) Bharagava et al. (2020)
Toxic carcinogens such as benzene, vinyl chloride, etc.	Cancer	Uccello et al. (2012)
Presence of particulate matter, gaseous pollutants, etc.	Intensifies asthma	Guarnieri and Balmes (2014)
Lead disposal in open fields and water bodies	Nervous system failure, low IQ, stunted growth, etc.	Wani et al. (2015)
Methylmercury discharged from chemical plants in water	Minamata disease	Rai et al. (2019)
Heavy metal disposal on land	Accumulation of heavy metals in the food chain	Bharadwaj et al. (2015)

Table 3.2 Increasing trends of publications in eco-friendly approaches for waste management from 1990–2000, 2000–2010 and 2010–2020 (Source: Google scholar)

S. No.	Eco-friendly approach for waste treatment	Number of publications in years		
		1990–2000	2000–2010	2010–2020
1.	Biomethanation	628	1600	6670
2.	Bioremediation	13,100	28,400	38,900
3.	Composting	15,800	42,100	54,800
4.	Enzymatic degradation	15,300	30,700	35,200
5.	Phytoremediation	2540	13,200	20,400

and energy requirements, they are less preferred nowadays. Biotechnological approaches like enzymatic degradation, bioremediation, phytoremediation, etc. are highly eco-friendly, low cost and efficient methods which possess great potential to convert waste to valuable products. Conversion of waste to biofuel and biogas by using biological methods is of great interest to scientific society in the current scenario and is being enormously investigated (Ezeonu et al. 2012; Martínez et al. 2015; Berhe and Sahu 2017). Numerous researches have been done till date to develop such eco-friendly approaches and the enormous increase in the number of publications related to these approaches provides great evidence for their viability (Table 3.2). The present chapter provides deep insights of these eco-friendly approaches which are being developed and utilized for waste management along with some conventional methods. Different types of waste, its impact on the environment and its global production trends have also been illustrated to put forward the scope of eco-friendly approaches at a global level to preserve the environment.

3.2 Global Trends in Waste Production and Management

Global waste index 2019 ranked Turkey, New Zealand, Latvia, Chile, Mexico, Italy, Canada, Estonia, Israel and the Slovak Republic among the top 10 waste producers of the world. Though the USA is the biggest waste producer accounting for 808 kg of waste produced by each citizen every year, its efficient waste management and recycling system leads overall reduction of waste and hence it is ranked 12th (https://sensoneo.com/sensoneo-global-waste-index-2019/). Statistical data supports the fact that most of the world's waste is being generated in East Asia and Pacific region followed by Europe and North America (Kaza et al. 2018). The global population has reached 7.6 billion, and the growth in the number of individuals has increased the level of consumption of goods and services, which constitutes the rise in total global waste.

The World Bank has classified economies on the basis of Gross National Income (GNI) per capita data, and facts reveal that higher-income countries are generating over 34% of the global waste.

Waste generation is not the major concern, but the way by which this waste is being processed is of more importance. Unethical management or mismanagement of waste can lead to environmental catastrophe. So, it is the need of the hour to treat waste in an eco-friendly and sustainable manner (https://www.epa.gov/).

In the following sections, we will deal with the types of waste produced around the globe and till date treatments which can be utilized to reduce it.

3.3 Waste Types and Their Effects on the Environment

Waste can be classified into several types based on its origin and the types of contaminants present in it. The major types of waste based on the source of production consist of domestic, industrial, municipal, agricultural, biomedical, radioactive and electronic waste (Velvizhi et al. 2020). Chemical and physical nature of waste plays a major role in its proper disposal and management. The biological aspects of degradability classify the waste into biodegradable and non-biodegradable waste which are of significant concern to develop eco-friendly waste management approaches (Kumar et al. 2017).

A large proportion of domestic and municipal organic waste which consist of vegetable, paper or human waste is being produced all around the world and can be degraded or broken down into simpler non-toxic substance by microbial action when left undisturbed for a long duration. But the plastic waste which is generated around the globe in millions of tonnes poses a great challenge and is classified as non-biodegradable. Polyethylene, polypropylene, polyethylene terephthalate, polystyrene, polyvinyl chloride are the major non-biodegradable forms of plastic used widely in present times. The characteristic features including high molecular weight, hydrophobicity, low number of functional groups which are favourable and crystallinity are highly responsible for the non-biodegradable nature of conventional plastic (Song et al. 2009). Today, variety of biodegradable plastic polymers like polylactic acid, poly-ε-caprolactone, polybutylene succinate, poly(butylene succinate-co-adipate), poly(3-hydroxybutyrateco-3hydroxy-valerate) are being synthesized and some have reached the market as well. They can be possibly degraded by microbial action. However, still, their usage is limited for the majority of purposes as they are unable to meet the desired level of characteristic durability and stress (heat, temperature and pressure) tolerance (Vroman and Tighzert 2009). Thus, the use of conventional non-biodegradable plastic is still highly prevalent, and accumulation of such plastic in environmental niches results in massive damage to environment and living species. Massive accumulation of plastic waste in oceans has grown up into a significant concern as the release of toxic substances into water bodies is causing massive loss of marine life (Hahladakis 2020).

Moreover, a considerable amount of agriculture waste, including cellulosic waste, is generated globally every year. India is ranked 2nd in agriculture-based economies and thus generates a large part of the world's agricultural waste. Burning of cellulosic waste is a common practice in a developing country like India and is a chief reason for excessive air pollution (Saini et al. 2015). The use of physical and

chemical treatments utilizes high energy and harsh chemicals, which in turn can cause environmental problems. The biodegradable nature of cellulosic waste provides an advantage, and thus such waste can be treated by composting, but complete degradation of cellulose to more straightforward form requires subjection to good cellulose-degrading microbial flora (Lynd et al. 2002). Further, conversion of cellulosic waste to bioenergy forms by utilization of microbial or enzymatic approaches is a significant concern being exhaustively investigated by scientific society (Robak and Balcerek 2018).

The heavy metal waste from industries and its accumulation causes a significant threat to the environment. Such waste is a common cause of soil and water pollution due to its highly toxic nature. At low concentration, these metals are highly reactive, and when accumulated, they can enter into the food web resulting in serious health hazards to the majority of life forms, including humans. Physical and chemical conventional methods for remediation have been utilized, but low economic feasibility and generation of toxic chemical by-products limit the utilization of these techniques. Biological approaches like bioremediation and phytoremediation utilize microorganisms and plants to absorb and extract heavy metals accumulated in soil and water and have shown promising outcomes in significant recovery of such metal forms in an eco-friendly mode (Barakat 2011; Rai et al. 2019).

Chemical waste from pesticide, food processing and packaging, pharma, petrochemical and textile industries land up in water bodies and soil causing great harm to the environment and variety of living species. Dye production for textile industry accounts for around 800,000 tons every year. Various organic and inorganic chemicals increase the chemical load in environment and accumulation of such huge chemical waste for long can build up highly toxic compounds which can enter into the food chain and cause numerous diseases like renal disorders, central nervous system disorders, etc. Physical and chemical treatment reduces chemical waste from the environment like filtration, adsorption, oxidation, ozonization, etc. are known, but their efficiency and by-products produced in turn pose some limitations. Biodegradation and bioremediation of chemical waste by utilizing microbes or enzymes produced by them have been investigated in many kinds of research and seem to possess great potential in this concern (Randhawa and Kullar 2011; Ali 2010).

However, conventional physical and chemical methods are widely being used, but most of them possess associated limitations and disadvantages. The following sections deal with a brief discussion of physical and chemical and thermo-chemical methods and their associated limitations for waste treatment and management.

3.4 Physical, Chemical and Thermo-chemical Methods of Waste Treatment

Various conventional methods based on physical, chemical and thermo-chemical principles have been developed to reduce a wide range of generated waste (Fig. 3.1). Some methods do possess the potential to convert waste into valuable products,

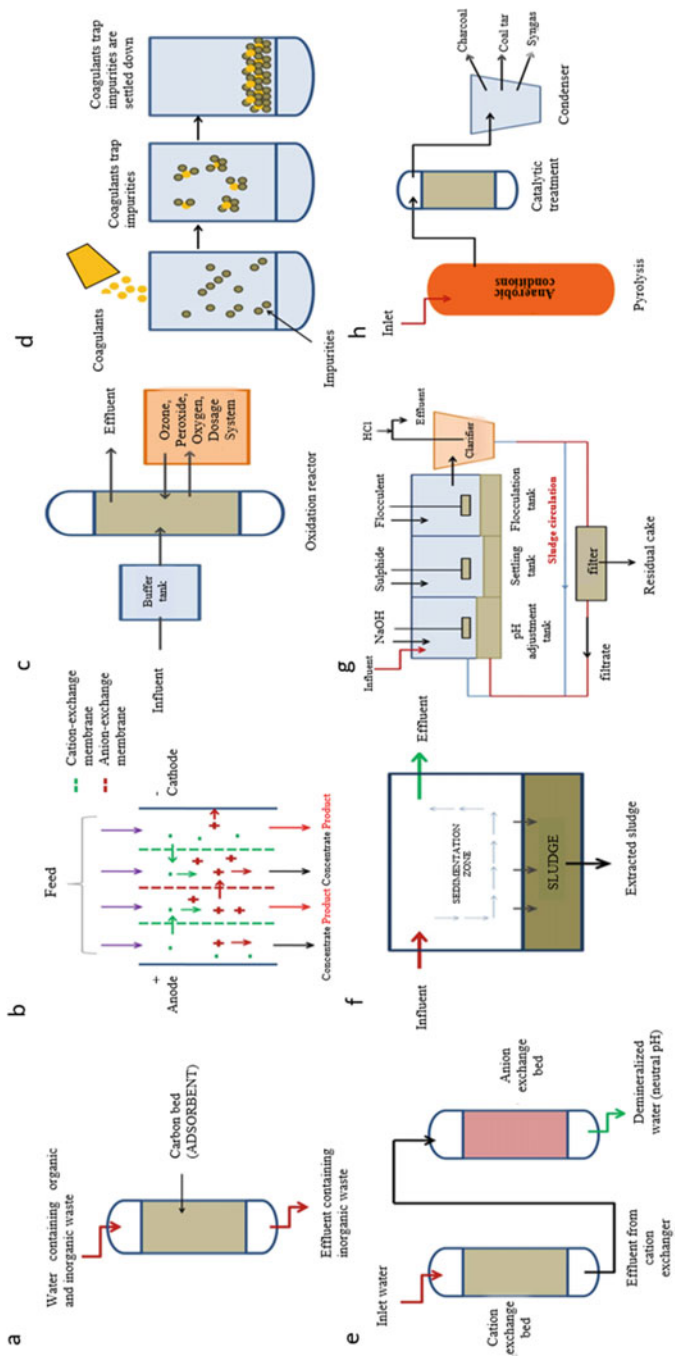

Fig. 3.1 Various physical, chemical and thermo-chemical treatments used for waste treatment: (**a**) Adsorption, (**b**) Electrodialysis, (**c**) Oxidation, (**d**) Coagulation, (**e**) Ion exchange (**f**) Sedimentation, (**g**) Chemical precipitation, (**h**) Pyrolysis

whereas others break the complex compounds into intermediate or simpler ones. The common physical methods utilized for waste treatment consist of adsorption, electrodialysis, ion exchange, sedimentation, photo-catalysis and membrane filtration. Chemical methods which have been utilized to treat waste in large quantities comprised of oxidation, advanced oxidation, coagulation and chemical precipitation. Some thermo-chemical methods include combined heat and power technique, gasification, pyrolysis, incineration, refuse derived fuel pallet formation and catalytic waste conversion (Krounbi et al. 2019; Santos et al. 2020).

3.4.1 Physical Methods

3.4.1.1 Adsorption

Among the various available techniques for the treatment of waste, adsorption is the most efficient process. It involves the use of solid adsorbent and segregates organic waste from inorganic waste. This is a physic-sorption method in which insoluble solid waste can be separated by passing it through an adsorbent which will bind the organic solid waste through a physical or chemical bond (Babel and Kurniawan 2004) as shown in Fig. 3.1a. This method is advantageous over other conventional methods because of its simple designing and low investment for operation. This process is widely being used for the treatment of industrial as well as household waste. The cheapest and readily available adsorbent is activated charcoal (Crini 2005).

3.4.1.2 Electrodialysis

The technique of electrodialysis is mainly used to extract heavy metals from different waste sources, mostly water polluted with heavy metals. Through this technique, ions are separated by passing them through the ion-selective membrane and are concentrated by applying an electric field. With the application of two oppositely charged electrodes on two sides of the membrane, the anions and cations are segregated (Yang and Yang 2006). The membranes are composed of polymers such as styrene or polyethylene, incorporating immobilized and mobile charges (Moreno et al. 2018). The separation is based on the attraction of oppositely charged ions towards the electrodes, and the efficiency of the separation process depends on the pH, flow rate, voltage, water ion concentration, etc. (Mohammadi et al. 2004).

3.4.1.3 Ion Exchange

Ion exchange is an attractive and easy process it employs ionic resins composed of carbon-based structures with attached functional groups. The functional group defines the selectivity of the resin as cationic resin exchange positive charge, whereas anionic resin exchange negative charge (Barakat 2011). The acidic functional groups exchange sodium and hydrogen and form cationic resins while the primary functional groups such as amines exchange hydroxyl ion and form anionic resins. As shown in Fig. 3.1c, the heavy metal ions such as Cr^{+6}, Pb^{+2}, Zn^{+2}, Cu^{+2} and Cd^{+2} can be removed by this technique (Bose et al. 2002).

3.4.1.4 Sedimentation

The heavy waste particles suspended in the slurry waste can be removed by gravitational settling. The waste is blocked into large settling tanks known as clarifiers so that the particles settle down and then can be removed as shown in Fig. 3.1d. The force that drives this clarification process is gravitational force and density gradient difference (Duncan et al. 2018).

3.4.1.5 Photo-Catalysis

Photo-catalysis is a very promising technique that is used for the treatment of waste released from chemical industries. This technique is based on nano-catalysts which absorbs UV radiations from the sun and degrades the toxic pollutants. Pollutants such as organic acids, dyes, pesticides, crude oil, inorganic molecules, etc. are broken down by this energy into simpler substances (Skubal et al. 2002).

3.4.1.6 Membrane Filtration

The waste released from different industries and household contains toxic solids, organic matter and microbes, which can be removed by membrane filtration. This is an advanced filtration technique and employs thin layered semi-permeable membranes. These membranes are composed of organic polymers such as polysulphones, cellulose esters and acetates, polyethene, polyimide/polyamide, polypropylene and polyether ketones, etc. Based on the type of pore size, nature of membrane and charge used for segregation, membrane filtration can be categorized as reverse osmosis, ultrafiltration, nanofiltration and microfiltration (Zhou and Smith 2002). Generally, for the treatment of industrial discharge waste nanofiltration and ultrafiltration are preferred because of high flux, good chemical resistance and pore size. Inorganic contaminants are removed by reverse osmosis where ionic diffusion takes place when the size of the ions of the inorganic contaminants is less than the narrowest part of the pore at low-pressure conditions, and low membrane thickness to porosity ratios (Van Der Bruggen et al. 2003).

3.4.2 Chemical Methods

3.4.2.1 Oxidation

Chemical oxidation of plastic waste along with phenols, chlorides and organic pollutants in large proportion is commonly used chemical treatment to degrade waste (Fig. 3.1e). Fenton's oxidation treatment is very effective in degrading such pollutants. This method works at low pH and degrades organic halides, polyphenols and total organic carbon. The various associated advantages of this method are easy handling and utilization of non-toxic reagents which are safe for the environment (Gogate and Pandit 2004). Advanced oxidation method break down toxic chemicals by the formation of hydroxyl radicals generated during oxidation. This method uses vigorous oxidizing agents like ozone, H_2O_2, catalysts like Fe^{+2}/Fe^{+3}, Mn, TiO_2, $NiSO_4$, CCl, $CuSO_4$ and higher-energy radiations (Dixit et al. 2015; Gogate and Pandit 2004).

3.4.2.2 Coagulation

Coagulation is a primary treatment given to waste slurry to segregate solid waste. In this process, the smaller waste particles present in the slurry are polymerized together to form big particles which are then filtered and subjected to secondary waste degrading treatments (Fig. 3.1f). The coagulants used for this purpose are salts of iron, aluminium, titanium and zirconium (Wan et al. 2019; Lofrano et al. 2013). These coagulating agents are susceptible to pH change.

3.4.2.3 Chemical Precipitation

Chemical precipitation is the method used to separate pollutants such as chlorides, sulphides and heavy metals by using different metallic salts (Mirbagheri and Hosseini 2005). Generally, the metals are precipitated as hydroxides by adding lime, and the other contaminants are removed by adding aluminium sulphate and ferric chloride (Nassef 2012) (Fig. 3.1g). This method is quite economical and also efficient enough to clean large volumes of waste. The major drawback of this method is its pH specificity.

3.4.3 Thermo-Chemical Waste Treatment

3.4.3.1 Combined Heat and Power

Combined heat and power (CHP) technology or cogeneration is used for simultaneous generation of heat and electricity by heat engines or power plants. Cogeneration allows efficient and effective use of primary energy resource (Kalam et al. 2012). It involves the conversion of all primary resources like vegetable oil, coal, biomass, bio-ethanol, heating oil, natural gas, biogas, municipal solid waste, etc. into electricity and heat by using circulation fluidized bed gasification technology. This technology is considered better for fuel conversion than direct combustion. It is highly beneficial for the industries or areas where, along with electricity, heat is also required (Roddy 2012). The deployment of CHP is very cost-effective, requires limited geographic manipulations and is less time-consuming. CHP plants have about 80% efficiency, which is relatively more than the conventional power plants. CHP not only solve the fuel wastage issue, but it also mitigates the harsh climate changes.

3.4.3.2 Pyrolysis

Pyrolysis is a process of decomposing organic waste by application of heat in strict anaerobic condition. As oxygen is absent in this process, no combustion of organic matter occurs, but the matter converts into combustible gases and charcoal. Pyrolysis of biomass gives out the three major products, e.g. bio-oil(liquid), bio-char(solid) and syngas(gas) (Fig. 3.1h). Bio-oil finds its application as low-grade diesel, bio-char increases the fertility of the soil and syngas can be used as fuel or be converted to ethanol by the action of specific enzymes (Devarapalli and Atiyeh 2015). In this process, destruction of the contaminants into smaller particles with lower molecular weight occurs, followed by separation of the contaminants from

organic matter. It is highly useful for organic substances like polychlorinated biphenyl (PCBs) and polycyclic aromatic hydrocarbon (PAHs) that are susceptible to cracking at high temperature.

3.4.3.3 Gasification

Gasification is a sustainable process which partially oxidizes carbonaceous matter like coal, pet coke, biomass, etc. into hydrogen, carbon monoxide, carbon dioxide, synthetic fuels, etc. which are collectively called syngas. Syngas is used as a feedstock to chemical industries and can be used to generate power. Waste biomass can be easily converted into energy source by this method with great ease (Farzad et al. 2016). It is a five steps process where firstly the organic matter is dried using high-temperature conditions (100–150 °C). Then, by the process of pyrolysis, the dried mass is converted to charcoal by heating it in the absence of oxygen at 200–500 °C. After charcoal formation, the air is sparged in the gasifier to burn and crack tar into lighter gases at a very high temperature of around 8000–1200 °C. These gases can then be reduced to flammable gases by passing carbon dioxide or water vapour across the bed of red-hot charcoal at 650–900 °C. The residual waste is collected at the landfill, while wastewater is first treated and then discharged into sewage or evaporated in the cooling tower (Breault 2010).

3.4.3.4 Incineration

Incineration is a commonly used process of waste management in which combustion of organic matter takes place. This technology helps in the conversion of waste into ash, flue gas and heat energy. The ash comprises the inorganic matter, and flue gas is released in the atmosphere only after treatment while heat can be utilized in thermal power plant for electricity generation. Though incineration is an exhaustive process, complete decomposition of waste does not occur, and only 80–85% of waste is reduced (Seltenrich 2016). The steps followed in this process include storage of waste and its conversion into feed for incinerator then burning of feed leading to the production of ash and hot gas. The temperature of hot gas is reduced to generate steam, and thus heat energy is recovered. Further, this gas is treated with cold air and then released into the atmosphere. Concludingly, temperature, turbulence and time act as key factors which significantly affect the process efficiency (Kuo et al. 2008).

3.4.3.5 Refuse Derived Fuel Pellet Formation

Refuse derived fuel is composed of combustible waste which cannot be recycled like plastic, label, paper cardboard, etc. This waste is transformed into pellets by compaction of garbage and is rich in organic matter. The moisture and inorganic matter are removed. Because of the reduction in the size of particles, the RDF pellets are homogeneous and are easier to use as compared to municipal solid waste feedstock (Brás et al. 2017).

3.4.3.6 Catalytic Waste Conversion

Waste biomass can sustainably replace fossil fuels for the generation of energy. This strategy includes a reduction in oxygen content of the feedstock for better energy

density and formation of C–C bond in the intermediates to increase the molecular weight of the final product. In this process, the waste biomass that is removed in the pyrolysis and gasification process is converted into energy. This whole process is known as aqueous phase reforming. The various catalysts like Pt black, ZrO_2, Al_2O_3, TiO_2, K_2CO_3 convert the biomass into energy. Furthermore, Pd can also be used, but it has less activity as compared to Pt (Park et al. 2012).

Plastic wastes have posed a serious environmental issue. They are non-biodegradable and are synthesized from non-renewable resources. The catalytic cracking of polyethylene and polypropylene can be done quickly by the use of selective catalysts to generate fuel. The various stimuli that are used for this process include Pd, Pt, Rh, Ni. Pd is highly active in this process, but it loses its activity with time-on stream (Keane 2009).

However, various above mentioned physical, chemical and thermo-chemical processes are being used for waste treatment, but the typical limitations of low efficiency, less process versatility, high energy input, harsh working conditions and increased usage of chemicals having toxic effect pose a significant challenge not only to the economy but also to the environment when implemented at large scale. The infrastructures needed for physical treatment methods have low economic feasibility (Garrido-Baserba et al. 2018). The chemical by-products released from chemical treatments of waste too have toxic effects if released in the environment. Complete degradation of conventional plastic is highly problematic even when subjected to high temperature and pressure and low pH conditions thus lead to the release of toxic by-products which have potential to cause significant damage to the environment as well as to associated living being. Chemicals like phthalates and bisphenol A or polychlorinated biphenyls released upon physical and chemical degradation result in highly toxic conditions. Such degradation coverts plastic into particles of microplastic, which spread over long distances and in turn increase pollution (Gallo et al. 2018).

To overcome these challenges, eco-friendly methods are of utmost importance. Microbial or enzymatic treatment of the enormous amount of waste can be a practical approach to deal with the current scenario. Wide variety of microbial genus and species have been investigated in a variety of studies for their ability to treat, reduce and completely biodegrade the hazardous waste. Different microbial flora from various sources has been implemented for the process of waste degradation. Numerous microbial pathways have been revealed which consumes waste components and converts them into valuable products. Scientists have developed leading approaches for treatment and management of waste to value products. The upcoming section discusses in detail the most efficient eco-friendly techniques which can be effectively utilized to treat and manage a considerable amount of waste produced around the globe. Moreover, corresponding researches conducted by numerous research groups have been illustrated to prove the high efficiency of these eco-friendly biological processes (Shalaby 2011; Le et al. 2016).

3.5 Eco-friendly Approaches to Waste Treatments

Though the exponentially growing global waste production is a tremendous challenge to the whole planet, the diverse microbial population present on the earth is compatible with fighting the challenge in a highly productive manner. Enormous biochemical pathway related to the wide range of microbes provides excellent scope to deal with the massive waste produced, with little or no harm to the planet "Earth". The huge piles of biodegradable and non-biodegradable plastic, highly toxic chemicals and heavy metals and lands filled form cellulose waste all can either be reduced to non-toxic forms or into valuable products upon subjection to diverse microbial flora. Activated sludge process, biomethanation, composting, bioremediation and phytoremediation are the leading eco-friendly approaches being implemented and further investigated for treating and reducing loads of waste being generated every year (Fig. 3.2) (Singh et al. 2019; Doiphode et al. 2016).

The conventional activated sludge process is based on the aerobic microbial treatment of solid and organic waste. Leading microorganisms such as bacteria (Bacteroidetes, Acidobacteria, chloroflexi, etc.), algae (Chlorella), fungi (Ascomycetes) are allowed to grow in large aeration tank containing dissolved oxygen and organic waste where they feed on waste and lead to floc formation (Rajasulochana and Preethy 2016). These flocs settle down at the bottom of the tanks and thus can be removed off in later stages (Fig. 3.2a). Though the conventional form of this process was not as efficient, recent developments have linked additional advancement to this process. Guven et al. (2019), in their study on high rated activated sludge process investigated that addition of food waste to municipal waste can further enhance the recovery of energy (Guven et al. 2019).

Biomethanation a multistep process of conversion of organic waste to methane has been well investigated (Fig. 3.2b). Large digester filled with hydrolysed organic waste and microbial flora in anaerobic conditions releases methane (55–75%), carbon dioxide (30–40%), and traces of hydrogen, hydrogen sulphide, ammonia, water vapour and nitrogen (Angelidaki et al. 2011). The two by-products, namely biogas and manure produced by this technology can be directly utilized as biofuel and fertilizer, respectively (Table 3.3). Two stages of biochemical conversions which take place in this process include hydrolysis, acidification and liquefaction at the first stage, and the transformation of acetate, carbon dioxide and hydrogen to methane at the second stage. Mishra et al. (2018) in their biomethanation study of recalcitrant crystalline cellulosic and lignin agricultural waste investigated microbial pretreatments like composting, microaerobic treatment, digestion stage separation and lignocellulolytic fungal treatment to have great potential in increasing methane yield with very low-cost inputs (Mishra et al. 2018). Dahiya and Joseph (2015), designed a high-efficiency biomethanation digester for treatment of food waste giving high biogas yield with around 60% methane content (Dahiya and Joseph 2015). In a recent study, Yang et al. investigated enhanced biofuel production from food waste when syngas biomethanation was integrated with anaerobic digestion of waste and digestate pyrolysis. The production of biomethane increased by 22%

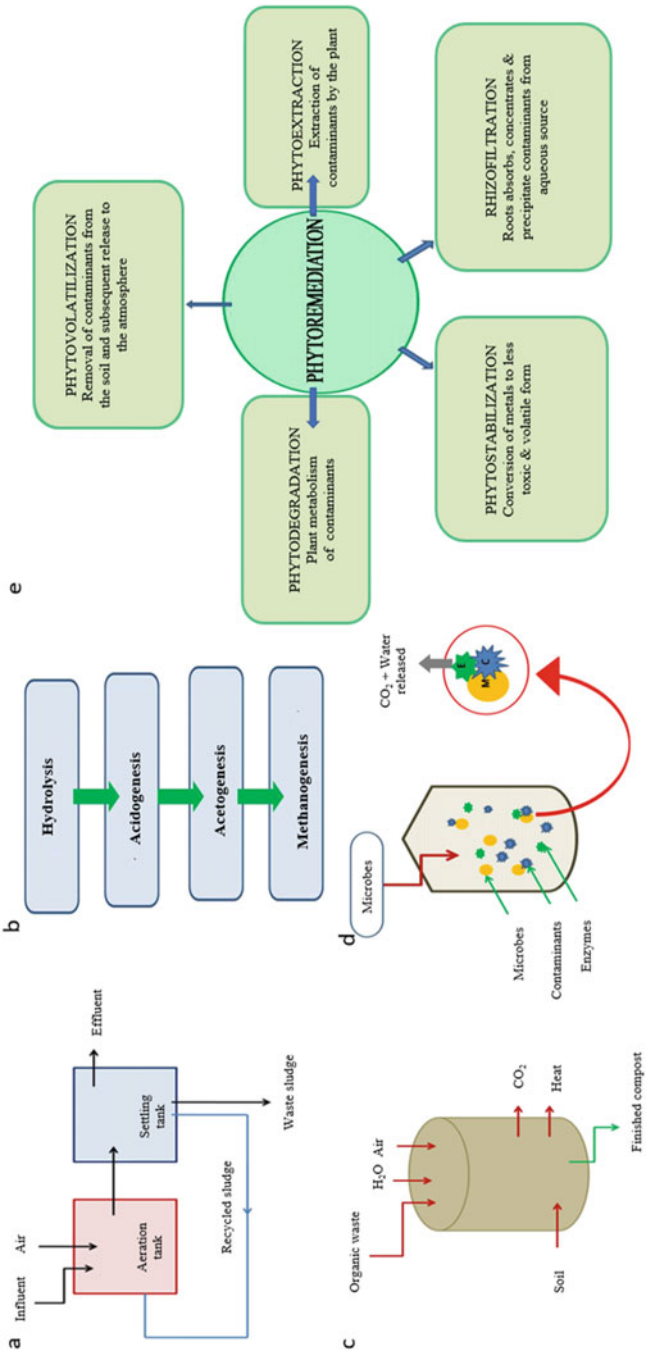

Fig. 3.2 Eco-friendly methods for waste treatment (**a**) Activated sludge process, (**b**) Biomethanation, (**c**) Composting, (**d**) Bioremediation, (**e**) Phytoremediation

Table 3.3 Advantages and disadvantages of biomethanation

Advantages	Disadvantages
It generates gaseous fuel.	The investment is more as compared to landfill and composting.
It can be carried out at a small scale.	The destruction of pathogens in the digesters operating at moderate temperature is less as compared to aerobic composting.
It does not require any external power supply like aerobic composting.	The process is not favourable for waste containing less biodegradable stuff.
The green gases produced in this closed container are collected for use, avoiding emission to the atmosphere.	
For establishing this plant, less land area is required.	
The end products, biogas and manure, are eco-friendly.	

when the gas volume increased from 560 to 5300 ml in heterogenotrophic methanogens containing thermophilic reactors (Yang et al. 2020).

Composting is an effective, eco-friendly, simple biological approach of aerobic degradation of organic waste by microbes (Fig. 3.2c). The compost produced is rich in nutrients which can immensely increase soil fertility, add humus to the soil and act as a natural pesticide as well. It prevents soil erosion by covering landfill areas and land and stream reclamation. The process of decomposition depends on proper air, water, decomposers and physiochemical conditions of temperature and pH. Bacteria and fungi are the main decomposers that produce heat, carbon dioxide and ammonia from the input (Debertoldi et al. 1983). Cellulose present in organic waste is highly recalcitrant in nature and requires specific microbial flora for its digestion. The major bacterial species which poses cellulose-degrading ability are *Cellulomonas*, *Bacillus sp*., *Pseudomonas* and *Thermoactinomycetes,* while the counterpart fungal species are *Trichoderma*, *Aspergillus*, white-rot fungi and *Sclerotium* (Gupta et al. 2012).

Vermicomposting by using earthworms as decomposers is an advancement in composting technology. The vermicast produced by earthworms is known to have high levels of plant nutrients and does not contain pathogenic microbes. Thus, utilization of this technology can not only reduce the waste load but can also provide high-quality manure for cultivation. Pirsaheb et al. (2013) in their vermicompost study on solid waste management depicted high ease of vermicomposting by using a native earthworm species *Eisenia fetida* and suggested vermicomposting can be employed by the homeowner for their produced waste (Pirsaheb et al. 2013).

Enzymatic degradation of solid waste is a highly efficient approach which can be utilized to degrade highly recalcitrant and toxic waste types. Wide range of enzymes can be isolated from microbes and can be immobilized or encapsulated into different substrates or matrices and then can be used for waste degradation. Such immobilization and encapsulation will increase the enzyme efficiency by providing favourable local conditions along with reusability (Feng et al. 2013). The common

enzymes employed for this purpose are cellulases, oxidoreductase, ligninase, laccases, peroxidases, tyrosinases, lipases, etc. These enzymes can even degrade resistant polymers like lignin, cellulose as well as phenols and chlorinated and non-chlorinated toxic substances. Alrumman (2016), optimized the process of fermentation and saccharification of cellulosic waste of date palm to lactic acid and glucose by utilizing cellulases produced by *Geobacillus stearothermophilus* and reported effective pH and temperature conditions of 5 and 50 °C, respectively (Alrumman 2016). Another resistant natural polymer present in large proportion in agricultural waste is lignin, which can be effectively degraded by ligninolytic enzymes like lignin peroxidase, laccase, peroxidase and manganese peroxidase (Kumar and Chandra 2020). Past study has reported the effectiveness of above-mentioned lignin-degrading enzymes in the degradation of petroleum-based polyethylene plastic. *Rhodococcus ruber* C2088 strain producing laccase was reported, as an effective strain for degradation of UV irradiated polymer of polyethylene (Gómez-Méndez et al. 2018). Moreover, proteases and esterases have been reported to degrade polyester polyurethane. PET or polyethylene terephthalate is a widely used plastic polymer which was known to be highly resistant to degradation. Several studies have reported significant degradation of PET by bacterial carboxylesterases produced by *B. subtilis, B. licheniformis* and *T. fusca* and cutinase enzyme from *T. insolens* (Wei and Zimmermann 2017).

Bioremediation is a highly effective, economically feasible and eco-friendly technique for degradation of a wide range of waste. This process converts the toxic chemical into simpler non-toxic substances by utilization of different microorganisms. This process consists of three stages, namely biostimulation, bioaugmentation and natural attenuation (Fig. 3.2d) (Jeyasingh and Philip 2005). Commonly used microbes for bioremediation of petroleum-based waste are *Pseudomonas, Bacillus, Alcaligenes, Micrococcus*, etc. Also, the biofilm-forming bacteria provide suitable micro-environment for soil decontamination (Solanki et al. 2020). Hazardous heavy metals which can enter food web and are highly reactive even at low concentration can be effectively absorbed by specific microbial species. These species have high metal holding capacities due to the presence of specific metal sequestrating mechanisms. Zhao et al. (2019) identified four strains, namely CZW2, CZW9, CZW5 and CZW12, which were having high urease activity, from abandoned mine and revealed their high bioremediation efficiency for cadmium removal. These strains remove cadmium by biomineralizing the toxic metal, thus posing no harm to the environment (Zhao et al. 2019). Further, the mechanism of biomineralization of calcium carbonate by various ureolytic bacteria is highly established and is being utilized to reduce high metal loaded toxic waste to a non-toxic form. Bioremediation has also been reported for the treatment of waste from pharmaceutical industries, petrochemical industries, as well as pesticide industries (Dhami et al. 2013). Randhawa and Kullar, in their study on high-efficiency bioremediation approach, using cow dung and its microbiota proved high scope for degradation of toxic chemicals from above-mentioned industries. Another study by Hossain et al. provided strong evidence for textile industry dye wastewater decolourization by white-rot fungi (Randhawa and Kullar 2011). Further, Dussud et al. (2018), while

studying biofouling mechanism of marine biofilms over different non-biodegradable and biodegradable plastics, revealed abundant progressive colonization of hydrocarbonoclastic bacteria (Aestuariicella hydrocarbonica, Alcanivorax sp., Marinobacter sp., and Neptuniibacter sp, Lutibacterium anuloederans) over all types of plastics including polyethylene, polyethylene with prooxidant, OXO (artificially aged), polyester and poly 3-hydroxy butyrate co-3-hydroxy-valerate providing evidence for the plastic degrading and colonizing ability of the associated microbes (Dussud et al. 2018). Bryant et al. (2016), in their study on active communities inhabiting on marine plastic debris identified *Bacteroidetes, Cyanobacteria, Alphaproteobacteria* and *Bryozoa* as dominant species inhabiting on plastic debris and seems to be very well adapted to their substrate (Bryant et al. 2016). Oberbeckmann et al. (2016), revealed that Bacteroidetes like *Cryomorphaceae, Flavobacteriaceae, Saprospiraceae* are capable to abundantly colonize non-biodegradable PET plastic bottles. Thus, all these studies depict great scope for bioremediation of plastic by bacterial species capable of degrading the most massive forms of non-biodegradable plastic waste (Oberbeckmann et al. 2016).

Phytoremediation is closely associated with bioremediation and utilizes plants for detoxification of waste and that of the environment. In this technique, the whole plant or specific parts of plants are used to remove pollutants. It is a green technology that converts toxic pollutants into harmless substances (Chaney et al. 1997). Phytoremediation is a combination of five environment-friendly approaches, phytovolatilization, phytodegradation, rhizofiltration, phytoextraction and phytostabilization as described in Fig. 3.2e. It works on the smooth plant–microbe association in rhizospheric region and converts hazardous pollutants to simpler non-toxic ones. Various plants such as *Arabidopsis sp., Thlaspi sp., Sedum alfredii sp.* have great potential in accumulating the toxic components and translocating into other parts of the plant which can be removed off easily (Kavitha et al. 2016). Several researchers have utilized phytoremediation technology for the removal of heavy metals from water and soil (Lone et al. 2008). Liu et al. in their bioremediation study of soil contaminated by petroleum oil suggested ornamental plants of *Fire Phoenix, G. aristata, Fawn, M. sativa* and *E. purpurea* species as highly potent remediators (Liu et al. 2012).

All the above-mentioned eco-friendly approaches provide strong evidence for the high effectiveness of biological treatments for waste treatment, utilization, degradation and reduction. The major advantages associated with these approaches are high economic feasibility, production of valuable by-products, low or null toxic effects on the environment, less space and infrastructure requirement and easily available input materials. Hence, further research to improve these bio-based approaches for large scale waste treatment and enhancement of produced valuable by-products can be of great benefit to society and is urgently demanded.

3.6 Conclusion & Future Prospects

A huge amount of waste is being produced around the earth. The growing urbanization and industrialization have improved the living standard and the choices of the population, which are the significant reasons for the overexploitation of resources and thus enhanced waste production. The increasing waste loads demand for high-efficiency approaches which can be easily practised without any harm to the environment. The conventional physical, chemical and thermo-chemical treatments used for waste management are unable to meet the requirement due to low efficiency and non-environment friendly nature with high associated cost and infrastructure need. Bio-based or biological treatments like composting, bioremediation, enzymatic treatment, biomethanation and phytoremediation are highly eco-friendly and cost-effective approaches and cause very low or null adversity to the environment. Although much research has been conducted to develop these approaches for large scale and for a wide variety of waste produced, further improvement is required to investigate higher efficiency versatile microbes to degrade till date non-biodegradable waste. This chapter provides insights about the conventional as well as recent eco-friendly approaches developed to date for waste treatment to improve awareness and evoke the interest of the readers in this concern.

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Eco-friendly Microbial Biofuel Production from Waste

4

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Abstract

The rapid consumption of liquid fossil fuels not only causes depletion of energy source but also gives rise to the pollution problem of air, land and water increasing greenhouse gases which is concerned with climatic changes like global warming which in turn raises the sea level. An eco-friendly alternative of energy, i.e. biofuel, is required, which is a promising technology as it reduces the problems of production of eco-friendly energy, zero CO_2 emission and cost-effective fuels. This makes it of high demand across the world over conventional fuels. Scientists are concentrating on biomass-based biofuels, especially agricultural biomass and wastes which can transform into liquid biofuels with the versatile use of microbes. Production of renewable energy biofuels with the versatile utilization of microbes from the biological waste and biomass can reduce this threatening concern to a massive extent. Over the past few years, there has been a steady increase in the use of microbes as they have diversified metabolic activity, which enables substantial biofuels production utilizing different substrates. For the production of ethanol, bacteria utilize sugars, and cellulolytic microbes utilize substrates which are driven from plants. Atmospheric CO_2 is

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reduced to biofuels photosynthetically by *Cyanobacteria* and microalgae; methane is used by methanotrophs for the production of methanol. Electrons are explicitly transported from the microbial outer-membrane to the conductive surfaces by *Geobacter sulfurreducens* and *Shewanella oneidensis*. This molecular mechanism of these microbes was set up in bioelectrochemical devices for the production of biohydrogen and bioelectricity. In the coming days focus should be on the confrontations related to the facilitation of the microbes and develop them a clear choice to replace the typical fossil fuels. Globally, there is a need for the versatile utilization of microbes in producing biofuels using several substrates has been increased due to their metabolic diversity. Among biofuels, bioethanol is gaining importance. It could be generated from numerous feedstocks (sucrose and starch, lignocellulosic and algal biomass) by fermentation through microbes. A total of 30 billion litres of biofuel are utilized annually. Production of ethanol includes pretreatment, hydrolysis and fermentation and depends on various factors, viz., inoculums size, fermentation time, pH, temperature, sugar concentration, and agitation rate, etc. Among microbes, microalgae should provide an alternative for biofuel production, replacing all renewable energy from biological wastes and biomass is gaining importance shortly.

Keywords

Microbes · Renewable energy · Biofuels · Microalgae · Biohydrogen

4.1 Introduction

A billion tonnes of carbon emission ejected into the environment due to the peak production of oil worldwide. This causes global warming to change the climate, and it is also exhausting future conventional energy resources. Hence it is essential to prevent future energy crisis by exploring to eco-friendly energy reserves. Reducing the concentration of global greenhouse gas to below 550 ppm carbon dioxide (CO_2) corresponds to severe emissions decrease equally to phase out all fossil fuel released in a developed country by 2050, if the emissions in developing countries continue to grow up as expected (Flannery and Malin 1998; Kheshgi et al. 2000; Wei 2020). Greenhouse gases emission is due to the use of energy (Boerrigter 2006; Marchi et al. 2019; Delucchi 1997). However, in developed countries, all energy-related emissions are due to electricity and transportation fuels. At present, 11% of global primary energy contributed by current renewable energy, but shortly 60% of energy comes from current renewable sources. A total of 21% of global CO_2 emission into the environment is by the transportation sector. The emission from power production accounts to second. By 2030, average financial growth maybe 3.2% annually. The increase in energy demand for transportation is about 2.1% over the same period at an average annual rate. Total anthropogenic greenhouse gases (GHG) emissions from the transport sector are projected to be about 23% in 2030 (Kheshgi et al. 2004; Boden et al. 2017; Prasanna Choudhury 2018). Renewable sources for biofuel

production can limit fossil fuel utilization and maintain eco-friendly as well as economic sustainability. Hence there is a need for alternative fuels by replacing conventional fuels.

This chapter focuses on a broad view of the versatile utilization of microbes for the replacement of conventional fuels by their diversified metabolic pathways. Among microbes, microalgae should provide an alternative for biofuel production, replacing all renewable energy from biological wastes and biomass is gaining importance shortly.

4.2 Types of Biofuels

Organic matter derived biofuels are named as solid, liquid or gaseous fuels (Nigam and Singh 2011; Koskin et al. 2020; Sindhu et al. 2019). Biofuels are classified as (i) Natural, (ii) Primary and (iii) Secondary biofuels. Organic sources (landfill gas, vegetable, animal waste) derived biofuels are natural biofuels. Fuel-woods derived biofuels are primary biofuels used mainly for general purpose (electricity production, heating, cooking or brick kiln). Biomass derived from biofuels is secondary biofuels (biodiesel and bioethanol) used in transportation (Loy et al. 2015; Sindhu et al. 2019). Based on processing technology, feedstocks and their development stages, secondary biofuels are divided into three generations such as (a) 1st generation biofuels, (b) 2nd generation biofuels and (c) 3rd generation biofuels (Kumar et al. 2020).

The first-generation biofuels are derived from human feedstocks and CO_2 free biofuels. Major drawbacks of first-generation biofuels are that more agricultural lands are required for biomass production, and this limits the production of human and animal feedstocks. This causes severe food shortage in countries like South America, Africa, Asia, where arable land is reducing and but 800 million people are suffering from hunger. The decrease in food production lands along with environmental pollution is due to extensive usage of fertilizers, pesticides and freshwater (Deknock et al. 2019; Schenk et al. 2008). Increasing in food price beyond is due to an increased usage of first-generation. Hence, scientists focused on 2nd generation biofuels (Ayodele et al. 2020).

Secondary biofuels are derived from lignocellulose, woody part of plants. Biofuel production using lingo-cellulose, agricultural deposit and waste includes pruned branches, leaves, forest harvesting residues, wood chips, wood processing residues like beet sugar cane, sawdust and non-edible components of corn, etc.

The second-generation biofuels do not contest with human and animal feedstocks (Vázquez et al. 2020). Sophisticated and expensive tools are required for the converting woody biomass into fermentable sugars. The major drawback of second-generation biofuel is that pretreatment requires special enzymes which make non-profitable economically for marketing (Vázquez et al. 2020; Lara et al. 2020).

Third-generation biofuels include microalgae as the alternative energy resource can overcome drawbacks of 1st and 2nd generation biofuels (Sadatshojaei et al.

2020). Biodiesel production from microalgae is efficient because of its short life cycle about 1–10 days for harvesting depends on multiple or continuous harvesting process (Muhammad et al. 2020) for accumulating triacylglycerides (TAGs) and 300 times efficient than conventional crops on region basis (Jeslin et al. 2020) and can be harvested. For biomass production elevated valuable land is not essential (Ingle et al. 2020).

4.3 Microbes as Biofactories for Biofuel Production

Microbes consume organic substrates and convert them into useful products for humankind in their metabolic process for the generation of biofuels. For biofuel production microbial strain, substrates and production process selection play an important role. Bioethanol production from corn requires more amount of fossil fuel in comparison with the method, which includes sugarcane as it is a substrate (Jugwanth et al. 2020; Hoffstadt et al. 2020). For commercialization, biofuel with additional net balance energy is appropriate for consideration.

Efficient substrate selection for microbes is an essential concern in biofuel production. The organic substrates include lignocellulose, waste from agriculture and plant biomass are the most vital alternative feedstocks. But lignocellulosic substrates are not entirely degraded into their fermentative substrates by yeast (*S. cerevisiae*) (Chang et al. 2013; Claes et al. 2020). Various types of microbes involved in biofuel production from various precursors were listed (Table 4.1).

In the biofuel production process, lignocellulosic biomass of plants can be converted into simple sugars employing pretreatment succeeded by hydrolysis with enzymes or by combining bioprocessing (Chew et al. 2018; Verardi et al. 2020). Cellulolytic hyphal penetration may be biological, chemical, and physical or combination of these methods. Use of either non-complicated enzymatic cocktails or by cellulolytic microbes for the hydrolysis of penetrated biomass is preferred (Adsul et al. 2020). From anaerobic digestion of organic substrates or through landfills, CO_2 is produced in more quantity, followed by methane in less quantity (Yvon-Durocher et al. 2014). Natural gas majorly consists of methane, but the production is ceased. Hence this causes the urgent need for a more proficient carbon source. Methanotrophs directly utilize natural gas wells or landfill for production of fuels or may be converted into methanol which was used for fuel generation by methylotrophs (Gautam et al. 2020). Methanotrophs reduce oxygen atoms to hydrogen peroxide (H_2O_2), in turn, oxidize methane in turn through methane monooxygenases (MMOs) (Fuerst 2013; Gautam et al. 2020). Methane is transformed into methanol. MMOs may be soluble and particulate type. Particulate type methane monooxygenase cells are dynamic growth and more affinity for methane than the soluble type of cells (Lee et al. 2020).

Table 4.1 List of various biofuel producing microbes from various precursors

S. No.	Microbes	Feedstock used	Technique used	Silent features	References
1.	*Clostridium acetobutylicum*	Sweet sorghum stalk	Fermentation	Sweet sorghum was used as feedstock for biofuel production as it does not interfere with food	Mirfakhar et al. (2020)
2.	*Clostridium thermocellum*	Switchgrass	Fermentation	Increasing solid concentration results in a reduction of solubilization of carbohydrates has been observed for switchgrass	Shao et al. (2020)
3.	*Escherichia coli* (strain TC4)	*Lignocellulosic sugars*	Fermentation	*Z. mobilis, as well as S. cerevisiae,* has the limitation of using pentoses for ethanol production, this was overcome by genetically modified organism (GMO) *E. Coli*	Shanmugam et al. (2020)
4.	*Saccharomyces cerevisiae*	Watermelon, waste bread and muskmelon	Fermentation	These feedstocks are potential precursors for bioethanol production waste and alleviate financial loss due to food waste	Ünal et al. (2020)
5.	*Saccharomyces cerevisiae*	Waste office papers	Fermentation	Hydrogen peroxide (H_2O_2) (0.5% v/v) pretreatment with results in increased cellulose and removal of lignin from waste, which further converts into bioethanol	Annamalai et al. (2020)
6.	*Rhodococcus opacus, Saccharomyces cerevisiae, Schizosaccharomyces pombe* and *Zymomonas mobilis*	Municipal solid waste	Fermentation	These microbes grow on organic fraction of municipal solid waste (OMSW) fibre hydrolysate for industrial bioprocesses development	Dornau et al. (2020)
7.	*Zymomonas mobilis*	Sugarcane bagasse	Co-fermentation	Continuous SHcF (separate hydrolysis and co-fermentation) and SScF (simultaneous saccharification and co-fermentation) ethanol production from	Wirawan et al. (2020)

(continued)

Table 4.1 (continued)

S. No.	Microbes	Feedstock used	Technique used	Silent features	References
				alkaline-pretreated sugarcane bagasse by PVA(polyvinyl alcohol)-immobilized microbes	
8.	*Zymomonas mobilis* and *Scheffersomyces shehatae*	Kans grass (Lignocellulosic biomass)	Co-culture fermentation	*Z. mobilis* and *S. shehatae* co-culture of microbes with 84.88% of sugar recovery from kans grass, resulting in high ethanol yield	Mishra and Ghosh (2020)
9.	*Caldicellulosiruptor bescii*	Black cottonwood (*Populus trichocarpa*)	Fermentation	*C. bescii* lignocellulosic fermentative microbe can use both hexoses and pentoses for biofuel production	Straub et al. (2020)
10.	*Aspergillus niger* MK543209	Paper waste	Submerged fermentation	*A. niger* (MK543209) converts paper waste to reducing sugars and finally produces bioethanol	Darwesh et al. (2020)
11.	*Trichoderma reesei* and *Aspergillus niger*	Dragon fruit peel	Fermentation	In the treatment, *T. reesei* and *A. niger* enzymes enhance the sugar levels reduction of about 49.68%. Highest bioethanol level was seen in *T. reesei*:*A. niger* (3:1) ratio	Widyaningrum and Parahadi (2020)

4.4 Upscaling of Biofuel Production by Metabolic Engineering

For biofuel, production microbes exhibit specific metabolic pathways and various catalytic enzymes (Fig. 4.1). In yeast (*Saccharomyces cerevisiae*) pyruvate is directly decarboxylated for the production of ethanol. In *Escherichia coli*, ethanol is produced from the decarboxylation of pyruvate followed by reduction, in which the acyl group was activated by coenzyme A (CoA). To increase the productivity of biofuels, metabolic engineering of pathways would be profitable, which can be applied in different ways.

Ethanol production in *S. cerevisiae* and in *E. coli* can be produced in two ways. Comparatively, CoA is considered as an effective method for producing ethanol (Liao et al. 2016). By using genetic engineering techniques, genes for a pathway of producing ethanol were expressed in other microbes. Thus, microbes were deficient in metabolic pathway for certain biofuel, hence they were transformed with genes from microbes capable of biofuel production, results in non-biofuel microbes to biofuel fuel generating microbes. For biofuel production, engineering of microbes with this approach is beneficial for exploiting various substrates.

In *E. coli*, fatty acid biosynthesis was inhibited by acyl-ACP (acyl carrier protein) (Misson et al. 2020; Davis and Cronan 2001). Synthesis of free fatty acids is allowed by the inhibition of overexpression of thioesterase in turn allows the synthesis of precursor (acyl-CoA, for fatty alcohol synthesis). Using advanced techniques and

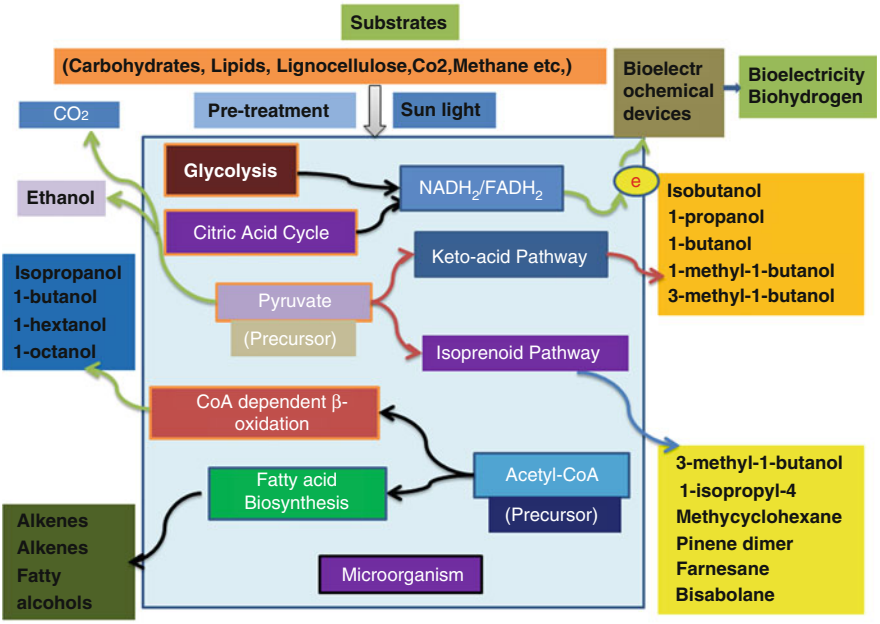

Fig. 4.1 Overview of microbial metabolic pathways for biofuel production (Kumar and Kumar 2017)

experimental tools catalytic activity and turnovers of substrate-specific enzymes can be improved by manipulation of the genetic material of enzyme. To produce artificial enzymes with the desired function is achieved by a computational approach to structure unnatural amino acids for biofuel production. Artificial metabolic pathway synthesis is a challenging task that requires sophisticated tools to control the mRNA protein level for the proper functioning of the artificial pathway.

4.5 Microbes as a Source of Bioelectrochemical Devices for the Production of Bioelectricity and Biohydrogen

Generating bioenergy from bioelectrochemical cells (BEC) by using organic biomass and wastewater has gained significant interest in recent years. For bioelectricity and biohydrogen production, microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) are employed (Ramanjaneyulu and Reddy 2019; Sangeetha et al. 2020). For bioenergy production, common microbes can be employed because both these kinds of fuel cells employ on a parallel principle.

The BECs are exoelectrogens or electricigens that exhibit particular molecular machinery which aids in transporting electrons from the microbial outer-membrane of microbe towards the conductive surfaces (Ramanjaneyulu and Reddy 2019) to produce electrical energy and other products. However, the power generated by MFCs is about 1.2 V, and MECs is 3.4 mol H_2/mol-acetate (Logan et al. 2015) which is inadequate and not economically viable. A significant drawback of the real challenge to BEC is cost-effective on a large-scale production. To upscale the BECs, efficient and appropriate BEC design is essential. BECs are in their infancy stage, towards improving in the bioenergy production. Electroactive biofilm formation, mechanisms of transferring electrons help in improved perception of microbial pathways that are crucial in BECs performance, and further manipulation of this mechanism helps in progressing the energy from these systems.

For the exploration of microbial physiology and microbe metal surface interactions, bioelectrochemical cells offer a considerable opportunity to understand biofilm development and mechanisms of transferring electrons between bacteria and electrode surfaces and amongst bacteria.

Electrons are transferred from microbial outer surface to electrode surface in exoelectrogens utilizing specific redox proteins/molecules (Kracke et al. 2015), which may enhance more electrons to move at a more rapid rate. This is accomplished by genetic engineering techniques, by incorporating the genes of redox proteins into the exoelectrogen's hereditary material. Towards improving the performance of BECs this method would be enormously efficient in terms of diminishing the start-up time. In scaling up of this technology using genetically modified exoelectrogens could be beneficial.

4.6 Microalgae as a Source for the Production of Biofuel

The idea of biofuel production from microalgae is not a new one (Nagle and Lemke 1990). Algae are the oldest life-forms with the shortest life cycle with globally fastest-growing plants. In developing nations, the use of human feedstock as biomass for the production of biofuel is assumed to cause food shortage and global dissatisfaction. Hence, using of microalgae in biofuel production is an alternative with advantages like zero CO_2 emission, rapid doubling time, high lipids production capacity and no competition of feedstocks of humans and animals by microalgae, grown on non-agricultural land and saltwater. Biofuel production from microalgae will not compete with arable lands, humans and animal feedstocks. But some species of algae has the potential for biofuel production. Microalgae are phototrophic organisms that require CO_2, sunlight, water, inorganic salts like nitrogen, potassium and phosphorous and temperature of 20–30 °C. Microalgae fix CO_2 from the environment, release gas from industry and soluble carbonates (Kassim et al. 2020; Wang et al. 2008) and the biomass accounts for about 50% of carbon by dry weight (Porcelli et al. 2020; Mirón et al. 2003) by producing 100 t of biomass from 183 t of CO_2. Microalgae produce various types of lipids, hydrocarbons and other complex oils (Priharto et al. 2020) ranging from 20 to 50% of biomass by dry weight. Microalgae fatty acid and lipid composition depend on culture conditions and optimization of growth determining factors (Hu et al. 2008), algal growth rate and oil content of biomass. Oil production yield by microalgae is more about 100,000 L/ha compared to other crops. Algae use photoautotrophic, heterotrophic and mixotrophic mechanisms for its production but photoautotrophic production is the cost-effective, a practical and technically feasible method for significant production of algae biomass for non-energy production (Borowitzka 1997). Open and closed pond bioreactor systems are employed (Borowitzka 1999) for algae biomass production, but open pond systems achieve more biomass. One or additional solid-liquid separation steps are required for the recovery of biomass which accounts for 20–30% of the total costs of production (de Carvalho et al. 2020), and it is a critical phase of the algal biomass process (Wang et al. 2008). For economic feasibility, selection of harvesting technology is essential for algal biomass production (Alhattab and Brooks 2020).

Microalgae have rapid doubling time, less complicated structure and high oil content. Various types of microalgae involved in biofuel production were listed (Table 4.2). Many algal strains differ in their composition of oil, carbohydrates and proteins species are known. Algal strain with high carbohydrates and oils produces starches which can be fermented to ethanol and proteins are converted into animal grains. The mass oil-producing strains include (i) *Dunaliella tertiolecta,* (ii) *Chlorella,* (iii) *Botryococcus braunii*, (iv) *Pleurochrysis carterae* (also called CCMP647) and (v) *Gracilaria.*

Recently microalgae importance in biofuel production has observed and extensively cultured and reaped at a huge scale (Lee et al. 2019). Microalgae are rich in lipid content of about 5% dry weight of oil compared to terrestrial crops (Chisti 2007). Microalgae have a rapid doubling time of about 24 h, but some show

Table 4.2 List of various types of microalgae in biofuel production

S. No.	Microbes	Feedstock used	Technique used	Salient features	References
1.	*Selenastrum capricornutum*	Formulated media for microalgae	Photobioreactor	*S. capricornutum*, a novel strain for biodiesel production	Pugliese et al. (2020)
2.	*Chlorella sorokiniana* pa.91 and *Chlorella vulgaris*	Dairy wastewater treatment plant effluents	Photobioreactor	For high algal biomass production, high amount of N and P in wastewaters provides nutrients further results in production of biofuel	Asadi et al. (2020)
3.	*Chlorella vulgaris* 31	Glucose, maltose and sodium acetate	Photobioreactor	The feedstocks significantly promoted the growth of *C. vulgaris* 31 results in algal biomass production further used for biofuel production	Kong et al. (2020)
4.	*Chlorella pyrenoidosa*	Wastewater pretreatment by the magnetic field selenite enrichment (SE) medium	Bottom-magnetic field (MF) pretreatment mode	Bottom-MF and bypass-MF equipment wastewater pretreatment result in high algal biomass production further used for biofuel production	Feng et al. (2020)
5.	*Haematococcus pluvialis*	Bold's Basal medium (BBM)	Hydrolysis	The high ethanol yield and high methane yield with *H. pluvialis* as a proper candidate and astaxanthin production	Hosseini et al. (2020)
6.	*Scenedesmus obliquus*	Fish meal diet	Fermentation	Algal-assisted aquaculture algal biomass production further used for biofuel production	Ansari et al. (2020)
7.	*Haematococcus pluvialis*	TAP medium	Stirred tank photobioreactor	*P. tricornutum* for fucoxanthin production	Guler et al. (2020)
8.	*Dunaliella salina*	Organic solvents	In situ transesterfication	Enhancing the protein production in Dunaliella salina by optimization of culture condition	Sugumar et al. (2020)
9.	Marine *Chlorella* sp	Sugars	Direct transesterification	Oleaginous microalgae are promising for pigments, biodiesel, zero-waste biorefinery	Mandik et al. (2020)

doubling time for every 3.5 h during the peak growth phase (Włodarczyk et al. 2020). The oil content of microalgae is generally between 20% and 50%, while some strains can range as high as 80%. Microalgae biotechnology hinges on the progress of large-scale photobioreactors with zero contamination risk, which can operate under distinct optimal conditions. Closed air systems are better than open systems to evade mostly every single problem; but more economical and effectual closed culturing system development is vital (El-Dalatony et al. 2016; Pattiya and Suttibak 2017). The microbial biodiesel/ biofuel production includes algae cultivation, collection as well as harvesting. Biodiesel was produced by the transesterification of microalgal lipids. Triglyceride forms the backbone of lipids, in which 10% of the original weight of the oil contains glycerine, which was removed in the presence of a catalyst.

4.7 Challenges and Prospects

The application of modern different omic approaches—metagenomics, metatranscriptomics and metabolomics and their integrated analysis provides important data to analyse the microbes. By applying these technologies, a great amount of data in the environmental biotechnology field is provided, and there is a prerequisite to organize the data in a step-wise pattern within the database. All these modern techniques throw light upon the biodegradation pathways, the structure and functions of key proteins and molecular characterization of microbes. Both metabolic and signalling pathways in gene regulation, protein–protein interaction are used to study the model interactions among biological constituent entities. To analyse the interrelationships of microbiomes and so as to integrate data from omic sources, heterogeneous networks would consent researchers to engender a new exciting hypothesis. Heterogeneous networks have been considered in other applications, such as associations among genetic interactions and protein–protein interactions for cellular function. Hence by applying these integrated metadata analyses, we will engineer microbes to alter their pathways for the production of biofuel in enhanced amounts, which replaces the conventional biofuels usage.

4.8 Conclusion

Scientists are concentrating on biomass-based biofuels, especially agricultural biomass and wastes that can transform into liquid biofuels with the versatile use of microbes. Scientific reports suggest that it is possible to produce bioenergy from a wide variety of biomass residues and biological waste. The efforts of ongoing research on biofuel production technologies make them cost-effective. Biomass residues and biowaste can be transformed into transportation fuels and bioelectricity by diversified metabolic pathways. Production of renewable energy biofuels with the versatile utilization of microbes from the biological waste and biomass can reduce this threatening concern to a large extent (Liao et al. 2016). Microbes have

diversified metabolic activity, which enables significant biofuels production utilizing different substrates; this has been growing progressively in current times. Bacteria utilize sugars and convert them into ethanol, and cellulolytic microbes can use substrates taken from plants. Cyanobacteria and microalgae photosynthetically reduce atmospheric CO_2 into biofuels, and methanotrophs utilize methane for the production of methanol (Liao et al. 2016). *Geobacter sulfurreducens* and *Shewanella oneidensis* specifically transport electrons from microbial outer-membrane to conductive surfaces. This molecular mechanism of these microbes was set up in bioelectrochemical devices for biohydrogen and bioelectricity production. The awaiting necessity to focus on the confrontations related to the facilitation of the microbes develops them as a clear and possible choice to replace the typical fossil fuels that have discussed in this review. They are generating a more significant number of biofuels with cost-effective, and high efficiency using microbial cells as factories is a significant hurdle compared to conventional fossil fuels. Petrol is replaced by bioethanol which is cost-effective in terms of daily usage. Hence, utilizing microbes should provide an alternative for biofuel production, replacing all renewable energy from biological wastes and biomass is gaining importance soon.

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Bioremediation: Current Research Trends and Applications

5

Ipsita Mohanty and Animesha Rath

Abstract

Environmental pollution due to heavy metals, nuclear wastes, pesticides, nuclear gases, hydrocarbons, etc. seems to be of significant concern. The only possible solution to this is remediation using microbial processes (bioremediation). Bioremediation employs microbial metabolism under optimum environmental conditions and sufficient nutrients to degrade contaminants. This has proven to be effective and reliable due to its eco-friendly nature. Both in situ and ex situ techniques of bioremediation can be employed to reduce pollutant concentration. A diverse range of methods and strategies like bioaugmentation, biostimulation, bioventing, bioattenuation, etc. with their own merits and demerits are in use for the bioremediation process.

Keywords

Bioremediation · Bioaugmentation · Biostimulation · Bioventing · Bioattenuation

5.1 Introduction

The past two decades have experienced advances in bioremediation, aiming to restore polluted environments using eco-friendly techniques in a cost-effective manner. Several bioremediation techniques are developed by researchers. The nature and type of pollutants make use of numerous combinations of techniques to restore

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polluted environments. The natural microbes present in the polluted environment solely solve the problems associated with biodegradation and bioremediation of pollutant (Verma and Jaiswal 2016) only when the environmental conditions are conducive for their growth and metabolism. The physical and chemical methods of remediation are harmful as compared to the eco-friendly and cost-effective bioremediation techniques. Biodegradation is involved in a process called bioremediation. In bioremediation, microbes are able to convert and modify toxic pollutants to obtain energy and biomass. These toxic pollutants are converted to less toxic or non-toxic forms. Thus microbes like fungi, bacteria and archaea are also termed as bioremediators (Strong and Burgess 2008).

The nature of the pollutant, i.e. agrochemicals, chlorinated compounds, dyes, greenhouse gases, heavy metals, hydrocarbons, nuclear wastes, plastics and sewage decides the process that is to be involved in the removal of pollutant. Bioremediation techniques are categorized to ex situ or in situ. This categorization is chosen to take into account the nature of pollutant, degree of pollution, type of environment, location, cost and environmental policies (Smith et al. 2015). Besides this, oxygen and nutrient concentrations, temperature, pH, and other abiotic factors are also given importance. The bioremediation techniques have a diverse application focusing on hydrocarbons polluting soil and groundwater (Frutos et al. 2010; Firmino et al. 2015). These techniques may be economical and efficient. This review focuses on the brief knowledge of remediation techniques highlighting their principle, merits, demerits, applications and prospects.

5.2 Principle

The principle of bioremediation is the biological degradation of organic wastes to an innocuous state or to levels below concentration limits as established by regulatory authorities (Mueller et al. 1996). This biological degradation of contaminants is carried by naturally occurring bacteria, fungi or plants to substances nonhazardous to human health and the environment. These microbes are either indigenous to a contaminated site or are isolated, cultured separated and then inoculated at the contaminated site for the remediation process. Practical bioremediation can be achieved by microbes through an enzymatic attack on the contaminants, thereby converting them to harmless products. The environmental conditions play an important role in microbial growth and activity, along with the degradation process (Kumar et al. 2011). Bioremediation can be carried under both aerobic and anaerobic conditions (Colberg and Young 1995).

However, some contaminants such as chlorinated organic or high aromatic compounds are resistant to microbial enzymatic degradation. They are either slowly degraded or not at all degraded, making it difficult to predict the degradation rate.

5.3 Factors Affecting Bioremediation

Bioremediation depends on factors like the chemical nature and concentration of the pollutants, the physicochemical characteristics of the environment and their availability to the microbes (Strong and Burgess 2008). The rate of degradation seems to be affected as microbes, and the pollutants do not contact each other and are non-uniformly spread in the environment. Efficient bioremediation is achieved by the following factors:

5.3.1 Biological Factors

Here, microbes play a key role (Fig. 5.1). The microbes of interest can be isolated from any environmental condition, cultured and inoculated at the contaminated site.

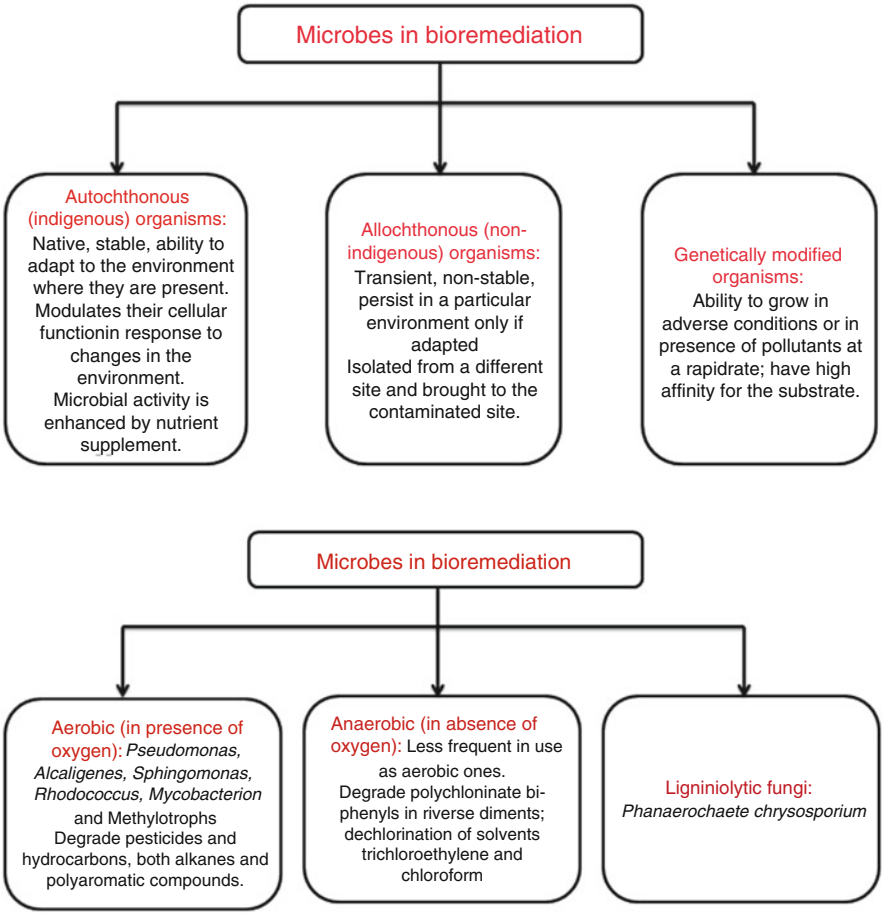

Fig. 5.1 Microbes used in the process of bioremediation

They have the adaptability to grow ranging from subzero temperatures to extreme heat from water to a condition in presence or absence of oxygen. They need a source of energy and carbon to sustain. Their adaptability to various conditions helps them degrade or remediate environmental hazards (Cheung 2013).

The interaction between biological, chemical and physical processes forms the basis of microbial remediation (Cheung 2013), the mechanism of which is depicted in Fig. 5.2.

5.3.2 Environmental Factors

The interaction between the microbes and the targeted contaminant depends upon the environmental parameters of the site of interaction. Microbial growth and activity are determined by pH, temperature, moisture, soil structure, solubility in water, nutrients, site characteristics, redox potential and oxygen and physio-chemic bioavailability of pollutants (Table 5.1) (Madhavi and Mohini 2012; Adams et al. 2015).

5.3.2.1 Nutrients

Addition of nutrients, especially nitrogen (N) and phosphorus (P) balances microbial growth and reproduction affecting rate and effectiveness of biodegradation. This optimizes the bacterial carbon (C): N:P ratio. The nutrient addition in appropriate quantity enhances the metabolic activity and biodegradation rate in a cold environment (Couto et al. 2014; Phulia et al. 2013).

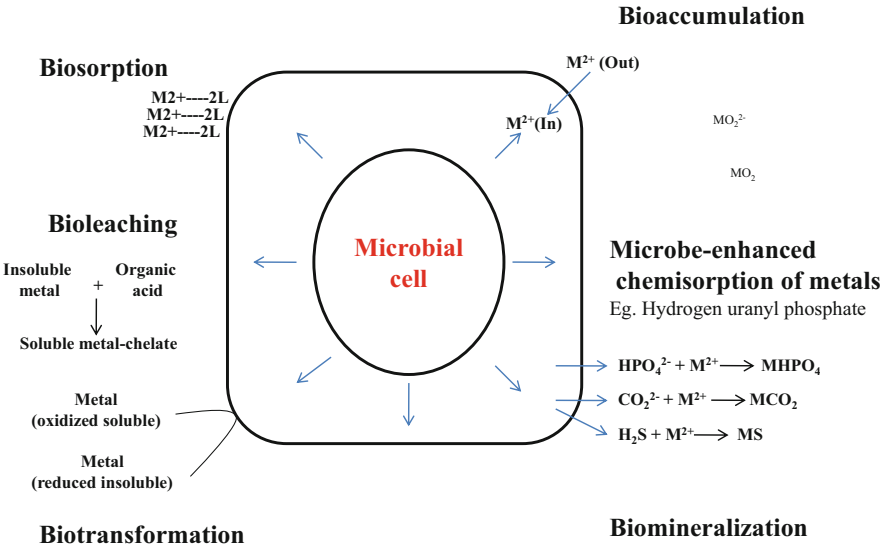

Fig. 5.2 Mechanism of microbial remediation (Cheung 2013)

Table 5.1 Environmental conditions affecting bioremediation (Sutar and Das 2012)

Parameters	Conditions for microbial activity
Soil moisture	25–28% of water holding capacity
Soil pH	5.5–8.8
Oxygen content	Aerobic, minimum air-filled pore space of 10%
Nutrient content	N and P for microbial growth
Temperature (°C)	15–45
Contaminants	Not too toxic
Heavy metals	Total content 2000 ppm
Soil type	Low clay or silt content

5.3.2.2 Temperature

Temperature is crucial to determine the survival of microbes and composition of hydrocarbons. A specific temperature is essential for the degradation of a specific compound. By affecting the microbial physiological properties, the temperature can accelerate and decelerate the bioremediation process. With an increase in temperature, the rate of microbial activities increases and reaches a maximum at an optimum temperature. However, it declines further with an increase or decrease in temperature and eventually stops after reaching a specific temperature (Macaulay 2014; Das and Chandran 2011).

5.3.2.3 Oxygen Concentration

Biological degradation can be carried out both in aerobic and anaerobic conditions. Aerobic conditions degrade hydrocarbons while anaerobic conditions degrade chlorate compounds (Macaulay 2014).

5.3.2.4 Moisture

Pollutant degradation is affected by moisture content. The moisture content has an adverse effect on biodegradable agents.

5.3.2.5 pH

It impacts microbial activity and influences the biodegradation. A measure in soil pH justifies microbial growth (Asira 2013). A slight change in pH affects the microbial process (Wang et al. 2011).

5.3.2.6 Metal Ions

Small quantity of metal ions in microbes directly or indirectly impact the degradation rate.

5.3.3 Types of Bioremediation

Bioremediation of pollutants is carried either in situ or in ex situ conditions (Fig. 5.3). Both these remediation strategies depend on community dynamics of organisms, their development and existence, structure and function.

5.4 Ex situ Bioremediation Techniques

It involves excavating pollutants from polluted sites for subsequent treatment in another site.

Factors considered:

1. The cost of treatment.
2. Type of pollutant.
3. Depth and degree of pollution.
4. Geographical location and geology of polluted site.

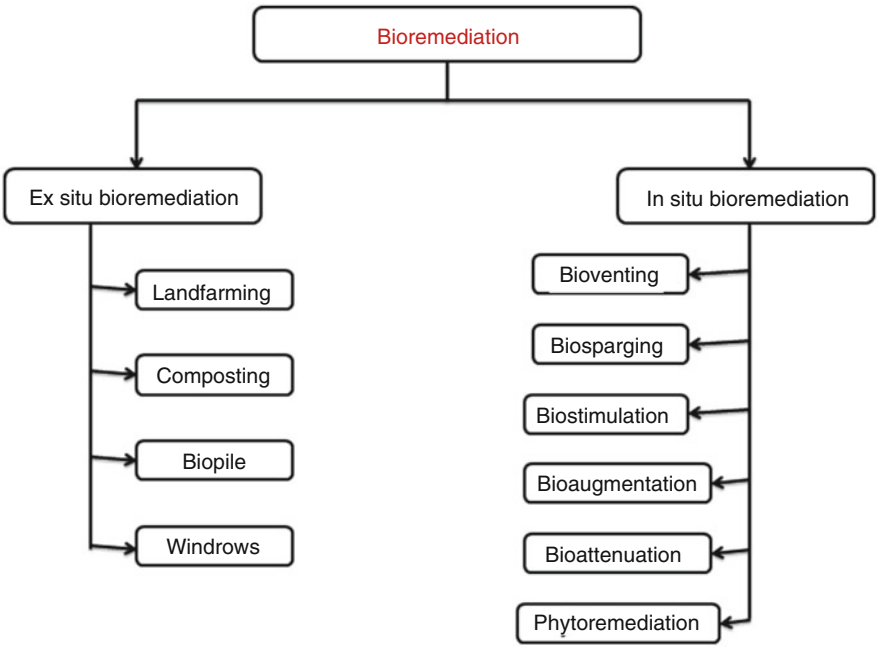

Fig. 5.3 Schematic representation of bioremediation techniques

5.4.1 Landfarming

It involves the excavation of contaminated soil to a prepared bed where it is periodically tilled to degrade pollutants. Here aerobic degradation of contaminants through indigenous microbes is achieved (Silva-Castro et al. 2015). Limitation involves the treatment of superficial (10–35 cm) soil. However, it is widely used as a disposal alternative as it needs less monitoring, maintenance costs and less operational equipment with low cleanup liabilities. This is used to treat voluminous polluted soil with meagre environmental impact and energy requirement (Maila and Colete 2004). This technique is employed for remediation of hydrocarbon polluted sites, including polyaromatic hydrocarbons (Cerqueira et al. 2014). This technique complies with government regulations and can be used in any climatic condition and location (Besaltatpour et al. 2011).

5.4.2 Composting

The contaminated soil is enriched with nonhazardous organic materials such as manure or agricultural wastes so as to develop a rich microbial population and enhance temperature suitable for composting.

5.4.3 Biopile

This technique involves above-ground piling of excavated polluted soil, followed by nutrient amendment and aeration to enhance bioremediation. This technique relies on enhancement in microbial activities through microbial respiration resulting in higher degradation of adsorbed petroleum pollutant (Emami et al. 2012). A system of pumps either forces air under positive pressure or negative pressure (Delille et al. 2008).

Components:

1. Aeration.
2. Irrigation.
3. Nutrient and leachate collection systems.
4. Treatment beds.

This technique (von Fahnestock et al. 1998) is cost-effective with an effective biodegradation provided nutrient, temperature and aeration are adequately controlled (Whelan et al. 2015). This technique is a hybrid of landfarming and composting. These treat surface contamination with hydrocarbons of petroleum. Biopiles conserve space compared to other ex situ techniques like landfarming. However, robust engineering, cost of maintenance and operation, lack of power supply at remote areas are significant drawbacks of this technique. Overheating of air can lead to drying of soil undergoing bioremediation, which will result in inhibition of microbial

activities and promote volatilization (Sanscartier et al. 2009). Indigenous aerobic and anaerobic microbes are favoured in this method.

5.4.4 Windrows

Here, bioremediation is enhanced by periodic turning of piled polluted soil to increase degradation activities of indigenous and transient bacteria residing in polluted soil. Furthermore, when water is added increases aeration, uniform distribution of pollutants, nutrients and microbial degradative activities speeding up the rate of biodegradation (Barr 2002). Higher rate of hydrocarbon removal was observed in windrows as compared to biopile treatment (Coulon et al. 2010). Windrows treatment has been used in greenhouse gas release (Hobson et al. 2005).

Ex situ bioremediation does not require an extensive preliminary assessment of polluted site before remediation making the preliminary stage short less labourious and less expensive.

5.5 In situ Bioremediation Techniques

These techniques are applied to soil and groundwater at site avoiding excavation and transport of contaminants with minimal disturbance to soil structure. It involves low cost making it the most desirable option. However, the cost of design and on-site installation of some sophisticated types of equipment to enhance microbial activities are of significant concern. It is limited by the depth of the soil that can be effectively treated.

Some in situ bioremediation techniques are enhanced using bioventing, biosparging and phytoremediation methods, while other techniques like intrinsic bioremediation or natural attenuation involve no enhancement.

These techniques treated chlorinated solvents, dyes, heavy metals, and hydrocarbon polluted sites (Folch et al. 2013; Roy et al. 2015). Some of the major factors taken into account for achieving a successful in situ bioremediation are electron acceptor, soil porosity, moisture content, nutrient availability, pH and temperature.

5.5.1 Bioventing

This process stimulates the growth of natural or introduced microbes through a vent that supplies oxygen by means of direct air injection to the soil. It is more functional in aerobically degradable compounds. The microbial activities are sustained through modulating airflow rates to provide the necessary oxygen for biodegradation minimizing volatilization and release of contaminants to the atmosphere. It also works for contamination deep under the surface. It is used to treat soil contaminated with fuels, non-halogenated volatile organic compounds, semi-volatile organic

compounds, pesticides and herbicides. This technique is used for biodegradation of petroleum-contaminated soil (Lee et al. 2006; Agarry and Latinwo 2015).

5.5.2 Biosparging

This process involves the injection of air under pressure below the water table to increase groundwater oxygen concentration which increases the rate of biological degradation of contaminants by indigenous bacteria. It treats groundwater contamination by fuels, non-halogenated volatile organic compounds, semi-volatile organic compounds, pesticides, organics and herbicides. It requires indigenous microbes, nutrients for their growth and specific containment availability.

5.5.3 Biostimulation

It deals with the injection of water-based solution carrying nutrients, electron acceptor or other amendments at the contaminated site (soil/groundwater) to stimulate the activity of indigenous microbes. Microbial activity is stimulated by supplying fertilizers, growth supplements, trace elements, environmental requirements like pH, temperature and oxygen to enhance their metabolism and pathway (Adams et al. 2015; Kumar et al. 2011). This is used to treat soil and groundwater contaminated by fuels, non-halogenated volatile organic compounds, semi-volatile organic compounds, pesticides and herbicides. Bioremediation process is enhanced when biostimulation is coupled with advanced tools and techniques.

5.5.4 Bioaugmentation

It involves augmenting the biodegradative capacity of indigenous microbial populations on contaminated site through the addition of natural/exotic/engineered pollutant degrading microbes. These microbes may include bacteria, protozoa, nematodes, rotifers and fungi. These microbes are collected from the remediation site, cultured, genetically modified and returned to the contaminated site for degradation. These in situ microbes can degrade sites contaminated with chlorinated ethenes to ethylene and chloride that are non-toxic (Niu et al. 2009; Malik and Ahmed 2012; Alwan et al. 2013; Gomez and Sartaj 2014). The genetically engineered microbes are more competent than that of natural species in degrading contaminants. These genetically engineered microbes can remediate soil, groundwater and activated sludge, exhibiting a higher degradative ability of chemical and physical pollutants (Sayler and Ripp 2000; Thapa et al. 2012).

5.5.5 Bioattenuation/Natural Attenuation

It is a proactive approach that monitors natural remediation processes (Khan et al. 2004). It is a method of eradication of pollutant concentrations from the surrounding environment. It uses natural methods to limit the spread of contamination, reducing the concentration and amount of pollutants at the contaminated sites (Boparai et al. 2008; Khan et al. 2004). It involves biological processes like aerobic and anaerobic biodegradation; physical phenomena like advection, dispersion, dilution, diffusion, volatilization, sorption/desorption; and chemical reactions like ion exchange, complexation and abiotic transformation. This process often involves terms like passive remediation, in situ bioremediation, intrinsic remediation or biotransformation (Mulligana and Yong 2004).

This process is categorized either as destructive or non-destructive (Gelman and Binstock 2008). This process is applied to fuels, halogenated organics, non-halogenated volatile organic compounds, semi-volatile organic compounds, pesticides, herbicides and hydrophobic contaminants.

For chemical pollutants in the environment, nature can clean up in the following ways (Li et al. 2010):

1. The chemicals are consumed by microbes dwelling in the soil and groundwater, converting them into the water and harmless gases.
2. The chemicals stick to the soil, holding them in the soil, preventing them from contaminating the groundwater.
3. The chemical pollutant on movement through the soil and groundwater dilutes it before mixing with the clean water.
4. Chemical pollutants in oil and solvents can evaporate and mix with the air where sunlight destroys them.
5. If the process of bioattenuation is not achieved, enhanced bioremediation techniques like biostimulation or bioaugmentation may be used.

5.5.6 Phytoremediation

The term "phytoremediation" was coined in 1991. It is an emerging technology where plants are used for treatment and mineralization of pollutants from contaminated sites (Raskin and Ensley 2000).

The overview below shows the applications of phytoremediation (Fig. 5.4).

This technique is used at the very large field site where other methods of remediation are not cost-effective or practicable; at sites with a low concentration of contaminants where only polish treatment is required for a more extended period and besides with other techniques where vegetation is used as a final cap and closure of the site.

Limitation:

1. Longer duration of time for remediation.

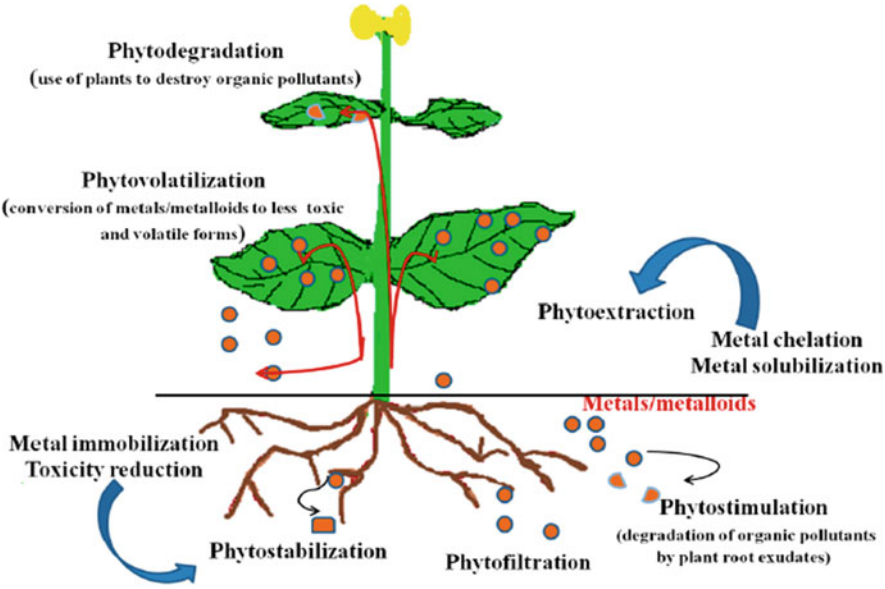

Fig. 5.4 Processes involved in phytoremediation of contaminants (heavy metals) (Ojuederie and Babalola 2017)

2. Potential contamination of vegetation and food chain.
3. Difficulty in establishing and maintaining vegetation at some sites that have high toxic levels.

5.6 Bioreactor

The bioreactor is an engineered device or vessel specifically designed and developed for abundant growth and metabolic activity of microbes through a biocatalyst, enzyme or microbial or animal or plant cells. The basic raw material is an organic or inorganic chemical compound. The internal condition of a bioreactor should support the growth of the cell, which is further enhanced by their biocatalytic activity (Singh et al. 2014). Bioreactors are much different from conventional chemical reactors supporting biological growth. Bioreactors should have a robust environment that must control the process upset and contaminants. It should enhance biological activities and minimize undesirable activities. Additionally, coenzymes are added that influence the kinetics of bioreactors (Singh et al. 2014). Most of the bioreactors operate by providing culture control, optimization, standardization, scale-up feasibility and automatic operation for cultivation of cells (Paopo 2014). Microbial bioreactors are classified to batch, fed-batch and continuous bioreactors (Figs. 5.5, 5.6 and 5.7).

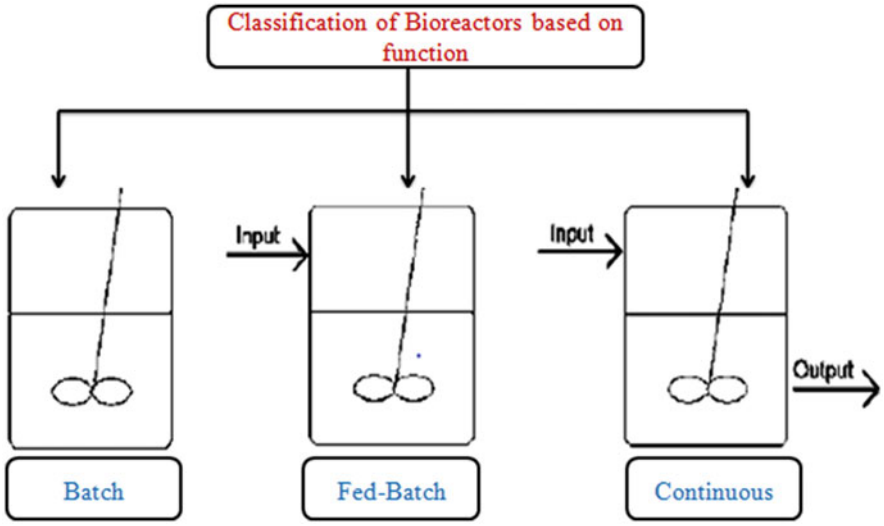

Fig. 5.5 Classification of bioreactors based on functions

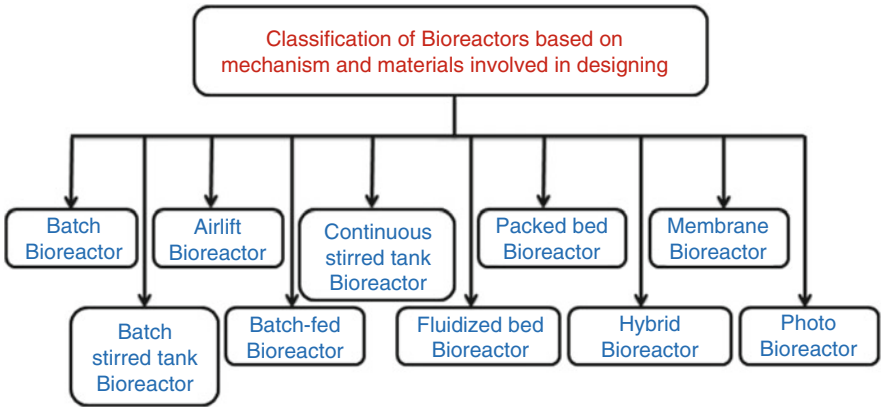

Fig. 5.6 Classification of bioreactors (Praharaj 2012; Sarkar and Mazumder 2014; Mandenius 2016)

5.7 Merits of Bioremediation

1. Effective and economical than that of other conventional technologies.
2. Less labour-intensive.
3. Complete destruction of pollutants.
4. Since there is no use of harmful chemicals, it is eco-friendly (as it releases harmless/less toxic by-product) and sustainable.

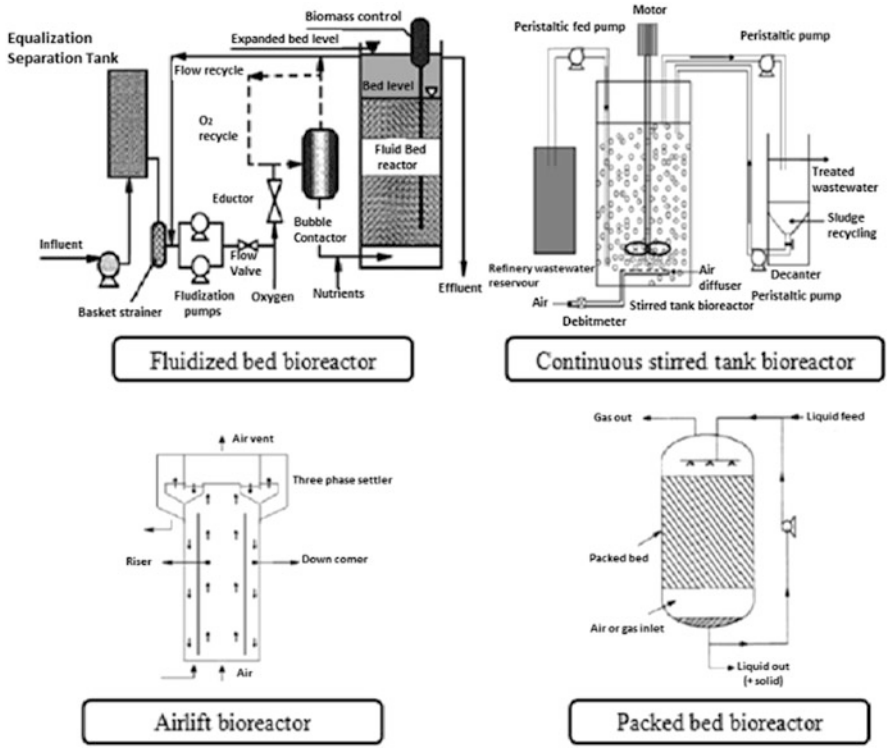

Fig. 5.7 Types of bioreactors

5. Relatively easy in implementing the technique.
6. On-site treatment possible without causing a major disruption of normal activities.
7. Do not affect natural flora, making it an effective way of remediating the natural ecosystem.

5.8 Demerits of Bioremediation

1. Biodegradable compounds can undergo biodegradation.
2. The specific environmental condition required.
3. Specific microflora required.
4. Appropriate levels of nutrients and contaminants required.
5. Long time required to remove or transform contaminant.
6. Some end products may be persistent or toxic than their parent compound.

5.9 Applications

5.9.1 Microbial Remediation of Contaminants (Heavy Metals)

Microbes can tolerate metal toxicity in numerous ways. Microbes have been used to sequester, precipitate or alter the oxidation state of numerous heavy metals (Gupta et al. 2016; Kang et al. 2016). A consortium of bacterial strain is more effective than a single strain culture for successful bioremediation. Wang and Chen (2009) studied that the bacterial mixture of *Viridibacillus arenosi* B-21, *Sporosarcina soli* B-22, *Enterobacter cloacae* KJ-46 and *E. cloacae* KJ-47 had more excellent resistance and efficiency than the single strain culture in bioremediation of a mixture of Pb (lead), Cd (cadmium) and Cu (copper) from contaminated soils. The microbial mechanism for bioremediation is given in the following flowchart (Jan et al. 2014) (Fig. 5.8).

Figueroa et al. showed that several microbes exhibited high resistance to 19 metalloids. These strains exhibited metal or metalloid, reducing capacity and have been used successfully to synthesize nanostructures. Tables 5.2 and 5.3 show different kinds of in situ and ex situ techniques that used to bioremediate of different contaminants.

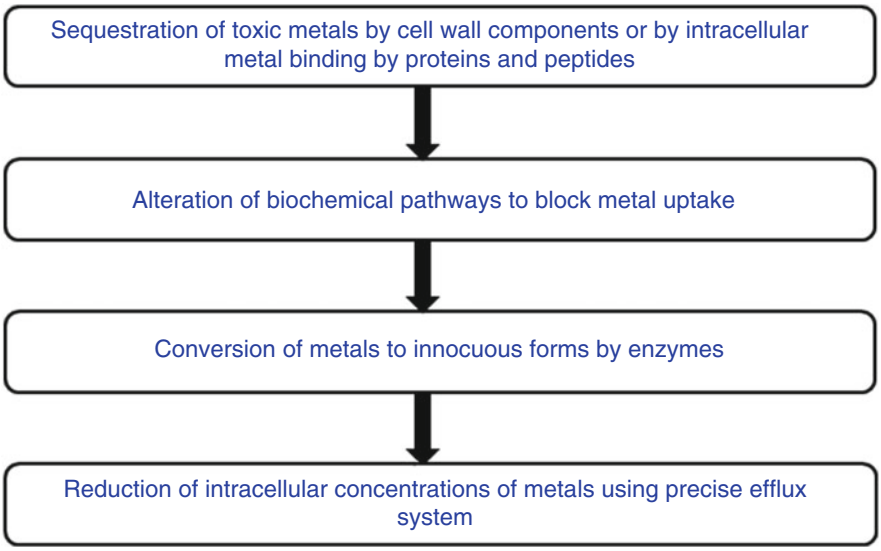

Fig. 5.8 Microbial mechanism for bioremediation

Table 5.2 Application of in situ bioremediation techniques

In situ technique	Contaminant	References
Bioventing	Diesel oil in the soil	Agarry and Latinwo (2015)
	Petroleum hydrocarbons	Mosco and Zytner (2017) and Xiao and Zytner (2019)
	Benzene and toluene	Amin et al. (2014)
Biostimulation	Chlorinated solvents	Henry (2010)
	TCE (Trichloroethane)	
	n-Alkane in diesel	Kahraman et al. (2017)
	Kerosene	
	Sulphur and metallic ions	Rodrigues et al. (2020)
Biosparging	Petroleum hydrocarbons	Shahsavari et al. (2017)
	Crude oil	Wang et al. (2019)
Bioaugmentation	Chlorpyrifos	Zhang et al. (2012)
	Chlorinated solvents	Mao et al. (2012)
Phytoremediation	Arsenic	Upadhyay et al. (2018)
	Mercury and lead	Kumar et al. (2017)
	Heavy metal	Yan et al. (2020)

Table 5.3 Application of ex situ bioremediation techniques

Ex situ techniques	Contaminant	References
Landfarming	Heavy metal	Kapahi and Sachdeva (2019)
	Hydrocarbon	Bergsveinson et al. (2019)
	Crude oil	Ali et al. (2020)
Composting	Heavy metal	Rahman and Singh (2020)
	Phenolic compounds	Tripathi and Dixit (2016)
	Hydrocarbon	Paladino et al. (2016)

5.9.2 Bioremediation of Pesticides

Pesticide usage has afflicted both the soil and water affecting many lives. Thus decontamination of these harmful pesticides is highly needed (Gavrilescu 2005) (Fig. 5.9).

5.10 Trends in Bioremediation

The bioremediation techniques effectively restore sites polluted with numerous pollutants. Since microbes are crucial in this process, their diversity, abundance and community structure in polluted sites are important only when the environmental parameters for their metabolic activities are maintained. Bioremediation can be

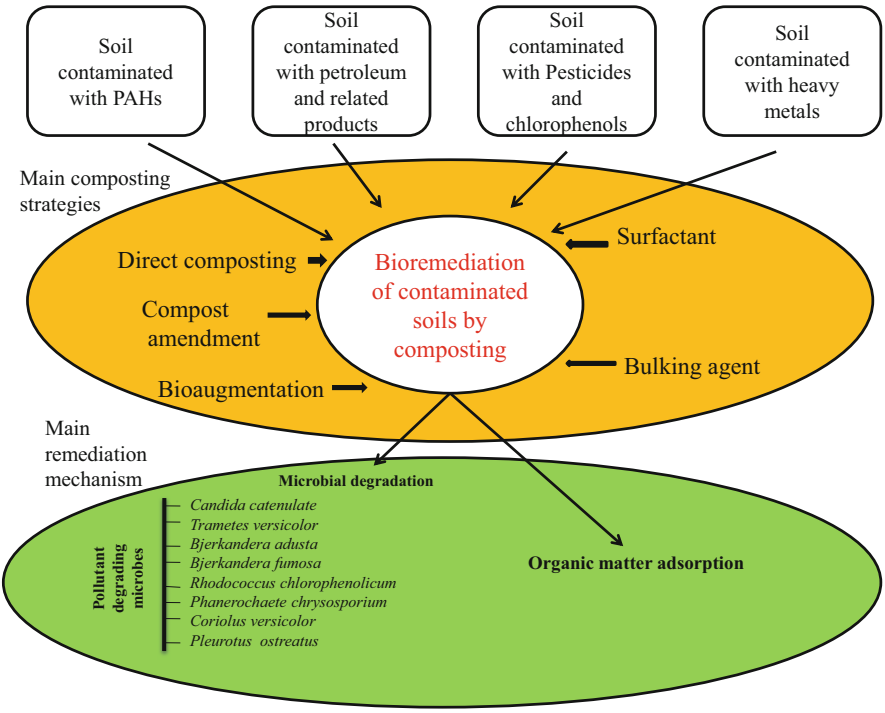

Fig. 5.9 Biodegradation of pesticide phenanthrene in compost amended soil (Puglisi et al. 2007)

enhanced through the application of several tools and techniques, as mentioned below (Fig. 5.10).

5.10.1 Biosurfactants

These are surface-active substances prepared by microbial cells. Their diversity, eco-friendly nature, large scale production, selectivity, performance under extreme conditions and usage in environmental protection have enhanced their interest (Rahman et al. 2002; Bodour et al. 2004). The usage will decrease the hydrophobic nature of the contaminant, making it readily available to the biological system for its remediation.

5.10.2 Oxygen Releasing Compounds

Aerobic condition ensures faster degradation of petroleum hydrocarbons than the anaerobic conditions. So the addition of oxygen or oxygen releasing compounds enhances bioremediation rate. These compounds release oxygen slowly when it

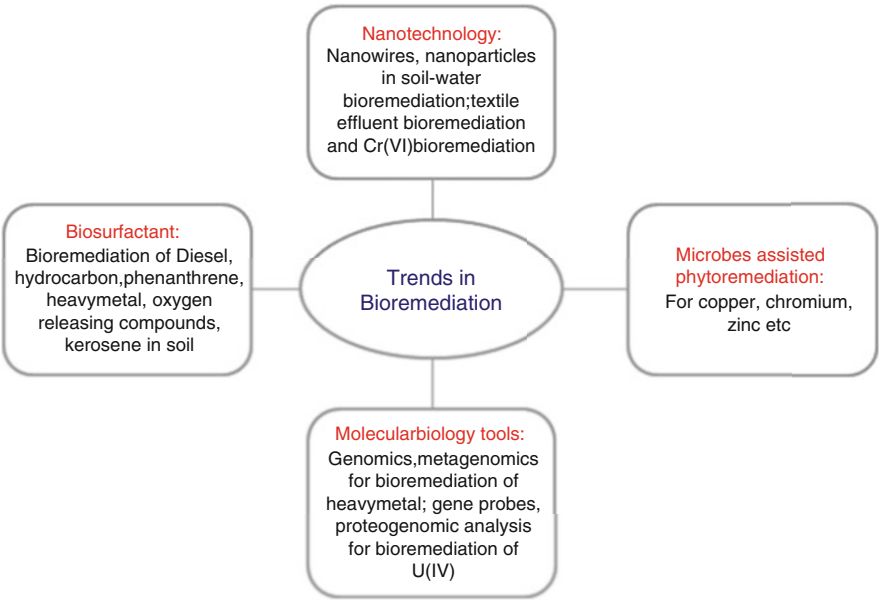

Fig. 5.10 Schematics of trends in bioremediation

comes in contact with water. Oxygen releasing compound product similar to Milk of Magnesia is used to address dissolved phase contamination, such as total petroleum hydrocarbons and BTEX (Benzene, Toluene, Ethylbenzene and Xylene).

5.10.3 Molecular Biological Tools and Techniques

Though many microbes are uncultivable in the laboratory, yet their importance cannot be neglected in the bioremediation process. Certain molecular techniques like genomics, metagenomics, gene probes, etc. are used to trace these microbes, their genes and enzymes involved in the bioremediation process. These techniques track the metabolic pathway employed by the microbe to degrade the contaminant.

5.10.4 Bioinformatics

The tools of bioinformatics helps identify, analyse various components of the cells, viz. gene and protein functions, interactions, metabolic and regulatory pathways. These tools facilitate the analysis of cellular processes to understand their cellular mechanism. Bioinformatics is helpful in structure-function determination and pathways of biodegradation of xenobiotics (Fulekar and Sharma 2008).

5.10.5 Nanotechnology

The use of nanotechnology seems like a fascinating and highly promising approach to clean the environment contaminated with pollutants.

5.11 Conclusion

Bioremediation is a green approach that cleans the environment. The microbial activity is employed in remediating, cleaning, managing and recovering techniques for degrading environmental contaminants. The rate of degradation depends on the biological agents, nutrient supply, external abiotic conditions and availability of the pollutant. Understanding microbial communities and their response to the natural environment and pollutant, studying the genetics of microbes to increase the capabilities to degrade pollutant and field trials of existing and new remediation techniques are the significant insights to be focused. It offers the advantage of being efficient, eco-friendly and cost-effective. Successful bioremediation depends upon site characterization, which establishes suitable bioremediation technique (ex situ or in situ). The efficient method to treat polluted site depends upon the geological feature of the polluted site (viz. soil type, pollutant depth and type, site location, etc.). The increasing popularity of these techniques shows that merits outnumber demerits. The advanced tools in bioremediation need field trials to be further implemented. Mostly, environmental factors and toxic nature of the contaminant hamper the usage of available technology urging the need for the development of hybrid technologies that can adjust to environmental parameters and toxicity of the contaminant.

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Bioremediation: An Approach for Environmental Pollutants Detoxification

6

Heena Shah and Shekhar Jain

Abstract

Pollutants are the substances or agents (biological, chemical, radiological, or physical) present in environments and cause potential harm to humans, animals, and plants by itself or through interfering with other components. Utilization of biological process for the elimination of these harmful pollutants and wastes from the environment is an absolute requisite to promote sustainable development with low environmental impact. Bioremediation plays a major role in the abstraction of contaminants and capitalizes the astonishing catabolic multifariousness of microorganisms to degrade/convert such compounds into less toxic materials. Practical bioremediation needs a combination of the optimum growth parameter physical and chemical, and addition of growth stimulators. These amendments complete bioremediation process by providing suitable growth conditions required for microorganism. Although some progress has been made in recognition of the importance of microorganisms for the decontamination of polluted waters, some important points still need to be addressed. Such as genetic engineering of microorganisms with a degradative pathway of a target compound, selection of appropriate approach, use of nanoparticles, etc., could improve bioremediation efficiency.

Keywords

Bioremediation · Microorganisms · Toxic materials

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6.1 Introduction

The world is facing many problems in the modern era. Pollution is one of the most critical environmental issues. Various types of activities such as setting up of new industries, urban sprawl, transportation, poor agricultural practices, including use of the intense amount of chemical fertilizers, pesticides, etc., resulted in exponential growth in the increase of waste products and also added a wide range of xenobiotic compounds to the environment (Dinka 2018). Soil, water, and air have traditionally been used as sites for the disposal of all these wastes. These disposed of materials have longer sustainability and can convert into toxic recalcitrant upon combining with non-toxic material or human-made products (Gursahani and Gupta 2011). The use of living organisms to degrade environmental pollutant or to clean up of a contaminated medium such as soil, sediment, air, or water is called Bioremediation (Kumar et al. 2011). Bioremediation utilizes the application of biological agents prominently microorganisms, e.g. bacteria, fungi to clean up soil and water contamination (Strong and Burgess 2008). This technology is based on enhancing the growth of specific microflora or microbial consortia to eliminate toxic material from the environment or to convert them to less toxic material. Microbial degradation of xenobiotics is one of the fundamental ways to remove environmentally harmful compounds. The establishment of such microbial consortia can be done in several ways, e.g. by promoting growth through the addition of nutrients, by adding terminal electron acceptor, or by controlling moisture and temperature conditions (Hess et al. 1997; Smith et al. 1998). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Tang et al. 2007). Microorganisms serve as an effective means of xenobiotic degradation and toxic waste removal.

6.2 Causes and Consequences of Environmental Pollution

Environmental pollution is a global problem, and it causes moderate to severe effects on humans and natural resources. Pollution causes very dangerous and hazardous effects on the environment, which depletes the ecological balance of the environment. The environments get besmirched due to the addition of pollutants in natural resources such as soil, water, and air. The entry of pollutants, either directly (release of effluents on land) or indirectly (use of polluted water as irrigation to crops) leads to contamination of a vast area of soil resources and groundwater bodies which results in inadequate quality crop production that affects human and animal health through food contamination. Environmental pollution caused by the release of a wide range of compounds such as persistent organic pollutants from industries is creating a disturbance to the ecosystem. It leads to climatic changes, global warming, reduction of groundwater level, melting of icecaps, ozone layer depletion, etc., and these made ecologists focus more on impacts of pollution and its amelioration (Gursahani and Gupta 2011; le Mellec et al. 2010; Sharma et al. 2011).

6.2.1 Intense Use of Chemical Fertilizers

Agriculture is an important sector of the Indian economy as it is a significant source of subsistence of population. The absolute agricultural production has increased almost continuously over the years, mainly due to technological innovations, and intensive use of agricultural inputs. Many times, the safety of the environment has been compromised with the quality of these inputs due to pressure of enhancing the productivity of the land. (Gupta et al. 2019). Chemical fertilizers have been considered as an essential input to agriculture as these play an important role in achieving the higher crop production worldwide to feed the ever-increasing population and to meet their daily needs of food, fuel, and fibre. Consumption of chemical fertilizers and organic manure bear a direct relationship with food grain production. Generally, fertilizers are manufactured from the raw materials that are collected from underground natural resources through mining like rock phosphate, sulphates, etc. Therefore, fertilizers contain a highly variable amount of heavy metals as impurities. Among the fertilizers, use of rock phosphates and phosphate-based fertilizers in agriculture is considered as an environmental concern due to their potential for enhancing heavy metal levels in soil and contaminating food crops (Rai et al. 2019).

6.2.2 Indiscriminate Use of Pesticides and Insecticides

In addition to fertilizers, a large number of pesticides are also in agricultural practice to ensure a good yield of crops. Most part of the applied pesticide, irrespective of crops, ultimately get released into the soil. Though a large part of these is degraded by soil microorganisms or inactivated by soil matrix through absorption, these affect the functioning of non-target microbes and other soil organisms adversely before inactivation. (Jørs 2018; Prakash et al. 2004) studied the presence of hexachlorocyclohexane (HCH) isomers residues in the soil sample collected from agricultural sites of Delhi, Haryana, and Uttar Pradesh as well as around the HCH manufacturing plant of Indian Pesticide Limited and found most of the sample containing HCH isomer residues. He also reported the presence of residue of HCH isomer in branded drinking mineral water sample collected from Delhi. Random monitoring of pesticides in the water had also detected residues of persistent organochlorines in many rivers like Ganga, Yamuna, Ulsoor, Mandori, Hoogly inflicting damage to aquatic life and health of fish using human population in food.

In India, several cases of residues like parathion, endosulfan, DDT, etc., were reported in food samples. Several investigators reported the presence of pesticide residues in samples of fruits, vegetables, cereals, pulses, grains, wheat flour, oils, eggs, meat, fish, poultry, bovine milk, butter, and cheese in India. During the analysis of the presence of possible residues of agrochemicals like organochlorine in various samples of vegetable, fruits, spices, cereals, pulses, milk, animal feed, fish/crustacean, tea, honey, meat, egg, soil, and groundwater by 21 reputed laboratories across the country (during the period of April 2011 to March 2012), most of the samples found contaminated with heavy metals. Excessive use of

fertilizers and pesticides, antibiotics and hormones in livestock and irrigating farms with contaminated wastewater are agricultural factors affecting soil pollution (Saha et al. 2017a).

6.2.3 Industrial Pollution

A significant part of the pollutant loaded effluents generated particularly from small-scale industries are released untreated into land and water bodies. In most of the cases, metals are present in dilute and small quantities in polluted water bodies and may not cause any harm to plant growth immediately when used for irrigation. However, their immobility and consequent persistence imply that concentrations may become elevated in the long run to such an extent that they begin exhibiting toxic effect on the plant, soil microorganisms, and food chain. Long-term exposure to heavy metals has been reported to affect human and animal health adversely. Among the heavy metals, Ni, Co, Cr, and Cu are relatively more toxic to plants and As, Cd, Pb, and Hg are relatively more toxic to higher animals (McBride 1994; Dangi et al. 2019). Build up of different pollutants in Indian soils and their impact on soil quality, agricultural productivity, and food quality as well as the impact on organisms (Saha et al. 2017b). Textile manufacturing industries utilize thousands of different types of dyes containing heavy metals for various fabric-dying processes. Particularly in India and Bangladesh, where textile manufacturing is a prevalent industrial practice, lead to a high level of soil contamination by metals such as lead, cadmium, and chromium (Sun et al. 2019a).

In some cases, effluent released from industries is well treated like industrial emission, while in other cases like chemical or oil spills, it happens accidentally, which may result in lethal effect and can persist in terrestrial and aquatic environments. Substances in the environment are derived either by biogenic or anthropogenic sources. Chemically synthesized compounds play a significant role in polluting the environment (Rhind 2009). Xenobiotics are compounds found in living systems or in the environment which are not natural and are unusually present in high concentrations. The potential health hazard of a xenobiotic compound is a function of its persistence in the environment as well as the toxicity of the chemical class (Varsha et al. 2011). The issue of xenobiotics (and their metabolites) in the environment has been a growth area for research in environmental chemistry for several years.

6.3 Role of Biotechnology in Pollution Management

The use of biotechnological approaches or processes to treat/remove hazardous contaminants has now been considered as a handy and vital tool in pollution management. Biotechnology has shown a great promise in solving a plethora of environmental problems. Biological processes rely on useful microbial reactions including degradation and detoxification of hazardous organics, inorganic nutrients,

metal transformations, applied to gaseous, aqueous, and solid waste (Eglit 2002; Evans and Furlong 2003; Gavrilescu 2004).

Pollution caused by the accumulation of toxic chemicals or other waste and pollutants could be reduced or removed by the process of bioremediation. Bioremediation reduces the environmental burden of toxic substances using microorganisms. The application of biotechnology has helped to assess the well-being of the environment, by the transformation of pollutants into useful products, mineralization, develop environmentally safe manufacturing and disposal processes and, generate biodegradable materials from renewable sources. Advanced techniques or technologies are now possible to treat waste and degrade pollutants assisted by living organisms or to develop products and processes that generate less waste and preserve the natural non-renewable resources and energy (Olguin 1999; Gavrilescu and Chisti 2005; Chisti 2007).

Several methods, like biological methods and physicochemical, have been employed in the treatment or elimination of xenobiotics. The physicochemical methods are costly and often produce unwanted toxic products, requiring further treatment steps (Sridevi et al. 2011). Such type of techniques often adds fragmented elements which cannot be degraded easily and will make the environment still worse. To overcome these problems, many other eco-friendly techniques have been reported such as bioremediation, phytoremediation, etc. Microbial degradation of xenobiotics is one of the crucial ways to remove environmentally harmful compounds (Surani et al. 2011; Polewczyk et al. 2020). Biodegradation can be done by taking the waste product of one process and using it either as a fuel or as food for another process. Microorganisms also apply the same strategy; they utilize these residual compounds as a substrate by degradation and fragmentation for their growth, which is highly recommended in case of bioremediation. (Iyovoet al. 2010; Varsha et al. 2011).

6.4 Bioremediation

Bioremediation is an attractive technique for the restoration of the contaminated environment and sustainable development. “Remediate” means to treat and “bioremediate” means the use of living organisms such as bacteria, fungi, yeasts, etc., to clean up environment. Bioremediation can be defined as the process in which living microorganisms are used to reduce or eliminate pollutants from the environment (Abena et al. 2019). King et al. (1997) defined it as a treatability technology which utilizes biological activity to reduce pollutants concentration or toxicity. Bioremediation process involves detoxification and mineralization, where the waste is converted into inorganic compounds such as carbon dioxide, water, and methane (Reshma et al. 2011). Microbes are present in almost everywhere in the environment and may live as free individuals, in symbiotic association or in consortia. These microorganisms utilize the organic and inorganic waste for their growth and energy requirement, and the phenomenon is known as microbial degradation. Due to this potential of metabolizing xenobiotic compounds, microbial degradation has been

recognized as an effective means for the removal of toxic and hazardous waste (Agarry et al. 2008; Sridevi et al. 2011). The use of organisms for the removal of contamination is based on the concept that all organisms could remove substances from the environment for their own growth and metabolism (Wagner et al. 2002; Doble et al. 2004; Gavrilescu 2004). Bacteria and fungi are very good at degrading complex molecules, and the resultant wastes are generally safe; Protozoa, algae, and plants proved to be suitable to absorb nitrogen, phosphorus, sulphur, and many minerals and metals from the environments (Gavrilescu 2005).

6.4.1 Principle Involved in Bioremediation

The principle involves microbial degradation of xenobiotics compounds. Organic wastes are biologically degraded by the action of living organisms such as bacteria, fungi, or their enzymes under regulated conditions to maintain the concentration below limits set by regulatory authorities. Numerous opportunities for more efficient and advanced biological processes are offered by current studies in molecular biology and environmental science. These studies include notable accomplishments in cleaning up polluted water and land areas (Dixit et al. 2015). Microorganisms used in bioremediation may be native to a contaminated area or maybe isolated and carried to the contaminated site from elsewhere. Living organisms transform these contaminants or toxic compounds metabolically. Certain microbes secrete extracellular enzymes to degrade or metabolize substances that are harmful to human health and the environment. Biodegradation of a compound has often involved the actions of multiple organisms, to enhance degradation process for completion of this, microbes are imported to the contaminated site, and this process is known as bioaugmentation (Hatzikioseyian 2010). Because bioremediation can only be effective when environmental conditions are favourable for microbial growth and activity, its application often involves manipulating environmental parameters to permit for faster microbial growth and degradation. Bioremediation, like other systems, has its drawbacks. Many contaminants show resistance to bacterial attacks, such as chlorinated organic or high-aromatic hydrocarbons (Kumar et al. 2011). The potential use of microorganisms to metabolize toxic pollutants has been recognized as a useful tool for waste disposal, and some microorganisms can be mutated and genetically engineered for significant bioremediation capabilities, including the ability to degrade recalcitrant pollutants, guarantee better survival and colonization, and achieve enhanced rates of degradation in target polluted niches (Gavrilescu and Chisti 2005; Abena et al. 2019).

6.4.2 Microbes in Bioremediation

The generic term “microbe” includes prokaryotes (bacteria or archaea) and eukaryotes (yeasts, fungi, protozoa, algae, and rotifers). Bioremediation involves the applications of various microorganisms that are able to degrade and reduce the

toxicity of environmental pollutants. Microorganisms can easily grow in different environmental conditions. Environmental pollutant contains a large amount of organic waste in the form of hydrocarbons, plastic, pesticides, insecticides, dyes, paints, etc. Microorganisms utilize these organic matters in order to obtain nutrition and energy and produce biomass (Abatenh et al. 2017).

The potential of microorganisms to adapt nutritionally can also be exploited for pollutants biodegradation. Many bacteria, fungi, and some group of archaea are widely employed in bioremediation due to their metabolic potentiality to break down or convert contaminants into non-toxic or less toxic substances (Table 6.1). Microorganisms serve as essential tools for eliminating toxins present in the soil, water, and sediments; mostly because of their superiority over other systemic remediation protocols. By preventing further pollution, microorganisms are restoring the original natural environment. Some have been isolated, selected, mutated, and genetically engineered for effective bioremediation capabilities, including the ability to degrade recalcitrant pollutants, guarantee better survival and colonization, and achieve enhanced rates of degradation in target polluted niches (Gavrilescu and Chisti 2005). Extremophiles are the extensively studied microorganism used for the degradation of different types of xenobiotics, including metal and radionuclides. They are able to survive in hostile environments with high concentrations by using their genetic and metabolic mechanisms and play a significant role in environmental clean-up (Donati 2019).

Microorganisms used in bioremediation are-

6.4.2.1 Aerobic

Microorganisms that can grow and survive in the presence of oxygen are known as aerobic microorganism. Examples of aerobic bacteria recognized for their degradative abilities and used in bioremediation are *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus,* and *Mycobacterium.* These microbes possess the ability to degrade pesticides and both types of hydrocarbons, i.e. alkanes and polyaromatic compounds (Sharma et al. 2011).

6.4.2.2 Anaerobic

Microorganisms that do not require oxygen to grow; they cannot grow in the presence of oxygen is known as anaerobic microorganism. Anaerobic bacteria are not very often used as aerobic bacteria. There is a growing interest for anaerobic bacteria used in bioremediation of compounds like chloroform, polychlorinated biphenyls in river sediments and dechlorination of trichloroethylene solvent (Aken et al. 2009).

6.4.2.3 Psychrophiles

They are cold-loving organisms and grow best at 20–30 °C. Their optimum temperature for growth is 4 °C and 12 °C for reproduction. Organisms included in this group are algae and some bacteria. Psychrophiles have been reported with the ability to mineralize dodecane, as well as to degrade n-alkanes and diesel fuel at low temperature (Hamdan 2018).

Table 6.1 Microorganisms involved in environmental bioremediation

Microorganisms	Degrading abilities	References
Pseudomonas putida, P. aeruginosa	Monocyclic aromatic hydrocarbons, e.g. benzene and xylene.	Safiyanu et al. (2015)
P. alcaligenes, P. mendocina P. veronii, *Achromobacter Flavobacterium, Acinetobacter*	Petrol and diesel polycyclic aromatic hydrocarbons toluene	Sani et al. (2015), Safiyanu et al. (2015)
P. cepacia, B. cereus, B. coagulans, Citrobacter koseri, Serratia ficaria	Diesel oil, crude oil	Kehinde and Isaac (2016)
Streptococcus	Hydrocarbon-degrading bacteria, heavy oil, degrade dairy industry waste (whey)	Blonskaya and Vaalu (2006), Kumar et al. (2007), Mohana et al. (2007)
Brevibacillus borstelensis Rhodococcus ruber Pseudomonas aeruginosa	Degrade plastics	Abatenh et al. (2017), Gavrilescu (2010)
B. subtilis, B. cereus, Alcaligenes odorans, Corynebacterium propinquum, P. aeruginosa	Degrade crude oil, Bioremediation of chlorpyrifos contaminated soil Polycyclic aromatic hydrocarbons	Eglit (2002), Das and Mukherjee (2007), Lakshmi et al. (2009), Sun et al. (2019b)
Acinetobacter sp., Pseudomonas sp.*, Ralstonia* sp.*, Microbacterium* sp.	Aromatic hydrocarbons	Raquel et al. (2013)
Penicillium chrysogenum	Monocyclic aromatic hydro carbons, benzene, toluene, ethyl benzene and xylene,phenol compounds	Pereira et al. (2014)
Penicillium ochrochloron	Industrial dyes	Shedbalkar and Jadhav (2011)
A. niger, A. fumigatus F. solani P. funiculosum	Hydrocarbon, crude oil	AI-Jawhari (2014), Burghal et al. (2016)
A. versicolor, A.fumigatus Paecilomyces sp. *Trichoderma* sp. *Microsporum* sp. *Cladosporium* sp.	Utilize heavy metals cadmium	Fazli et al. (2015)
B. firmus, B. macerans, S. aureus Klebsiella oxytoca	Vat dyes, textile effluents	Adebajo et al. (2017)
Saccharomyces cerevisiae Cunninghamella elegans Rhodomicrobium Beggiatoa thiothrix	Heavy metals, lead, mercury, and nickel, Phototrophic oxidize H2S to S0	Guest and Smith (2002), Reddy et al. (2003), Bitton (2005)

(continued)

Table 6.1 (continued)

Microorganisms	Degrading abilities	References
Archaea: Thermophiles Hyperthermophiles Psychrophiles Acidophiles Alkaliphiles Halophiles	Use organic compounds as a source of carbon and energy (organotrophs), use CO_2 as a carbon source (chemoautotrophs)	Burton et al. (2002), Dunn et al. (2003), Bitton (2005), Doble and Kumar (2005)
Mushrooms—*Agaricus*, *Amanita* (poisonous)	Wood-rotting fungi play a significant role in the decomposition of cellulose and lignin	Hernández-Luna et al. (2007), Bitton (2005)
Cyanobacteria, green algae and diatoms, and *B. licheniformis*	Naphthalene	Lin et al. (2010)
Serratia marinorubra *Bacillus* sp. YW and YDLK consortia *Acinetobacter* sp. *Thermophilic, Anoxybacillus pushchinoensis* *Anoxybacillus kamchatkensis* *A. flavithermus*	Dyes (textile effluent)	Abatenh et al. (2017)
Enterobacter	Degrade pesticide, Chlorpyrifos	Niti et al. (2013)
Bacillus, Staphylococcus	Endosulfan	Mohamed et al. (2011)
P. putida, Pseudomonas A3 *P. aeruginosa* *Serratia marinorubra*	Pesticides and fungicides	
Bacillus cereus sp. *Lysinibacillus boronitolerans* sp.	Utilize arsenic compounds	Pushkar et al. (2019)
Sphingomonas *Idiomarina* *Phenylobacterium*	Petroleum hydrocarbons and polycyclic aromatic hydrocarbons	Huang et al. (2019)
Clostridiaceae, *Peptococcaceae,* *Veillonellaceae,* *Christensenellaceae,* *Lachnospiraceae,* *Bacillaceae*	Reduce sulphate and iron	Gupta et al. (2018)
Streptomyces sp.	Petroleum hydrocarbons, n-alkanes, and aromatic hydrocarbons	Baoune et al. (2019)
Acinetobacter sp. *Bacillus sp.* *Enterobacter* sp.	Mercury	Pushkar et al. (2019)
Stenotrophomonas maltophilia	Polycyclic aromatic hydrocarbons	Liu et al. (2020a)
Paenibacillus sp.	*Diesel oil and crude oil*	Jimoh and Lin (2020)
Acinetobacter radioresistens	Degrade phenols	Liu et al. (2020b)

6.4.2.4 Ligninolytic Fungi

Ligninolytic fungi are those, which degrade lignin and cellulose in wood. *Phanerochaete chrysosporium* a white-rot fungus has the ability to break down a wide range of toxic environmental pollutant. Some common substrates used by ligninolytic fungi are sawdust, straw, and corncobs (Girma 2015).

6.4.2.5 Methanogenic Bacteria and Methanotrophs

Methanogens are a group of bacteria that degrade hydrocarbons resulting in methane gas and carbon dioxide as a product, and the process is called Methanogenesis. On the other hand, bacteria that utilize methane and carbon for growth and energy requirements are called methanotrophs. Methanotrophs are anaerobic and contain enzyme methane monooxygenase, which reacts with methane and is active against a wide variety of compounds including the chlorinated hydrocarbons, for example, aliphatic trichloroethylene and 1, 2-dichloroethane (Zhao et al. 2015).

6.4.2.6 Halophiles

Microorganisms that are able to live in salty conditions through a fascinating adaptation is known as halophiles. Bertrand et al. (1990) observed that the halobacteria strain EH4 isolated from a salt-mark could degrade alkanes and other aromatic compounds in the presence of salt. Four extreme halophilic strain of archaea were studied to evaluate their potential to biodegrade crude oil and hydrocarbons. The research demonstrated the vital fact that archaea have potential to carry out biodegradation at high temperatures, in the range of 40–45 °C, which is advantageous because hydrocarbons have higher solubility and bioavailability at these higher temperatures. The four strains studied were resistant to six different antibiotics, including penicillin, streptomycin, cycloheximide and this gave them the potential to carry out biodegradation in conditions unfavourable for bacteria (Al-Mailem et al. 2010).

6.4.2.7 Cyanobacteria

Cyanobacterial consortia are generally used for degradation of oil derivatives. Cyanobacteria possess the capacity to oxidize and degrade a variety of complex organic compounds in the environment like catechols, crude oil, naphthalene, phenanthrene, pesticides, phenols, heavy metals, and xenobiotics (Kumar et al. 2016; Varsha et al. 2011).

6.4.2.8 Microalgal

Various species of microalgae have shown tolerance and biodegradation properties against different harmful contaminants. In situation, where the contaminated site contains more than one specific type of pollutant of concern, application of bioremediation using microalgal treatment process provides efficient and cost-effective remediation system. *Chlorella sorokiniana, Scenedesmus obliquus*, and *Chlorella vulgaris* are the most frequently reported microalgal species that can be used for bioremediation (Sutherland and Ralph 2019).

6.4.3 New Technologies

Genetic modification offers new hope for microbial remediation as microbes can be used to overexpress the enzymes involved in the existing microbial metabolic pathways or to introduce new pathways into microorganisms. Selection of gene responsible for specific compound degradation would be beneficial to develop the Genetically Modified Organisms (GMOs) for the bioremediation of xenobiotic compounds. Such advancements will always be a helping hand to already existing techniques. Many such efficient strategies are evolved to replace the less eco-friendly physicochemical approaches (Perpetuo et al. 2011).

6.5 Methods of Bioremediation

Bioremediation technology is principally based on biodegradation. The current study deals with the important microbial bioremediation and approaches used for xenobiotic degradation. Microbial bioremediation involves the use of such microorganisms that occur naturally or introduced intentionally at the site of contamination to completely eliminate or deplete toxic organic pollutants into compounds that are harmless or occurred naturally. Microorganisms such as fungi and bacteria use these waste residues as one of their substrates and colonize on them. The degraded product can be further utilized in the form of raw material for other processes (e.g. energy-generating process, etc.), and it makes intelligent use of resources for reducing the pollution. The bioremediation processes are broadly classified as in situ or ex situ (Fig. 6.1).

Some examples of bioremediation-related technologies are microbial remediation, which includes venting, bioleaching, land farming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation. In addition, phytoremediation is another essential technique of bioremediation. For the biodegradation of a wide variety of organic compounds numerous methods and pathways have been elucidated (Thapa et al. 2012; Mani and Kumar 2014; Brindha and Elango 2015; Chen et al. 2015). In this section, we will focus on microbial methods of bioremediation.

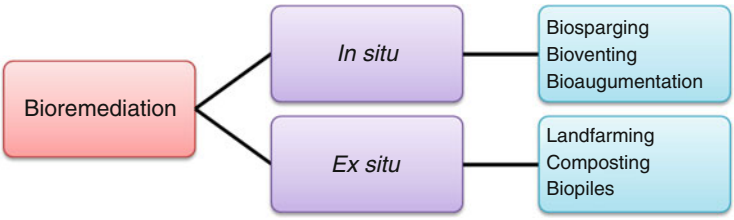

Fig. 6.1 Types of microbial bioremediation techniques

6.5.1 In Situ Bioremediation

In situ bioremediation involves the encouragement of indigenous bacterial populations to metabolize target contaminants through the addition of various amendments to the site of pollution. In addition to amendments selected strains of bacteria may be added to help treat some sites bacteria perform coupled oxidation/reduction (redox) reactions to live, and bioremediation exploits these reactions to remove contaminants from contaminated media (soil, air, or groundwater). Bacteria can use different electron acceptors (oxidized compounds) and donors (reduced compounds) in the three major oxidation pathways—aerobic respiration, anaerobic respiration, and fermentation (Mani and Kumar 2014; Azubuike et al. 2016). In situ, bioremediation can use all of these pathways, and contaminant degradation may occur through direct metabolism, co-metabolism, or abiotic transformations that may result from biological activities. This technique is generally used for bioremediation of soil and groundwater. In situ bioremediation does not require the excavation or removal of soils or water in order to accomplish remediation; in other words, the treatment of pollutants can be done at the site of contamination. In situ bioremediation includes various approaches for the treatment of waste such as:

6.5.1.1 Bioventing

Bioventing is widely used as in situ treatment method. It is an air-based remediation technology in which native or naturally occurring microbial species are stimulated by feeding them nutrients and oxygen to increase their metabolic activity. Bioventing uses low airflow levels to provide only adequate oxygen to sustain microbial activity. Oxygen can be added directly, or chemical oxidants can be applied into residual contamination by means of wells, which release oxygen as they dissolve or decompose. In addition to biodegradation of adsorbed fuel residuals, volatile compounds are also biodegraded as vapours move slowly through biologically active soil. Bioventing has been shown to be an effective means of bioremediation of petroleum-based contaminants in soil (Kumar et al. 2011).

6.5.1.2 Bioaugmentation

This approach deals with the addition of a group of indigenous microbial strains or a genetically modified strain for the treatment of contaminated soil or groundwater. If appropriate biodegrading microorganisms are not present in the soil or if microbial populations have been reduced because of contaminant toxicity, specific microorganisms can be added as "introduced organisms" at the site of contamination to enhance the existing populations, to improve contaminant clean up, and reduce clean up time and cost. Microbes from the soil or groundwater are isolated and are added to media containing the chemicals to be degraded. Only microbes capable of metabolizing the chemicals will grow on the media. This process isolates the microbial population of interest. Reductive dechlorination of solvents such as perchloroethylene and trichloroethylene is one of the leading environmental applications for bioaugmentation. It can completely degrade these contaminants into their non-toxic forms, i.e. ethylene and chloride in the environment. It is

possible that this process could expand the range of possibilities for future bioremediation systems (Das and Dash, 2014).

6.5.1.3 Biosparging

Biosparging technology uses indigenous microorganisms to degrade organic constituents. It is used in both saturated and unsaturated soil zones. In biosparging, air (or oxygen) and nutrients (if needed) are injected under pressure below the water table to increase groundwater oxygen concentrations to increase the biological activity of microorganisms (the United States Environmental Protection Agency 2017). The biosparging process is similar to air sparging. However, while air sparging removes constituents primarily through volatilization, biosparging promotes biodegradation of constituents rather than volatilization. Biosparging can be used to reduce the concentration of petroleum, that are dissolved in groundwater, adsorbed to the soil below the water table, and within the capillary fringe. A case study performed in the Damodar Valley in Eastern India showed that biosparging was effective at removing 75% of contaminants present within a one-year time period. The first results were obtained in the field, but these results were enumerated using laboratory tests and computer programs. The results from the study were used to set the optimum conditions for remediation, including appropriate moisture content, pH, temperature, nutrients, and carbon sources. The field tests used six separate tests sites. Different parameters were tested in each site in order to investigate the optimum conditions (Gogoi et al. 2002).

6.5.2 Ex Situ Bioremediation

This technique requires excavation of contaminants from polluted soil or groundwater pumping to facilitate microbial degradation and subsequently transfer the contaminated material to the site of treatment. As the technique requires extraction or removal of contaminated soil from the site of contamination, there are some limitations or drawbacks of using this process. According to the state of the contaminants to be removed, ex situ bioremediation is classified as (Azubuike et al. 2016):

6.5.2.1 Composting

Composting is a biological decomposition process of organic waste by microbial consortia such as bacteria, actinomycetes, and fungi, under anaerobic conditions. In this technique, contaminated soil is combined with a mixture of non-hazardous decayed organic matter, manure, agriculture waste, paper and pulp waste, etc. The effective recycling of bio-waste through composting or digestion can transform a potentially problematic "waste" into a valuable "product": *compost.* Almost any organic waste can be treated by this method, which results in end products as the biologically stable humus-like product for use as a soil conditioner, fertilizer, biofilter material, or fuel (Gavrilescu 2010; Kutzner 2000; Schuchardt 2005). A large fraction of the degradable organic carbon in the waste material is converted into

carbon dioxide (CO_2). Methane is formed in anaerobic sections of the compost, but it is oxidized largely in the aerobic sections of the compost. The estimated CH_4 released into the atmosphere ranges from less than 1% to a few percent of the initial carbon content in the material (Beck-Friis 2001). Composting can lead to waste stabilization, volume and mass reduction, drying, elimination of phytotoxic substances and undesired seeds and plant parts, and sanitation. Composting is also a method for restoration of contaminated soils.

6.5.2.2 Biopiles

Biopiling is a large scale ex situ bioremediation technology, also known as the heap technique that has been extensively used for remediation of petrochemical contaminants present in the soil. This technique involves the combination of two approaches, i.e. land farming and composting in which excavated soils are mixed with soil amendments and assembled into compost piles. Static piles are usually in the form of pyramids or trapezoids. Their heights vary between 0.8 and 2 m depending on the type of aeration used (either passive or active). Dynamic biopiles are consistently ploughed and turned to maximize their exposure to increasing the bioavailability of the contaminants by constantly exposing them to oxygen, water, nutrients, and microbes (Koning et al. 2000). A simple biopile system consists of a treatment bed, an aeration system, an irrigation/nutrient system, and a collection system for leachate. There is also the regulation of water, temperature, nutrients, oxygen, and pH to promote biodegradation (Fig. 6.2). The irrigation/nutrient network is buried under the soil for vacuum or positive pressure to pass air and nutrients. Biopiles provide suitable conditions for aerobic and anaerobic native microorganisms (Das and Dash 2014).

6.5.2.3 Land Farming

In this process, the contaminated soil is excavated and mechanically separated by sieving. The contaminated soil is then spread over a prepared bed of a 0.4 m thick layer of soil and periodically tilled until pollutants are degraded. It stimulates the

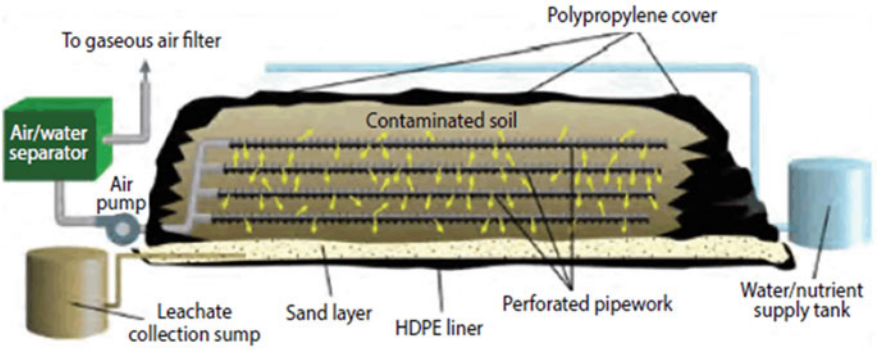

Fig. 6.2 Diagrammatic view of biopiling (Paudyn et al. 2008)

activity of indigenous biodegradative microorganisms and facilitates their aerobic degradation of contaminants. A synthetic, concrete, or clay membrane is used to cover the contaminated soil layer. Oxygen is added, and mixing occurs via ploughing, harrowing, or milling. Nutrients and moisture may also be added to aid the remediation process. The pH of the soil is also regulated (keeping it near 7.0) using crushed limestone or agricultural lime (Williams 2006).

6.6 Factors Affecting Microbial Bioremediation

Effective bioremediation approach depends upon the activities of aerobic, anaerobic, and heterotrophic microorganisms. Several studies showed that the efficiency of bioremediation could be influenced by various factors such as microbial factors, environmental factors, and contaminants (Fig. 6.3; Caliman et al. 2011; Gavrilescu and Macoveanu 2000; Gavrilescu 2005; Gavrilescu 2010).

6.6.1 Microbial Factors

A successful bioremediation effort relies on the utilization of the appropriate microorganisms (Neilson and Allard 2008). Bacteria, especially Gram-positive and Gram-negative, possess useful bioremediation capabilities including degradation of toxic substances. Gram-positive bacteria, e.g. *Staphylococcus* sp., *Rhodococcus, Bacillus, Arthrobacter, Gordonia, Streptomyces*, etc., are metabolically diverse, colonize on the site of contaminated with xenobiotic hydrocarbons and can degrade various toxic materials such as benzene and naphthalene (Narancic et al. 2012). In comparison, Gram-negative bacteria are effective against heavy metal pollution and show better toxicity tolerance for metal pollutants (La za roaie 2010).

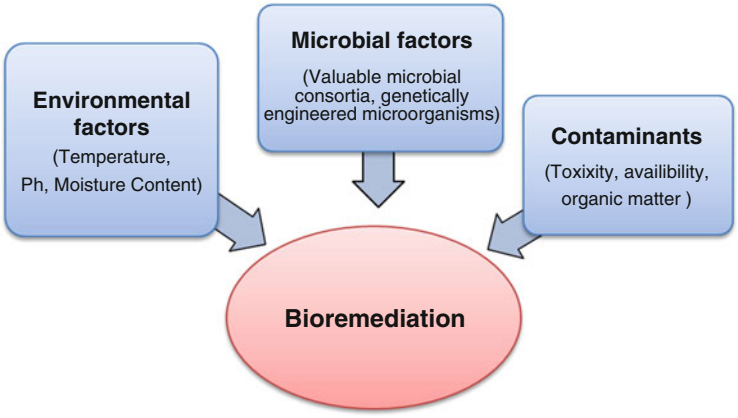

Fig. 6.3 Factors affecting the process of bioremediation

6.6.2 Environmental Factors

Environmental factors include a set of parameters such as environmental conditions, pH, temperature, environmental component characteristics, dissolved oxygen, energy sources, electron donors; electron acceptors, carbon, nitrogen, phosphorus, sulphur, trace compounds, moisture contents and inhibitory substrates or metabolites, solubility, etc., greatly influenced the process of bioremediation (Fortuna et al. 2011).

6.6.3 Contaminants

These factors include the characteristics and types of contaminant/contamination, concentration of contaminants, chemical and physical properties, aggregation state, an environmental component that contains it, scale and extent of contamination, successive use of the site oxide reduction potential accessibility of resources, etc. (Gavrilescu 2010).

6.7 Effectiveness of Bioremediation and its Limitations

Bioremediation is an environmentally benign, powerful, and effective technology of waste treatment. However, there are some drawbacks also that can limit the effectiveness of the process. Some of the advantages which make it a useful tool for the waste treatment and disadvantages which delimit its effectiveness or posses challenges are as follows.

6.7.1 Advantages

- Bioremediation is a biological process in which a specific microbial population is used to degrade a wide variety of pollutants.
- It involves the complete destruction of pollutants or transforms the properties of toxic materials into harmless or less toxic substances by enzymatic metabolic pathway metabolic ability of bioremediators is very impressive.
- It is a less time-consuming process and widely used technology over a decade for the treatment of contaminated material such as soil and water.
- It is environment-friendly, cost-effective, and more efficient technology, hence requires less effort than the other conventional methods used to treat hazardous waste.
- Bioremediation is emerging as a potential tool to address the problem of soil pollution from crude oil. Many microorganisms, e.g. *Pseudomonas, Aeromonas*, *Nocardia*, *Beijerinckia*, *Flavobacteria*, *Corynebacteria*, *Acinetobacter*, *Mycobacteria,* *Modococci*, *Streptomyces*, *Bacilli*, *Arthrobacter*, and

Cyanobacteria can be employed for the removal of contaminants of petroleum hydrocarbon from soil (Thapa et al. 2012).
- It does not include any harmful chemicals. Microorganisms use organic waste present at the site of pollution for their growth and eliminate them from the environment.
- Source separated bio-wastes can be converted into valuable resources like fertilizers, biogas, animal feed, etc., by composting or anaerobic digestion.

6.7.2 Disadvantages

- The major disadvantage of bioremediation process is that it is restricted to biodegradable compounds. Not all contaminants are easily degraded by bioremediation using microorganisms.
- Enzymatic degradation of complex compounds is a highly specific process, which requires suitable conditions for growth, presence of metabolically active microbial population, an appropriate amount of nutrient and optimum physicochemical condition at the site of contamination. There are some concerns related to the products of biodegradation that if the organic broken down completely, the resultant contaminant product may be more persistent or toxic than the initial contamination (Bisht et al. 2015).
- Another obstacle hindering bioremediation application is regulatory factors.
- Effectiveness of bioremediation technology depends on extensive monitoring of the process, site-specific treatment, e.g. ex situ, in situ, the addition of specific microbial population able to degrade contaminants, use of genetically engineered microorganism.

6.8 Conclusion

Bioremediation is nowadays considered as one of the most efficient environmental clean-up techniques. This technology utilizes elimination, reduction, and transformation of organic and inorganic pollutants. Today, the experience accumulated over the last decades has improved our understanding of many aspects of this multidisciplinary technology. Research is needed to develop an understanding of microbial interaction communities with environment and contaminants, exploring the knowledge about the genome of microorganisms to increase their abilities to degrade pollutants, isolation and identification of potential microbial consortia that have the ability to degrade almost all organic and inorganic contaminants. Research continues to verify the bioremediation potential of microorganisms. For example, a recent addition to the growing list of bacteria that can reduce metals is *Geobacter metallireducens*, which removes uranium, a radioactive waste, from drainage waters in mining operations and from contaminated groundwaters. Even dead microbial cells can be useful in bioremediation technologies. These discoveries suggest that further exploration of microbial diversity is likely to lead to the discovery of many

more organisms with unique properties useful in bioremediation. A further selection of appropriate bioremediation method depending on the type of contamination is necessary for successful application of remediation.

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Bioethanol Extraction and Its Production from Agricultural Residues for Sustainable Development

7

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Abstract

Bioethanol is a reasonable alternative option, its generation from readily available in an enormous amount and eco-friendly bio-resources, i.e., agricultural residues, one of the most suitable and renewable alternative options in place of fossils fuels resources, which being to deplete in an upcoming day. According to the recent statistical analysis report of Economic Co-operation and Development (OECD) and the Food and Agriculture Organization (FAO), for the 2017–2026 year, discussed the cereal feedstocks availability like Wheat and Rice across the world for biofuel production. Wheat and rice cereal cops contribute approximately, 742 Mt and 495 Mt million hectares annually, instead of other cereal crop residues such as Pearl millet (*Pennisetum glaucum*), Barley (*Hordeum vulgare*), Gram pea (*Cicer arietinum*), Sugarcane (*Saccharum officinarum*), and Great millet (*Sorghum vulgare*), abundantly available in Asian countries only. Instead of agricultural residues, municipal sewage waste (MSW) can also be utilized as organic biomass for bioethanol production in Mediterranean countries. As per advanced technologies concern, all of these are described in this chapter, they are direct combustion, combustion after physical processing, thermo-chemical and biological processing followed by the simultaneous saccharification and co-fermentation (SSCF) and consolidated bioprocessing (CBP). In addition, other applications of recycled wastes were also discussed. The foremost aim of the chapter was too focused on the latest development with respect to technologies related to the biofuel sector and sustainable development of the agricultural sector as well.

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Keywords

Agricultural residues · Bioethanol · Biomass · Simultaneous saccharification and co-fermentation · Consolidated bioprocessing

7.1 Introduction

Fossil fuels are known to be a finite source of energy, but now it is slowly depleted, and possibly vanishes in a couple of next few decades. The possible explanation of this current problem is to replace fossil fuels with the eco-friendly biofuels that have become a realistic, focused area of research in the last few decades. Another problem of climatic and global warming issues arises due to the burning of fossil fuels, which warn us to take strict initiative steps to look forward to substituting fossil fuels, that will be, replaced by biofuels in an upcoming day, so as to meet our energy demands. Biofuels produced from biomass (Abdeshahian et al. 2010). The term biomass (Greek bio meaning life + maza meaning mass) refers to non-fossilized and biodegradable organic material originating from plants, animals, and microorganisms. The biomass comprises of by-products, residues, and waste derived from agriculture, forestry, and related industries, as well as, and these are non-fossilized and biodegradable organic fractions of industrial and municipal solid wastes (Williams et al. 1997). Moreover, the biomass originated from the decomposition of non-fossilized and biodegradable organic materials (Demirbas 2009) had gases and liquid fractions. Thus, the biomass considered as bioenergy resources and alternative options, in terms of, sufficient energy value per unit mass, but its energy value is significantly lower than that of fossil fuels. Till date, several researchers focused on biomass consumption as a valuable energy source and acting as a promising research area in a couple of decades. Biomass is a renewable feedstock, accustomed to producing biofuels (in a solid, liquid, and gas forms) for sustainable development in an upcoming year (Stocker 2008). However, a variety of crop residues are being produced (Pedersen and Meyer 2010; Kumar et al. 2019) around the world, and these crop residues were utilized to produce biofuels by adopting a different pre- and post-processing steps of pretreatment and production methods, for, e.g. thermochemical conversion (e.g., combustion, pyrolysis, hydrothermal liquefaction, and gasification), biochemical conversion (e.g., microbial fermentation, enzymatic hydrolysis, and anaerobic digestion), and chemical treatment (e.g., transesterification).

According to the feedstock materials utilized and associated conversion methods adopted, biofuels are further classified into three biofuel generations: First-generation biofuels based on crop plants (Amartey and Jeffries 1994; Shukla and Cheryan 2001; Azeredo et al. 2006; Ganjyal et al. 2004; Kim et al. 2004; Jerez et al. 2005; Aithani and Mohanty 2006; Kılıc and Ozbek 2007; Singh 2008), Second-generation biofuel is sourced from lignocellulosic biomaterials or non-edible feedstocks materials (Brumbley et al. 2007; Nass et al. 2007; Nel 2010), Third

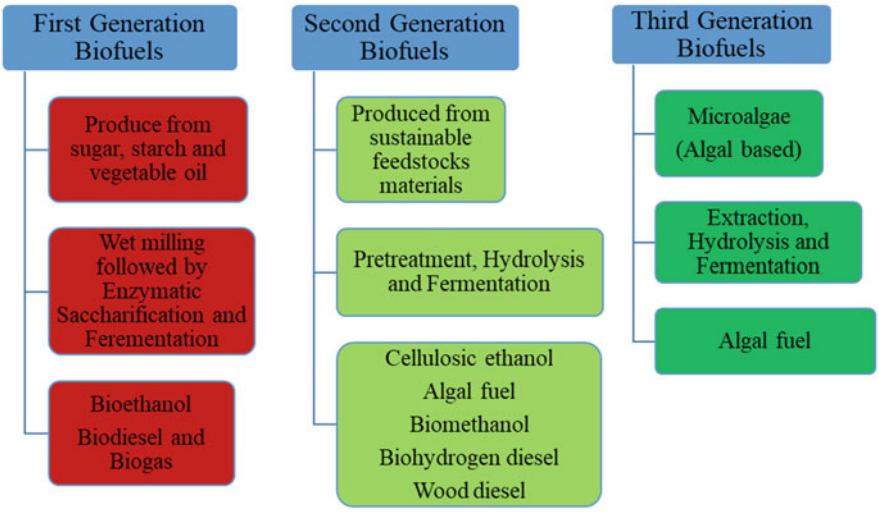

Fig. 7.1 Biofuels classification trends with pretreatment technologies

generation biofuels derived from algal biomass (Lakaniemi et al. 2013) (Fig. 7.1). However, an important concern to environmental forfeits of contending the use of crop residues should be evaluated on short-term and long-term perception basis, considering its impact on agricultural land and biofuel productivity. Several varieties of biofuels are available today, out of them, bioethanol is the most demanding, and thus, it required in a huge amount.

Traditionally, biomass-derived energy is utilized for the generation of liquid fuels: bioethanol and biodiesel. In India, bioethanol is mainly produced by the fermentation of molasses (Sengupta and Poddar 2013), a by-product of the sugar industry, and biodiesel, which is made from non-edible oilseeds. That is why Indian researcher's focused on the research, which is, based on the cellulosic biomass transformation into ethanol. Now, it can be possible to generate ethanol from a cultivated cellulosic biomass of Jatropha plants, for the local and municipal mass production of biodiesel, which is fit for the environment, and it can lead to better incomes generator especially, for a rural people. Meanwhile, with the establishment and continuous improvement of a Jatropha system, definitely, improves the four significant aspects of development and it ensures a sustainable way of life for the farmers, and their supporting land (Kapadia et al. 2019).

As per the report of (NUiCONE) Nirma University International Conference on Engineering Proceedings, India has now changed its strategies, in order to, enhance the biofuel production beyond to 1%. Thus, it has been observed that the 85% increment in biofuel production since 2009.

In the 2015 year, both the USA and Brazil collectively produced 85% of world ethanol. Instead of this, alone the USA becomes the largest ethanol producers in the world, with the 15 billion gallons production capacity as reported at the end of the 2015 year (Srivastava et al. 2019) and according to the current global biofuel demand, reached to 6.5% per annum. This current mandate reported that renewable

fuels predominate as transportation fuels in the USA by the end of the 2022 year. Its production is increased to 36 billion gallons per year, contribute at least 21 billion gallons of fuel derived from non-corn, cellulosic, and non-edible biomass resources (Ziolkowska and Simon 2014). Furthermore, the Agro-based industry has 51.3 gallon litre (GL) capacity of producing bioethanol, derived from the 180.73 million tons of available biomass of sugarcane bagasse (Saini et al. 2015). Other than cereal crops, industrially processed wastes, and various fruit wastes such as pineapple peel (8.34%), banana peel (7.45%), apple pomace (8.44%), palm oil empty fruit bunch (14.5%), and a mixture of apple and banana (38%) wastes are also employed to evaluate the quantification of bioethanol potential (Gupta and Verma 2015).

7.1.1 Agricultural Residue

Agricultural sector is one of the central integral parts of an economy, especially demonstrated in developing countries like India, Thailand, Indonesia, and Philippines, etc. Although, the crop itself, contributes a large number of residues, accounting to about 140 billion tons (Sugathapala and Surya 2013) of a generation of crop residues were produced every year. The term agricultural residue describes all the organic materials, which are produced as by-products, actually derived from the agriculture activities (Bertero et al. 2012). These crop residues constitute a significant part of the total annual production of biomass residues and become a leading source of energy (Prasad et al. 2007) at the domestic and industrial level. Agricultural residues can be further categorized into the field-based residues and process-based residues. Such categorization is essential, especially, under the context of energy application, availability and accessibility to these sources critically depend on this attribute. Availability of field-based residues for energy application is usually limited, since its collection for energy utilization is difficult, and therefore, it can be utilized for other alternative purposes, such as fertilizing and animal feed. However, processed based residues are usually available in a relatively bulk amount, and it may be utilized as an energy source for the same industry, holding little transportation with no handling cost.

7.1.2 Agro-Waste Composition

Several developing countries reported that the there is a scarcity of fossil fuels so, they rely on biomass-based bioenergy originated from agricultural residues such as Wheat straw, Rice straw, Stovers, Sugarcane bagasse, Coconut shell, and Cornstalk, etc. (Gaurav et al. 2017). Fig. 7.2 describes the major biomass resources of India. All these residues are lignocellulosic biomass, which consists of structural elemental polymers: cellulose $(C_6H_{10}O_5)x$, hemicellulose $(C_5H_8O_4)y$ (pentose sugar) such as xylan and lignin $(C_9H_{10}O_3(CH_3O)0.9\text{-}1.7)z$ (polyphenolic compounds), where x,y,z represents the composition of biomass materials. A cellulosic fraction consists of sugar molecules with longer chains of carbon, linked together to form a polymer.

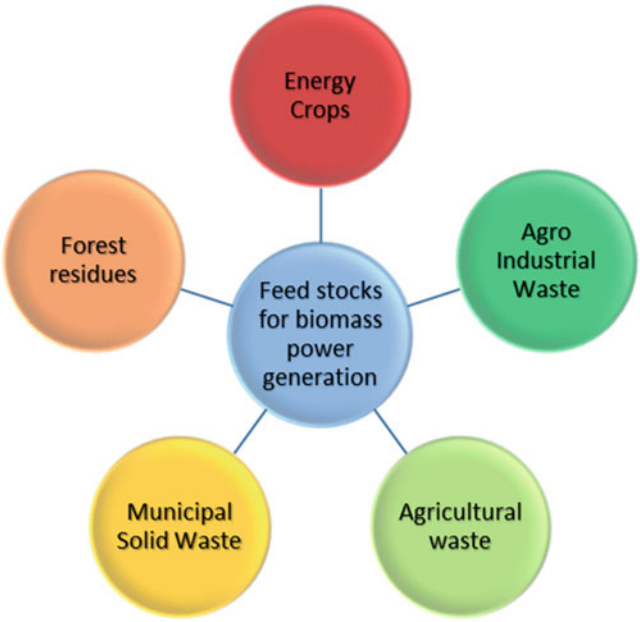

Fig. 7.2 Biomass resources available in India

The lignin fraction consists of non-sugar type moieties that act as a glue, holding together the cellulosic fibres, and contributes to structural rigidity to plant tissues. Lignin contains a very high energy structure, but due to its hardness, it is difficult to decompose, it means they are not easily breakable. For example, Woody plants have typically slow growth characterized, and composed of tightly bound fibres, giving a hard-external surface, while herbaceous plants are usually perennial, with more loosely bound fibres, indicating a lower proportion of lignin content, which binds together with cellulosic fibres tightly. Both the polysaccharides elementary materials are good examples of natural long-chain polymers. The relative proportions of cellulose and lignin content in biomass are one of the critical determining factors while demonstrating the choosing the plant species for subsequent processing or suitability as energy crops.

Biomass chemistry makes up the three essential elements: Carbon, Oxygen, and Hydrogen. In addition, Nitrogen, Sulphur, Chlorine, Potassium, and Silica can also be found in a sufficient quantity, usually, less than 1% of dry matter but well above this quantity is occasionally found. Because of the presence of carbohydrate structure in biomass, actually determines the highly oxygenated energy resources compared to conventional fossil fuels, includes the Hydrocarbons (HC) liquids and coals. Therefore, the bio-refineries industries should be developed, in order to, enhance the production of biofuels, that is, dependent on the efficient bioconversion technologies, the biochemical composition of biomass, incentives and government policies. Some agricultural residues are listed below in Table. 7.1

Table 7.1 Different agricultural residues with biochemical composition extracted from crops and plants mass type

Agro-residues	Hemicellulose (%)	Cellulose (%)	Lignin (%)	References
Rice straw	20.47–31.42	28.1–43.77	4.84–23.3	Mancini et al. (2018a), Shen et al. (2019), Xin et al. (2018), Negi et al. (2018)
Wheat straw	10.5–43	28.8–51.5	5.4–30	Tsegayea et al. (2019), Mancini et al. (2018b), Tian et al. (2018), Geng et al. (2019)
Almond shell	28.0–38.0	29.0–31.1	27.7–35	Queiros et al. (2020)
Cashew nut shell	18.6	41.3	40.1	Popa (2018)
Coir pith	0.15–0.25	36–43	41–45	Panamgama and Peramune (2018)
Corn straw	14.8–21.3	27.9–42.6	8.2–19	Liu et al. (2018)
Eucalyptus	11–18	45–51	29	Carrillo et al. (2018)
Groundnut shell	18.7	35.7	30.2	Ganguly et al. (2020)
Horticultural Waste	28.6	34.5	36	Li et al. (2018)
Jute fibres	18–21	45–53	21–26	Ahuja et al. (2018)
Millet husk	33.3	29.6	14	Packiam et al. (2018)
Nut shells	25–30	25–30	30–40	Shah et al. (2018)
Oat straw	27-38	31–39.4	16–19	Rattanaporn et al. (2018)
Palm fibre	19.9	35.4	27.3	Rattanaporn et al. (2018)
Mustard straw	21.5–30	30.9–35	16–25.3	Robak et al. (2019)
Sorghum straw	24.0–27.0	2.0–35.0	15.0–21.0	Almeida et al. (2019)
Wheat shell	30	10–15	4–8	Laca et al. (2019)
Wheat husk	22.3–50	29–49	5–21	Alonso (2018), Kumari and Singh (2018), Hysek et al. (2018), Lin et al. (2019), Bhatia et al. (2018)

7.1.3 Agricultural Residues Available for Energy Plantation

Usually, agricultural biomass is available in a considerable amount for a cattle feed, utilized for fuel generation and power production, especially fruitful in a rural area of India, and makes the rural people livelihood easier and more comfortable. Some crops like such as corn, sugarcane, grains, pulses, rubber, etc. are available today in a vast amount for a biomass power generation (Davis et al. 2014). In India, the requirement of enormous power demand is likely to be fulfilled by adopting the variety of readily available energy bio-resources in Indian villages. Some prominent biomasses such as burflower-tree (*Neolamarckia cadamba*), Gum arabic tree (*Vachellia nilotica*), Bamboo (*Bambusa vulgaris*), Julie flora, and *Melia dubia* are also acted as energy plantation resources (MNES. In: Ministry of non-conventional

Table 7.2 Indian zone wise available agro-feedstock materials and cost/t. (Kumar et al. 2015)

Indian regions	States	Available agro-waste	Approximate cost range of feedstock (Rs/t)
North-west	Rajasthan, Gujarat	Stalks of Mustard, Juliflora, Maize, Coriander, Soybean, Cotton, Pigeon pea, and Sesame	1300–2500
Central and South-west	Madhya Pradesh, Maharashtra	Cotton stalk, Soy husks, Mustards, Maize stalks, Chilly, Rice husk, Juliflora, and Bamboo	1500–2800
South	Andhra Pradesh, Karnataka, Tamil Nadu, Kerala	Rice husk, Juliflora, Groundnut, Coconut shell, Bengal gram, Chilly stalk, Cane trash, Maize, and Chickpea	1200–2500
North-East	Jharkhand, West Bengal	Wood chips, Rice husk, and Sugar cane	1100–2600
North	Punjab, Haryana, Himachal Pradesh, Uttaranchal, Uttar Pradesh	Rice husk & straw, Mustard stalk, Straw and Wheat husk, Juliflora, and Cane trash	1550–3000
Central and South-east	Orissa, Chhattisgarh	Rice husk, Cotton stalk, Sawdust, and Juliflora	1100–2600

energy sources. Government of India 1996), and their extensive usage for energy purposes, is because of having a drier matter content, high calorific value, and high carbon proportion, with low moisture and ash content, *etc*.

According to Ministry of New and Renewable Energy (MNRE), approx., 200million tonnes of agro-processing and domestic wastes are generated annually in India and disposed of in a regular manner, and these disposed of sites, are actually managed by local farmers and the unorganized sector, rural worker and the low income-based small agro-industries (Singh 2008). Since these processes entangled no or little production costs, therefore, they are totally ignored, and that is why, they are not utilized properly because the majority of wastes are amounts of leafy wastes, which are burnt out in these disposed of sites, and causes air pollution and even harm the soil fertility. A list of zone wise available agro-feedstock materials listed below in Table 7.2.

7.2 Global Scenario of Agro-Lignocellulosic Residues

Wheat and Rice are the main cereal crops of India, and its farming productivity is approx. 379 million hectares (Mha) per annum, but it has been contributed, in terms of, production per capita in the irrigated lands. During the harvesting of wheat and rice crops, their waste residues such as wheat straw and rice straw are leftover on

agriculture land. These left residues/it may be a hinge material, which is generally utilized for animal feed, house construction, and fuel generation. In a recent decade, mechanized harvesting machinery released an enormous straw residue in a considerable amount, but farmers prefer to burn in situ else the residues would interfere with tillage and seeding of next session crops (Chauhan et al. 2012). Incineration of crop residues should also be avoided because it leads to a severe environmental issue mainly concerning with accelerated decomposition of soil organic matter (SOM), resulting into reduced soil fertility by vanishing the soil microbial activity (Biederbeck et al. 1980), and other health hazards issues are also associated with it, which leads to a severe respiratory and eye irritation problems.

As per the OECD-FAO's recent statistical reports, for 2017–2026 year, recorded the world cereal production to be 2563 metric ton (Mt) annually, it was noticed that the annual wheat and rice production is 742 Mt and 495 Mt subsequently (Nilsson et al. 2015), that is, described in this report in a comprehensive manner. At the same time, the 301Mt annual production of other Coarse Cereals is also recorded in the same year.

Rice and wheat production rate considerably increased and reached an average level of production, indicated at an industrial scale. It indicates the residues to product ratio, which decides the exact agricultural practices, that would be lie 0.416–1.875 in the range that was recorded mostly in the Southeastern Asian countries (Bhattacharya et al. 1993). Thus, these residues can be utilized for the purpose of biofuel production. The statistical report of the OCED-FAO agricultural outlook for the 2017–2026 year depicts the annual wheat, and rice crop residues production (Carrillo et al. 2019) is approximately 1336 Mt and 891 Mt subsequently, produced throughout the world.

The Wheat and Rice residues majorly constitute the total biomass residues, which are produced yearly through the agricultural practices; therefore, these residues are widely available and a vital source of energy in a domestic and industrial scale. In 2017–2018 the report stated that the annual production of Wheat and Barley in Turkey, that is, approximately 19.5Mt and 7.0Mt, respectively. In the 2017–18, it was reported that the annual production of wheat and rapeseed in Australia is 23.5Mt and 3.2 Mt respectively. The annual productions of corn in Vietnam and Argentina, was approx. 5.6–42.0 Mt respectively, as per the reports of 2017–18.

An organization like Foreign Agricultural Services (FAS) and World Agricultural Supply and Demand Estimates (WASDE) recorded globally, the whole crop production in every year and announced the list of selected food crop residues such as Great millet (*Sorghum vulgare*), Pearl millet (*Pennisetum glaucum*), Maize (*Zea mays*), Finger millet (*Eleusine coracana*), Barley (*Hordeum vulgare*), Gram (*Cicer arietinum*), and Sugarcane (*Saccharum officinarum*), and acquire a unique position in Asian countries, just because of likely to be utilized as bio-resources for biofuel production. Indian Ministry of Agriculture, published a research article in the 2005 year, discussing the post-harvesting of sunflower seeds, and oilseed crops cultivated at a large scale in India. India occupies a unique position in oilseed crop production in the world, especially in case of sunflower seed. It accounts for 1250 thousand MT (4.77%) of total world production of sunflower in the 2004 year. This studied article

reports the Silver leaf sunflower, which has a unique property of drought-resistant rather than its wild species, which undoubtedly, produces a more extensive, and more solid stem, which grows up to 4.5 m tall (Picot et al. 1984). Because of these reasons, the post-harvesting practices were not concentrating much on the residue collection. At present, several researchers were planned to collect the Sunflower seeds, acting as a potential source for biofuel production.

7.2.1 Other Lignocellulosic Residues

The cellulosic fraction of the municipal solid waste (MSW) can be a potential source for the bioethanol production due to their easy availability, but difficulties are associated with processing and handling of MSW which represented an as challenging problem for researchers. Municipal solid waste (MSW) is an inexpensive source of organic biomass, and its categories include domestic and industrial waste as well. Hadar 2013 reported that the 30 metric tons of annual ethanol production could be achieved from 50% of the 180 million tons of MSW, which is, only produced in the Mediterranean countries.

Oil palm industries occupy a significant place in Island like Indonesia. The waste obtained from oil palm industries, that is, the waste of oil palm empty fruit bunches or frond, mesocarp fibre, and oil palm trunk, obtained after the milling and refining activities. In palm oil processing plants, only 10% of the total dry matter was converted to oil, remaining 90% being oil palm biomass that can be utilized to produce the biofuels and their by-products. Sukiran et al. 2017 reported that palm oil products obtained from palm oil industry waste reached up to a sum of 25.64MT/annum.

7.3 New Directions to Overcome the Problems of Agro-Industrial Waste Contamination

Most of the agro-industrial residues are recycled as animal's feedstock, but its bulk mass is burnt in an open field. In 2008 year, global biofuel production was reached to about 83 billion litres, contributing about 1.5% of the global transport fuel consumption. In 2016 year, transport biofuels consumption has been increased to 4% of the world demand. In this respect, the USA and Brazil are the two largest producers. While in 2018 year, Brazil, China, and Thailand are three largest producers outside the OECD region, with a share of about 40% of the total production. Biofuel production is expected to rise to 159 billion litres in the last 5 years' decades as reported by IEA agency Renewable information, 2018. Now, it is expected that biofuels contribution provides roughly 9% of the total transport fuel demand by the end of the 2030 year, and its extended analysis demonstrates that the biofuels could provide 26% of total estimated transportation fuel by the 2050 year, while the second-generation biofuels accounting for roughly make up 90% of all biofuels (Hafiz et al. 2019). Bioethanol is a renewable and sustainable biofuel, a second-

generation biofuel, with a promising future to compensate for the global energy crisis with the improvement in environmental quality (Aditiya et al. 2016). Second-generation bioethanol has excellent potential if implemented nationally. More than half of the second-generation biofuels production are still in the Blue-Map Scenario (oriented to reduce to a half of the CO_2 emissions, that is, related to the global energy by 2050 compared to 2005, already outlined in the World Energy Outlook, 2009 as announced by International Energy Agency 2010 which projected to occur in non-OECD countries, like China and India, accounting for 19% of the total production (Hafiz et al. 2019) globally. India position 13th place itself in world production of agricultural crops, alone Mexico generates large amounts of agro-industrial waste (AIW), which are usually raw unprocessed and discarded materials (Carrillo et al. 2019). Now Mexico could increase its ethanol production, in order, to reduce and replace the extensive current consumption of fossil fuels, and reduces the adverse impact of harmful environmental. Moreover, the presence of the wide variety of soils, climates, and ecosystems, Mexico has an ideal condition for growing the varieties of lignocellulosic biomass to produce a value-added commercial product. These agricultural residues generations are directly linked to the farming practices, and the technologies employed for cultivating, harvesting, transportation, storage, and processing. According to Thompson's Web of Science (web of knowledge.com), there have been 85 articles published in Mexico on the subject of biomass for ethanol production between 2010 and present. During the period of 2000–2009, for comparison, only 16 articles were published on the subject. This reflects the extensive research inputs in order to explore the high potential of second-generation bioethanol in Mexico. However, Second-generation bioethanol faces specific technical barriers in the sense of, economically non-competitiveness, pinpointed on a commercial scale. Therefore, an efficient process must be determined in order to define the heterogeneity of the lignocellulosic substrates without affecting the yield and productivity (Parisutham et al. 2014) of fuels.

India is the fifth largest consumer of energy after the USA, China, Russia, and Japan, accounting for 4% of the global energy consumption (as per the report of Federation of Indian Chambers of Commerce and Industry Price water house Cooperation 2013). With annual energy demand growing steadily at a rate of 4.8%, India is projected to become the world's third-biggest energy consumer by the end of 2030year. Now, India focused on the sustainable and renewable energy alternative options to fulfil their huge energy demands in order to save the petroleum and associated resources, the only reason of this hike in energy demand is because of a sharp increase in human population along with urbanization. The bioethanol market in India is expected to increase drastically, with steady growth in the transportation sector and stop the continuous consumption of petroleum products. The bioethanol is a cleaner and greener fuel because it reduces the carbon emission and makes our environment pollution-free. The market value of potable alcohol industry is reached to expected fuel price value of Rs 300 billion and has been growing at a fast rate of 7–10% per year, as discussed by (ICRIER) Indian Council for Research on International Economic Relations Policy Report, 2011.

India's bioethanol programme exclusively depends upon non-edible feedstock materials like sugarcane molasses, sugarcane bagasse. Although, India being the second largest sugarcane producer accounts for only 1% of the global biofuel production (Shinoj et al. 2011).

In India, most of the bioethanol production and its blending with gasoline has been largely driven by the National Biofuel Policies (http://mnre.gov.in/file-manager/UserFiles/biofuel_policy.pdf). In the 2003 year, India has started the Ethanol blended petrol programmes to promote bioethanol production and allows the 5% blending with gasoline, but this programme was failed due to the shortage of sugarcane molasses supply. In 2010 year, Government of India had made the National Biofuel Policy which changed the bioethanol blending target to 20% by 2017 and searches out the other alternative biomass and renewed their development program for innovative bioprocess technologies. Alone India produces of 686 million tones of crop biomass per year, out of them, only 34% surplus of biomass can be used for bioenergy generation (Hiloidhari et al. 2014). An alternative raw material i.e. cane juice, Miscanthus, Sweet Sorghum and other readily available lignocellulosic biomass, which are also renewable available need to be promoted for contented the blending targets. Now in India, it should also improvise new technologies, intended for better biomass conversion into biofuels, for the sustainable development of the bioenergy sector of the country.

7.4 Methods of Extracting Energy from Biomass

Methods explored are directly concerning with primary fuels generation or its biomass processing form depends on the size reduction, drying compaction (densification), and carbonization, *etc*.

Categories are as follows:

1. Direct combustion (simplest method).
2. Combustion after following physical processing such as sorting of raw biomass, chipping, compressing or air drying.
3. Thermo-chemical processing to upgrade the biofuel: further divided into pyrolysis, gasification or liquefication (Katyal 2007).
4. Biological processing: example: anaerobic digestion and fermentation.

7.4.1 Conversion of Agro-Residues into Bioethanol: Processes Involved for Bioethanol Production

One of the comfortable availabilities of Lignocellulosic biomass (LCB) in nature fascinates the possibilities of exploring and enhanced the production of second-generation fuels throughout the year. Conversion of biomass into biofuels can be made by the biomass processing unit, that is, applicable to the bulk mass of

biomasses as well as for the range of biomasses such as hardwood to straw residues. This LCB conversion to bioethanol generation is accomplished in three stages:

(i) Pretreatment, (ii) Saccharification, and (iii) Fermentation of sugar to ethanol recovery.

7.4.2 Pretreatment of Lignocellulosic Biomass

Recovery of sugar from lignocellulosic biomass is far more difficult due to its recalcitrant nature, structural characteristics of biomass including the, *i.e.,* heterogeneity of lignin polymers, toxic inhibitors generation, and high energy requirement to yield a low energy product. In this pretreatment stage, these highlighted factors are overcome by choosing a suitable pretreatment technology to circumvent the problems faced during lignocellulosic ethanol production. Pretreatment is a necessary step to alter some structural characteristics of lignocellulose (Garcia et al. 2009), without losing glucan and xylan content (Vandenbossche et al. 2014). The extent of lignin deformation and cellulose recovery depends upon the choice of pretreatment technologies utilized (Kumar and Wyman 2009). In a recent decade, there are many techniques that have been developed to encounter the problems faced during the pretreatment processing, but still, they are in demonstration level owing to lack of process intensification.

7.4.3 Saccharification

Cellulose hydrolysis, also known as saccharification, is the process in which the cellulose is converted into glucose. A variety of raw materials are utilized for bioethanol production. Mainly three types of materials are used for this purpose: sugars, starches, and cellulosic materials. A complex sugar like starches must be hydrolyzed to simpler sugar by the action of enzymes from malt or mould. This is an indirect method. Cellulose (from wood, agricultural residues, waste sulfite liquor from the pulp, and paper mills) must likewise be converted into sugars, generally by the action of acids or cellulolytic enzymes (Franks et al. 2006). An enzymatic saccharification is the quite cost-effective, less corrosive, and mild method as compared acid or alkaline methods in terms of ethanol production. Factors affecting enzymatic saccharification process involve substrate concentration, enzyme loading, temperature, and time of saccharification (Tucker et al. 2003).

7.4.4 Fermentation of Sugar to Ethanol Recovery

Sugars extracted from Cane or Sweet Sorghum juice (Kılıc and Ozbek 2007), molasses can be used directly for ethanol production via fermentation. This can be possible by incorporating the most common and robust fermenting microorganism such as *S. cerevisiae* and *Z. mobilis* are employed to produce ethanol. Actually,

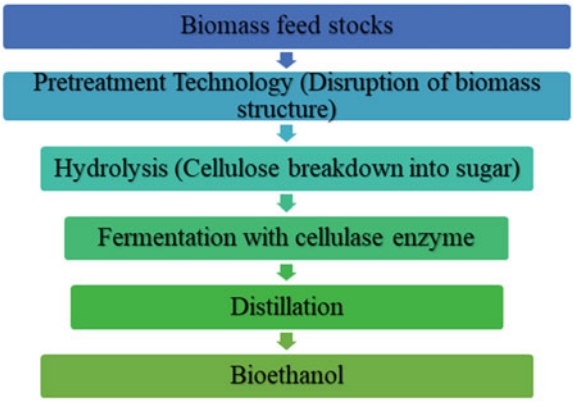

Fig. 7.3 Process flow diagram of biomass conversion process into bioethanol (Joshi et al. 2014)

produced ethanol is drawn out from sugars derived from starch and sucrose, which has been commercially utilized by yeast (Diaz et al. 2015). However, *S. cerevisiae* is capable of converting only hexose sugars to ethanol. In addition, other most promising yeast *Pichia stipitis*, *Candida shehatae,* and *Pachysolen tannophilus,* that have the capability to use both the pentoses and hexoses sugars simultaneously.

One of the Thermo-tolerant yeasts could be more suitable for ethanol production at an industrial level, because of the ability to ferment sugar even at higher temperatures. One of the significant limitations of using thermo-tolerant bacteria is lower tolerance to ethanol (>30 g/l) as studied by Talebnia et al. 2010. Furthermore, rest of the solid residue obtained during this process can be further utilized to feed cattle, while bagasse which is obtained from sugar cane can be further utilized for next gasification step or it can be used as a fuel for boilers (Das and Ghatnekar 1979). An above strategy can be represented in a simplified manner, as depicted in Fig. 7.3 highlighting the process flow diagram of biomass feedstock's conversion into bioethanol.

Another supportive strategy for ethanol production via fermentation is simultaneous saccharification and co-fermentation (SSCF) method, which outlined the simultaneous co-fermentation of hexoses and pentoses is carried out by microbial organisms. In SSCF, the co-fermenting microorganisms have to be compatible with operating pH and temperature (Neves et al. 2007) environmental conditions. However, the ability to ferment pentoses along with hexoses is not widespread among the microorganisms and lack of ideal co-fermenting microorganism, that is, one of the most significant obstacles in industrial production of second-generation ethanol (Talebnia et al. 2010). Sometimes co-culture technique proves to be a useful technology, thereby, a combination of hexose and pentose fermenting microorganisms is utilized for complete utilization of biomass sugars. Neves et al. (2007) studied report suggested that the co-culture of *Candida shehatae* and *Saccharomyces cerevisiae* are the two best organisms to conduct the SSCF process.

One of the most recent and advanced technologies for ethanol production via fermentation is consolidated bioprocessing (CBP), where ethanol and all necessary

enzymes are produced by a single microbial organism. Bacteria such as *Clostridium thermocellum* and some fungi like *Neurospora crassa*, *Fusarium oxysporum,* and *Paecilomyces* sp. had shown the kind of dual activity. However, bigger drawbacks of CBP technology are because of the less-efficient process with poor ethanol yields and longer fermentation time of more than 3–4 days. Significant cost reductions are also encountered while progressing from improved SSF *via* SSCF to CBP. Other beneficial factors such as broad range of multiple substrate utilization by the microorganism, actually represent the high yield (>90% of theoretical yield), the high titre value of ethanol and should have a high tolerance to ethanol (>40% bioethanol tolerance) to extreme pH and temperature (acidic pH or higher temperature) conditions, an actual inhibitors present in a hydrolysate while pre-processing. All of these above-highlighted factors are responsible for maximizing the recovery of the product like ethanol.

7.5 Agro-Industrial Wastes as Potential Substrates in India for Alternative Fuel Production

Today's scenario, organic wastes generated from agro-industries are considered as the primary sources of environmental pollution.

In general, organic waste mainly exists in two forms:

(i) Agricultural and forestry.

(ii) Industrial waste.

Wastes derived from agricultural and forestry activities include the livestock slurry, manure, crop remains, and some other wastes which are derived from pruning and the maintenance of woodlands (Yusuf 2017). Industries also generate the organic wastes, which includes the by-products of the agri-food industry such as coffee dregs, bagasse, degummed fruits and legumes, milk serum, sludge from wool, cellulose, etc. (Bhat 2019). The present scenario is changed, because of urbanization replacing the rural areas, just for economic development, usually, seen in developing Asian countries like India, Bangladesh, Nepal, Bhutan, Sri Lanka, etc.

Moreover, the quantity of the agricultural and industrial waste has increased to alarming state, leading to a serious concern associated with our environment and therefore, in this respect, several countries have made the legislation, in order to, prevent the serious issues concerning with our environment. However, several norms are made by the Indian government to follow the rules and regulation, in respect to the proper waste management, emphasizing on waste assessment and their removal from urban areas must be followed without disturbing the natural flora and fauna, and recycled the organic waste as fertilizer, in agricultural fields. Furthermore, it strictly imposes norms to control the industrial pollution, just because of the presence of organic and inorganic waste or Municipal solid waste (MSW) (Matsakas et al. 2017) in an environment in a huge amount, the only way to eradicate this arising problem recycle the wastes (Shafya and Mansour 2018), into the cleaner technology, i.e., biofuel production. Another prominent application of recycled wastes was also included in this report are enzyme production, organic acid

isolation, pigment extraction, food flavouring and preservative extraction, bioactive compound production, biodegradable Polyhydroxy-acetate (PHA) production, agricultural composting, *etc*. (Samarasinghe et al. 2008). Thus, it has to adopt the regulatory approval on capital investments, in order to, bring these value-added products in the commercial market with comfort.

7.6 Latest Research Studies on Bioethanol

Recent reported studies, on catalytic conversion of lignocellulosic biomass, to bioethanol G2 production, although, they are not yet mature to be utilized for an industrial purpose. This catalytic process has high selectivity, in terms of, product specificity, and it maintains the balance between the overall cost and effective use, whenever used in a bulk scale or industrial scale (Sudiyani et al. 2014). Looking towards the factors like efficient process design, and optimization of environmental conditions for bioethanol production, some significant obstacles are encountered during the pre-post processing steps, *e.g.,* pretreatment, enzymatic hydrolysis, and post-processing steps, *e.g.,* fermentation, and distillation, these observed methods can be chosen on the basis of efficiency of production in lesser time. For this purpose, the production of fermentable sugars, *e.g.,* pentose and hexose sugars, was done either in the presence of engineered microbial strains or it can be enhanced by adopting the hydrolytic process, which is yet to be achieved as biomass preprocessing step, that is, a major challenging task (Mariano et al. 2013) of any bio-refineries. One of the efficient processes observed during the bioethanol production is distillation, where too much energy is consumed, that is why an alternative green process such as per evaporation/Hybrid integrated membrane system should be commercialized widely on the industrial scale. Thus, in the near future, different types of biomass can be effectively utilized and optimized to produce bioethanol, along with the improvement of technologies (Kumar et al. 2019). However, extensive research work is still required to make the process cost-effective (energy return on investment) and efficient (high energy yields) for better ethanol production.

7.7 Research Gaps while Producing Bioethanol

Bioethanol production from edible bioresources like Sugarcane bagasse (Lisboa et al. 2011) was become a matter of discussion, as an alternative energy resource to reduce the dependencies of regional economies on fossil fuels. Even though the bioethanol generation from sugarcane is considered to be a beneficial and cost-effective strategy, so as to reduce greenhouse gaseous (GHG) emissions, but it is still a matter of controversy just because of insufficient knowledge of maintenance of total GHG emissions balance. Aside from these necessity to the account, another logical impact arises because of land-use change (LUC) causes large amounts of greenhouse gas emissions as well as soil N_2O emissions occurred during the processing of sugarcane. In contrast, GHG emissions are reported (Smith et al.

2019), during the pre-harvest and burning of left residues in an open area, which may adversely impact on the maintenance of total GHG balance. This research study represents the huge research gaps is created owing to the lack of knowledge and awareness of GHG emitting resources relevant to agricultural management while processing of sugarcane production (Smith et al. 2019). In addition to this, it also depends on the Biomass types, biomass cultivation practices, local irrigation practices, vinasse, and filter cake applications. Therefore, a still more strategic research should be planned before executing it, as taking an, *e.g.,* of bioethanol production from sugarcane bagasse, that is, an only viable option to mitigate the problem of energy-related GHG emissions (Bahl et al. 2011). Contrary to findings on GHG emissions, which ultimately affects an environment eco-toxicity and human health, it has also been reported with favourable topics relevant to acidification and eutrophication potential with the use of ethanol as biofuel while others not in favour of conventional gasoline usage (Borrionet al. 2012). Therefore, research is needed to obtain more accurate data to support modelling, analysis, and policies development across a wide range of biofuel areas.

7.8 Conclusion and Future Outlook

Waste to energy option is thus, considered to be the most satisfactory way of disposing of the unwanted waste. The feasible option of waste to energy conversion in any developing country that is greatly influenced by the waste collection, scavenging, and proper waste disposal practices adopted in a particular city/town/village. However, agro-wastes selection and utilization for biofuels generation depend on biomass composition, processing, and conversion technologies. More elaborately, biofuels yield can be enhanced by utilizing the co-substrate mixture, which depend on the agro-waste composition and the route of processing step following, that is, either through biochemically or thermochemically, instead of adopting the excess energy consumption process like distillation. In addition, during the bioethanol generation process, more advanced technologies are also included, they are direct combustion, combustion after physical processing, thermo-chemical and biological processing (*e.g.,* Fermentation of sugar to ethanol recovery) followed by simultaneous saccharification and co-fermentation (SSCF) and consolidated bioprocessing (CBP) methodology. The unwanted waste can be further recycled to generate the various kinds of value-added products such as organic acid production, pigment extraction, biodegradable plastic production, agricultural composting, *etc*. these recycled products, not only provide new ideas to researcher's, but it also reduces the current environmental health hazards, thus, make it eco-friendly in nature. However, still much research work is needed to explore out, in order to, accomplish the work extensively on the up-gradation of bioethanol generation by utilizing an engineered microbial strain to scale up productivity from pilot an industrial scale, instead of this, several researcher's focused on a critical purification step to recover ethanol by replacing the conventional distillation processing step, in order to

develop, a new hybrid integrated system of antifouling membranes by injection of the air jet while fermentation.

The significant advantage of this hybrid integrated system is to overcome the fouling problem found during the purification and ethanol recovery stages. Currently, intensive research is being conducted to improve the every processing steps of pretreatment to distillation, so as, to make processes eco-friendly and economical. This agro-industrial residues conversion to economically important substances may not only provide future dimension to researchers but also reduce the current environmental hazards.

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Part II

Biotechnological Approaches

Byproduct Valorization of Vegetable Oil Industry Through Biotechnological Approach

8

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Abstract

Vegetable oil industry produces oil cakes or meals as byproducts after the expulsion and/or extraction of oil from oleaginous materials including oilseeds. The oil cakes or meals can serve various needs of humankind once utilized properly not only in the form of food, feed, and/or concentrated manures but also as sources of various nutraceutically, pharmaceutically, and/or industrially important compounds or phytochemicals. The presence of protein, mineral, and special constituents in oil cake or meal makes it an important component of food and/or feed formulations, provided it is enabled by scientific and technological information and methodologies and supported by enabling policy ecosystem. In this context, there exists a need to review the latest literature on various technological approaches for the valorization of the byproducts of vegetable oil industry. The present chapter is an attempt to bring to the readers an up-to-date and comprehensive information on research and technology in the area of utilizing vegetable oil cakes/meals by way of harnessing the nutritional components and alleviating the problems of antinutritional and/or toxic (or poisonous) components. Though various approaches are discussed, a special emphasis is

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laid on biotechnological approaches for enhancing the value of byproducts of vegetable oil industry. Also, a special effort is made for providing future prospects, highlighting the key area of research for the future pursuit and prosecution. This chapter is addressed to the researchers and research managers from both industry as well as academia interested or involved in promoting the byproduct utilization of vegetable oil industry in meeting nutritional and/or food/feed security at global level in the scenario of never receding human population and ever-changing climatic conditions.

Keywords

Oil cake · Oil meal · Valorization · Utilization · Biotechnology · Fermentation · Bioprocessing · Food · Feed · Oilseeds · Oil crops

8.1 Introduction

The ultimate source of energy for the biome of the planet is sun from where the energy is harvested through photosynthesis and stored in form of energy-storing molecules, namely carbohydrate, protein and fat (oil). The highest efficiency of energy storage per unit quantity of molecules is in fat (oil), at 9 kcal/g, compared to carbohydrate and protein, each at 4 kcal/g (Ali 1995). Therefore, fat (oil) is the energy-rich biological macromolecules which is why some of the plant species store energy in form of seed oil to be utilized by the embryos of their progeny up till they attain photosynthetic autonomy. However, such seed oil has been the main source of vegetable oil for human consumption since the ancient time. In modern time vegetable oil is not only used for human consumption as nutrition but also for industrial purpose.

World is facing the mammoth challenges of energy crisis due to dwindling of depletable resources such as mineral oils. In addition to nutrition of human, vegetable oils provide an alternative resource both for industrial application as well as for fuel purpose (Awogbemi et al. 2019; Grönman et al. 2019). Vegetable oils refinery industry byproducts can be used as the sustainable, biodegradable and environment-friendly chemical feedstocks. In addition, they can be used as raw material for the production of soaps, detergents, resins, polyols, lubricants, polyols, and other products of chemical industry including pharmaceuticals and nutraceuticals (Yusuf 2018). There has been an increasing demand for vegetable oils both for human consumption and industrial application (Patel et al. 2016). According to the projections of the United Nations, by the turn of 2050, the world population is going to be 9.6 billion, placing more pressure on natural resources than at present (UN-Report 2016). It implies that vegetable oil industry occupies a very important place both at national and international levels, as a consequence of which significant amounts of byproducts are produced and they need to be valorized through technological interventions (Patel et al. 2016; Yusuf 2018).

This chapter attempts to bring to the readers a comprehensive and up-to-date coverage of research evidences to show that byproducts of vegetable oil industry can be valorized to meet the demand for nutritional, food, feed, and energy-security of the future. Towards this goal, we have discussed in the subsequent sections economic and technical issues, various utilities, nutritional and antinutritional factors, different approaches for valorizing oil cake/meal along with their limitations, and strategies and potentials of biotechnological approaches.

8.2 World Scenario of Vegetable Oil Economy

Botanical seeds of oilseeds are the most commercially important oleaginous material of plant origin, except oil palm and olive where the fruit pulps yield edible oils (Ali 1995). When oleaginous materials of plant origin are subjected to oil extraction, different kinds of byproducts are produced (Ali 1995). Glimpses of world scenario of vegetable oil production are given in Fig. 8.1 and Table 8.1. This indirectly provides insights into the volume of oil cakes/meals produced globally and their potentials as important resources for beneficial exploitation.

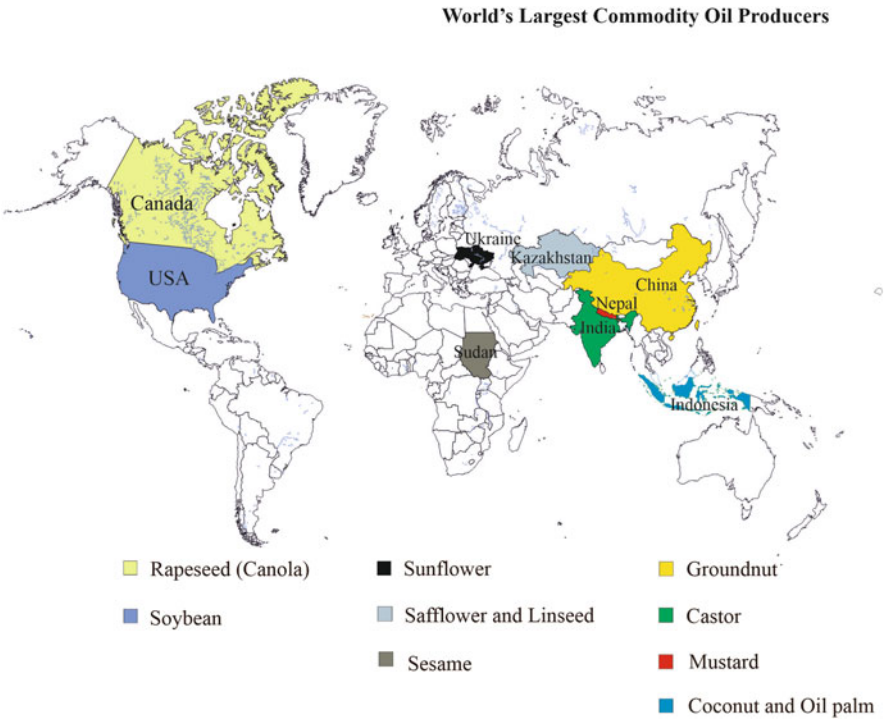

Fig. 8.1 World's largest commodity oil producers according to FAOSTAT 2018

Table 8.1 World scenario of oilseeds economy[a]

Crop	Scientific/ botanical name	Area[b] (Million ha)		Production (Million tons)	Yield (kg/ha)	World highest producing country[c]
Soybean	*Glycine max*	124.92	(107)	348.71	2791.4	USA (123.66)
Rapeseed	*Brassica napus*	37.58	(67)	75.00	1995.8	Canada (20.34)
Mustard	*Brassica juncea Brassica nigra*	0.93	(31)	0.71	763.6	Nepal (0.18)
Groundnut	*Arachis hypogaea*	28.52	(121)	45.95	1611.4	China (17.39)
Sunflower	*Helianthus annuus*	26.67	(78)	51.95	1948.2	Ukraine (14.17)
Castor	*Ricinus communis*	1.30	(48)	1.40	1076.5	India (1.20)
Safflower	*Carthamus tinctorius*	0.69	(26)	0.63	903.3	Kazakhstan (0.21)
Sesame	*Sesamum indicum*	11.74	(78)	6.02	512.3	Sudan (0.98)
Niger[d]	*Guizotia abyssinica*	0.624	(5)	0.19	368.0	India (0.13)
Linseed	*Linum usitatissimum*	3.26	(64)	3.18	975.1	Kazakhstan (0.93)
Coconut	*Cocos nucifera*	12.38	(97)	61.87	4996.8	Indonesia (18.56)
Oil palm	*Elaeis guineensis*	18.92	(45)	272.06	14381.2	Indonesia (115.27)
Olive	*Olea europaea*	10.5	(42)	21.07	2003.7	Spain (9.82)

[a]Based on FAOSTAT 2018
[b]Figure in parentheses indicates the number of countries
[c]Figures in parentheses indicates production in million tons
[d]Niger data is from ASAG 2018

Sunflower (*Helianthus annuus* L.) is the preferred source of oil for domestic consumption and cooking worldwide (Hu et al. 2010) and nutritionally ranks among the best edible vegetable oils in cultivation (Skoric et al. 2008). The seeds are also used for extraction of purple dye and have medicinal uses (Skoric et al. 2008); non-oilseeds are used in confectionary market and food industry (Grompone 2005; Van-der-Vossen and Fagbayide 2007; Skoric et al. 2008; Hu et al. 2010; USDA 2010; Palmieri et al. 2012; Adesina 2018). Sunflower meal is the byproduct of the extraction of oil from sunflower seeds. World production of the sunflower meal was 218.5 million tons in 2019 (IndexMundi 2019). Niger (*Guizotia abyssinica* (L.f.) Cass.) provides 50% of the Ethiopia's oilseed production (Getinet and Teklewold 1995; Getinet and Sharma 1996) and 3% of India's (Bulcha 2007). Safflower

(*Carthamus tinctorius* L.) is mainly grown for seed oil used for food and industrial purposes, and it is a minor crop (Mansouri et al. 2018). Castor or castor bean (*Ricinus communis* L.) yields major highly valued non-edible oil that uniquely has 80–85% of an unusual ricinoleic acid, i.e., hydroxyl fatty acid which is used for the development of several different industrial products, including high quality lubricants for aircrafts (Goa et al. 2006; Sousa et al. 2017). Drying or semidrying oil for paints, varnishes, and other substances of surface coating and heat-stable cooking oil require a high content (about 80%) of oleic acids; therefore, safflower cultivars with enhanced oleic acids have been developed (GRDC 2010; Oelke et al. 1992).

8.2.1 Oil Extraction Process

To meet the nutritional requirement for human consumption and chemical requirements of the industrial application in economically viable and technically feasible manner, extraction of vegetable oils from oleaginous material of plant origin has necessitated development and optimization of various methods—traditional as well as advanced and conventional as well as innovative. Type of the method used for oil extraction not only determines the recovery or yield of the oil but also its quality and purity—also decides those of corresponding oil meal and oil cake (Yusuf et al. 2015; Yusuf 2018).

Overview of the processes and methods used for producing oil cake/meal is illustrated in Fig. 8.2. Oilseeds are cultivated and harvested after ascertaining the proper maturity and harvesting stages and are subject to appropriate processing, on-farm or off-farm, including drying to retain appropriate moisture content (Yusuf 2018). In general, regardless of the type of the method used for oil extraction, specific pretreatment is deployed depending upon the nature of the oilseed and purpose of treatment. Pretreatment methods include dehulling, decortication, or removing seed coat; cleaning by winnowing or sieving; sorting and grading; breaking and reducing size through grinding or milling; and heat treatment, for enhancing the quality and recovery rate of the oil (Ogunniyi 2006; Yusuf et al. 2015; Patel et al. 2016; Yusuf 2018). Oil extraction methods can be broadly grouped into conventional and innovative approaches. Conventional methods include traditional methods such as *Ghani* (Alonge and Olaniyan 2006; Olaniyan 2010; Olaniyan and Yusuf 2012; Yusuf 2018), solvent extraction (Muzenda et al. 2012; Tayde et al. 2011; Dutta et al. 2015; Takadas and Doker 2017), mechanical expulsion (Olaniyan 2010; Arisanu 2013), and cold-pressing (Azadmard-Damirchi et al. 2011; Bhatol 2013; Kittiphoom and Sutasinee 2015). Conventional methods have several disadvantages, namely the high solvent requirement (Rassem et al. 2016; Takadas and Doker 2017), low recovery of oil (Olaniyan and Yusuf 2012), labor-intensiveness (Bhuiya et al. 2015), tedious, and time-consuming (Olaniyan and Yusuf 2012). To overcome these disadvantages, inventions were propelled and innovative methods were devised. Innovative techniques, for instance, are microwave-assisted extraction (Balasubramanian et al. 2010; Azadmard-Damirchi

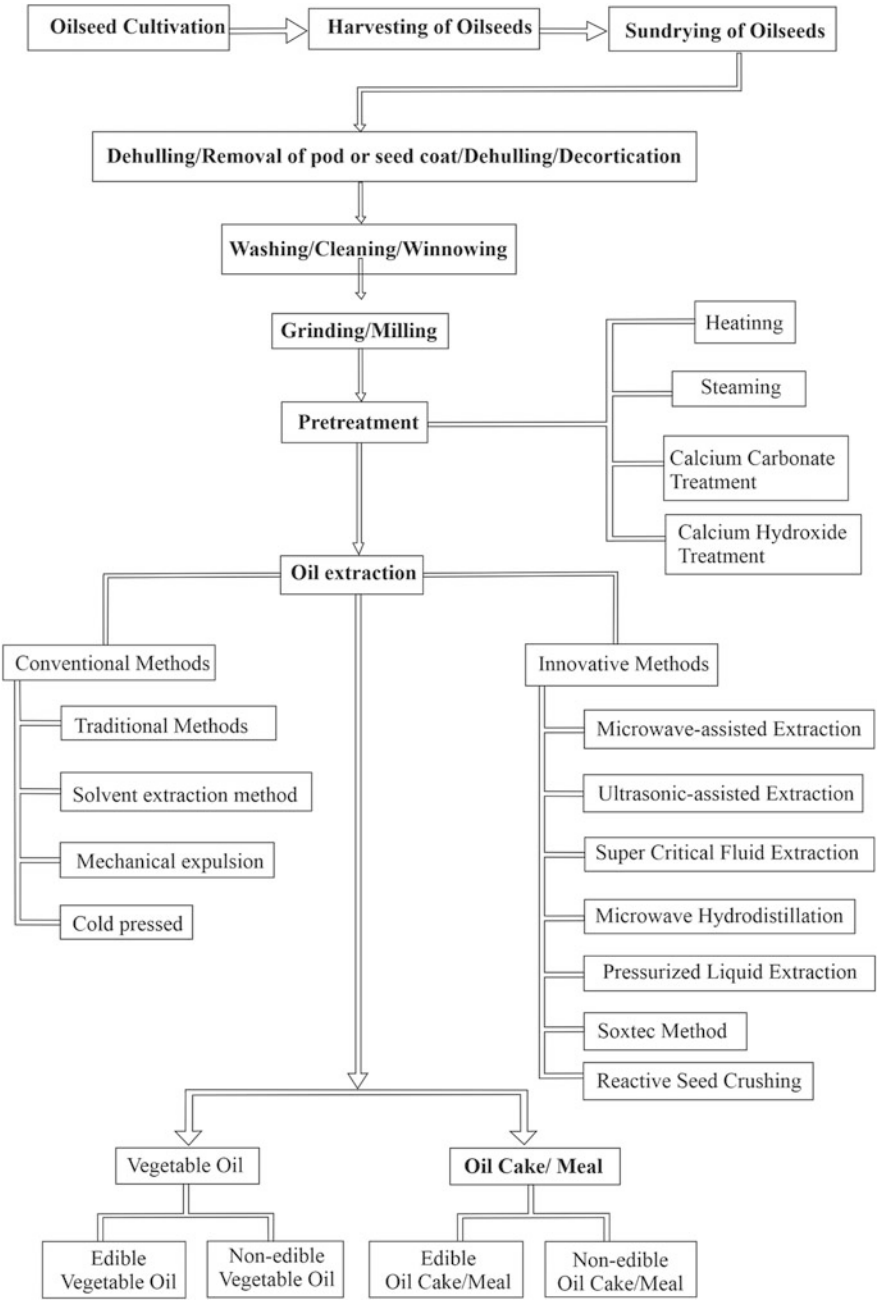

Fig. 8.2 A flow chart showing processes and methods leading to the production of oil cake/meal

et al. 2011; Mgudu et al. 2012; Kittiphoom and Sutasinee 2015; Rassem et al. 2016), ultrasonic-assisted extraction (Samaram et al. 2014; Takadas and Doker 2017), supercritical fluid extraction (Zhi-ling et al. 2011), microwave-assisted hydro-distillation (Lucchesi et al. 2004; Yusuf 2018), pressurized liquid extraction (Danlami et al. 2014), Soxtec extraction (Bampouli et al. 2014), and reactive seed crushing (RSC; Dubois et al. 2013).

8.3 Physicochemical Nature of Vegetable Oils (or Lipids)

Lipid refers to aggregate (bulk) of triacylglycerol/triacylglyceride (TAG) molecules: lipid in solid state is called fat and that in liquid state is called oil. Functional and industrial properties of lipids depend on their physical and chemical properties (Sciarini et al. 2013; Orsavova et al. 2015; Devi and Khatkar 2016).

8.3.1 Physical Nature of Lipids

Physical property of lipids refers to liquid or solid state at bulk level of triacylglycerides (TAGs) at room temperature (Sciarini et al. 2013). Physical property is dependent on the melting point of the lipid molecules (or TAG) which in turn is dependent on the following factors: (a) whether TAGs are homogeneous or heterogeneous; (b) whether TAGs are homoTAGs or heteroTAGs; (c) whether fatty acid constituents of TAG are saturated or unsaturated; (d) number and position of double bonds; and (e) whether double bonds are in *cis* or *trans* orientation (Sciarini et al. 2013; Devi and Khatkar 2016; Orsavova et al. 2015).

8.3.2 Chemical Nature of Lipids

Chemically each lipid (fat or oil) molecule is made up of two types of organic molecules: one molecule of alcohol called triglycerol and three molecules of hydrocarbon-rich organic acids called fatty acids. All the three hydroxyl moieties of triglycerol are bound to one fatty acid each through ester bonds, and the process is called esterification, to form triacylglyceride (TAG)—chemical name of fat or oil. TAG can be simple or mixed: if all the three fatty acid molecules are identical, then TAG is called simple (or homotriacylglycerides). If three fatty acid molecules in TAG are non-identical, then TAG is called mixed TAG (or heterotriacylglycerides; Devi and Khatkar 2016). Identity or non-identity of the constituent fatty acids is determined by three criteria: chain length of the fatty acid; number and position of the double bonds in the fatty acid chain. Based on their chain length, fatty acids are called by different chemical names (Table 8.2). Further, if all the carbon atoms of a fatty acid chain are completely saturated with hydrogen atoms, such fatty acid is called saturated fatty acid (SFA), whereas if there are one or more unsaturated positions (denoted by double bond) in the fatty acid chain, such fatty acid is called

Table 8.2 Names of fatty acids and their carbon chain length with/without modification (modified from de Man 1998; Orsavova et al. 2015)

Fatty acid	Carbon chain length	Fatty acid	Carbon chain length
Saturated fatty acids		Monounsaturated fatty acids (MUFA)	
Caprylic acid	8:0	Myristoleic acid	14:1
Capric acid	10:0	Palmitoleic acid	16:1
Lauric acid	12:0	Oleic acid	18:1
Myristic acid	14:0	Gadoleic acid	20:1
Palmitic acid	16:0	Erucic acid	22:1
Stearic acid	18:0	Nervonic acid	24:1
Arachidic acid	20:0	Polyunsaturated fatty acids (PUFA)	
Behenic acid	22:0	Linoleic acid	18:2
Lignoceric acid	24:0	Linolenic acid	18:3
		Eicosadienoic acid	20:2

It is the chemical and physical nature of the constituent fatty acids that decide those of TAG (de Man 1998; Orsavova et al. 2015)

unsaturated fatty acid (UFA). There are two types of unsaturated fatty acids: monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). As the name suggests, in MUFA only one double bond is present in the fatty acid, whereas in PUFA two or more double bonds (unsaturated points) are present (Orsavova et al. 2015).

8.4 Nutritional Boon of Vegetable Oil Cake/Meals

Nutritional composition of the oil cake/meal depends on the seed from which the cake/meal is derived. The extent to which certain nutrients are lost or enriched in the cake vis-a-vis the precursor oilseed determines its nutritional composition, and it depends on the genotype and type of the oil extraction method and the solvent/temperature used (Jain et al. 2015; Jain and Singla 2016; Alhassan et al. 2017; Aletor and Adegoke 2018). Cakes/meals obtained after extracting oil are rich in various nutrients as detailed in Table 8.3 and they can be commercially utilized for various purposes. The highest protein content of 35.6 % (w/w) is present in soybean meal (Uwem et al. 2017; Ogbemudia et al. 2018) among oilseeds and oil palm cake (20.55%, w/w, Atasie and Akinhanmi 2009; Akinyeye et al. 2011) among oil trees. Fiber content is highest in sunflower oil meal (65%, w/w, Mansouri et al. 2018) among oilseeds and olive meal (27.41%, w/w, Tiku et al. 2013; Ishfaq et al. 2015; Khan et al. 2015; El-Riachy et al. 2019) among oil trees. Ash content is the reflection of mineral composition and indicates the source of minerals for humans and other animals that feed on the oil cake/meal. Among oilseeds, linseed cake (Aslam et al. 2019) has the highest mineral content of 6.98% on weight by weight basis, whereas it is 5.69% in palm oil cake (Atasie and Akinhanmi 2009; Akinyeye et al. 2011) among oil trees. Even though non-edible, there is a wide variation in the nutrient content of castor seed meal, depending on whether it is decorticated or not. Protein

Table 8.3 Major oilseeds and average contents of oil in seeds[a] and protein, fiber, and ash in their cakes/meals

S. No.	Common name	Botanical name	Oil content (%, w/w)	Protein content (%, w/w)	Fiber content (%, w/w)	Average ash content (%, w/w)	Reference
Oilseeds							
1.	Groundnut	*Arachis hypogaea*	31–46	20.9–25.3	1.4–3.9	1.2–2.3	Jain et al. (2015), Alhassan et al. (2017)
2.	Soybean	*Glycine max*	18.5	35.6	6.27	4.92	Uwem et al. (2017), Ogbemudia et al. (2018)
3.	Rapeseed (Yellow)	*Brassica napus*	25.5	30.7	7.14	6.32	Święch et al. (2016)
	Rapeseed (brown)	*Brassica napus*	19.0	33.4	10.2	5.9	Święch et al. (2016)
	Mustard (black)	*Brassica nigra*	8.7	3.17	12.17	7.1	Sarker et al. (2015)
	Mustard (yellow)	*Brassica nigra*	15.67	28.80	14.8	5.9	Sarker et al. (2015)
	Mustard (brown)	*Brassica juncea*	51.6	23.11	9.34	3.22	Aletor and Adegoke (2018)
4.	Sunflower (dehulled)	*Helianthus annuus*	20.84	33.82	17.93	5.20	Adesina (2018)
5.	Safflower	*Carthamus tinctorius*	45	17	65	3.5	Mansouri et al. (2018)
6.	Castor	*Ricinus communis*	51.2	31.06	2.5	11.1	Annongu and Joseph (2008)
7.	Sesame	*Sesamum indicum*	40.97	33.91	5.63	4.04	Ogungbenle and Onoge (2014)
8.	Niger	*Guizotia abyssinica*	35	25	18	4	Jain and Singla (2016)
9.	Linseed	*Linum usitatissimum*	10.5	20	42	6.98	Aslam et al. (2019)

(continued)

Table 8.3 (continued)

S. No.	Common name	Botanical name	Oil content (%, w/w)	Protein content (%, w/w)	Fiber content (%, w/w)	Average ash content (%, w/w)	Reference
Oil trees (or tree-borne oilseeds)							
1.	Coconut Copra (Kernel)	*Cocos nucifera*	59.8 (63.6)	10.2 (8.4)	7.5 (6.7)	1.5 (2.13)	Obasi et al. (2012), Appaiah et al. (2014)
2.	Oil palm tree	*Elaeis guineensis*	42	20.55	9.01	5.69	Atasie and Akinhanmi (2009), Akinyeye et al. (2011)
3.	Olive	*Olea europaea*	69	5.83	27.41	1.57	Tiku et al. (2013), Ishfaq et al. (2015), Khan et al. (2015), El-Riachy et al. (2019)
4.	Jatropha	*Jatropha curcas*	47.25	25	40	6.45	Gupta et al. (2018)

[a]Oil from oilseeds is extracted from seeds but from oiltrees it is extracted from different parts: kernel/meat from matured coconut, mesocarp of the oil palm, and fruit of olive tree

content varies from 20.5% to 46% depending on the method of processing. Carbohydrates and fiber may range from 26% to 49%, and mineral content from 10.5% to 15% (Annongu and Joseph 2008). The ash of the castor cake is rich in minerals and contains CaO 17%, P20 S 25%, MgO 6%, K20 10%, and Fe203 6% (Kulkarni 1959).

Castor bean meal containing 35% crude protein and 25% fiber can be utilized as a source of protein for livestock feeding (Lade et al. 2013b) but it is constrained due to ricin toxin. Groundnut oil meal (Desai et al. 1999) and sunflower oil cake (Golob et al. 2002) are good sources of protein, fiber, and mineral nutrients suited for human and animal consumption (Robertson and Russell 1972; Bau et al. 1983; Berot and Briffaud 1983). In addition, sunflower meal is a valuable source of calcium, phosphorus, and B vitamins (Grompone 2005; Adesina 2018).

Niger oil cake/meal is not only a very good source of crude protein (32.3–36.1% of dry matter) but has superior digestibility up to 80% (Jain and Singla 2016). Compared to groundnut, available lysine (400 mg/100 g) and methionine content is better in niger oil cake/meal (Bhagya and Shamanthaka Sastry 2003; Gebremedhin et al. 2009). In addition to the type of the oil extraction methods, environmental factors, geographical location, agroecosystem, and genotypes (or varieties) cause variability in the composition of amino acids, proteins, fatty acids, and mineral contents (Bhagya and Shamanthaka Sastry 2003; Gebremedhin et al. 2009). Safflower seeds contain 28.3 ± 4.5% oil and 17.5 ± 1.8% protein, 3%, ash, and 30% fiber (DM basis) (Ligjue and Ramanatha Rao 1993) and hull portion ranges from thick (Hertarmpf and Diedad Pascual 2000) to thin (Baümler et al. 2006; Ecoport 2010; Oyen and Umali 2007). Hulls contain about 60% crude fiber and 21% lignin (Hertarmpf and Diedad Pascual 2000).

8.5 Antinutritional Bane of Vegetable Oil Cakes/Meals

The presence of the antinutritional factors in the vegetable oil cake/meal is the major constraints in the utilization of the latter as human food and/or animal feed. Major antinutritional/toxic factors present in vegetable oil cake/meals are given in Table 8.4.

The content of phenolic compounds in safflower oil cake/meal ranges from 3 to 4% (w/w) of which 79% is soluble and 21% is protein-bound (Dominguez et al. 1995). Chlorogenic acid (an ester of caffeic and quinic acids) and caffeic acid made up 70% of all phenolics (including chlorogenic, caffeic, p-hydroxybenzoic, p-coumaric, cinnamic, m-hydroxybenzoic, vanillic, syringic, transcinnamic, isoferulic, and sinapic acids) (Mansouri et al. 2018). Chemical composition of *Simarouba* meal has been analyzed with respect to its toxic factors, protein quality, and other chemical compositions (Govindaraju et al. 2009) and such attempts provide insights into possibility of using it in the formulation of food/feed. Based on the protein content (47.7 g/100 g) with high solubility (92%), amino acid-based computed nutritional indices and in vitro protein digestibility (88%) of deoiled meal, simarouba has potentiality to be used as a source of protein not only for feeding

Table 8.4 Major antinutritional/toxic factors in vegetable oil cakes

Oilseed crop	Major antinutritional/toxic factors in vegetable oil cakes/meals	References
Soybean	Trypsin inhibitors, raffinose, stachyose, phytic acid, lectins, and agglutinins	Clarke and Wiseman (2000)
Sunflower	Protease inhibitors, cyanogens, goitrogenic factors, and lectins	Gassmann (1983), Gonzalez-Perez and Arellano (2009)
Castor	Lectins, oxalates, phytic acids, tannins, ricin, ricinine, allergen, chlorogenic acid, and agglutinins	Lade et al. (2013), Taiwo et al. (2012)
Rapeseed and mustard	Sinapine, glucosinolate, and erucic acid	Halkier and Gershenzon (2006), Brand and Smith (2008), Kajla et al. (2017), Petersen et al. (2018), Nega and Woldes (2018)
Groundnut	Oxalates, phytates, cyanogenic glucosides, and trypsin inhibitors	Abdulrazak et al. (2014)
Safflower	Tannins, flavonoids, serotonin derivatives, and glycosides	Singhal et al. (2018)
Sesame	Phytic acids, oxalates, tannins, saponins, and hydrocyanins	Bello et al. (2013)
Niger	Tannins and phytates	Deme et al. (2017)
Linseed	Cyanogenic glycosides, phytic acids, tannins, trypsin inhibitors, and saponins	Russo and Reggiani (2017)
Olive	Phenolic compounds, tannins, and flavonoids	Chebaibi et al. (2019)
Pam oil	Tannic acids, phytin phosphorus, phytic acids, and oxalates	Akinyeye et al. (2011)
Coconut	Non-starchy polysaccharides (mannan and galactomannan), phytic acids, and tannins	Sundu et al. (2009), Tacon et al. (2009)
Jatropha	Cellulose, hemicellulose, lignins, phorbol esters, and lectins	Gupta et al. (2018), Moniruzzaman et al. (2016)

livestock, but also for human nutrition (Govindaraju et al. 2009). However, presence of toxic constituents renders simarouba meal unfit for feed/food. This necessitates further research for effectively detoxifying the simarouba meal to remove saponin, alkaloid, phenolics, and phytic acid through chemical processing or bioprocessing before exploiting its potential as source of protein and minerals in feed or food.

Even though groundnut oil cake (GOC) has good blending and pelleting properties, its usage as animal or aqua-feed has certain bottlenecks (Lovell 1989; Gosh and Mandal 2015). Sulfur containing amino acids, viz., lysine, cystine, and methionine is not present in GOC. However, it is highly rich in arginine (Green et al. 1988). Nutritionally more serious antinutritional factors, viz., trypsin inhibitor, tannin, and phytic acids are the major barriers in directly using GOC as food or feed (Nyina-Wamwiza et al. 2010). Animals feeding on GOC-based feed experience retarded growth caused by nondigestible complexes formed by interaction between dietary proteins and proteases due to their inhibition by dietary tannins (Krogdahl

1989). Several researchers reported the adverse effect of tannin on growth of aquatic animals, particularly fish (Hossain and Jauncey 1989; Bairagi et al. 2002, 2004; Maitra and Ray 2003; Mandal and Ghosh 2010). Tannins inhibit proteases by interfering with enzyme's activesite by way of altering or blocking it, ultimately preventing the accessibility of active site by the substrate (protein) and/or metal ions (Maitra et al. 2007), tannins also cause adverse effect on live stocks due to perturbation of biological membrane of gut flora and thereby reducing the gut flora populations (Scalbert 1991; McSweeney et al. 2001; Smith et al. 2005). Phytic acid readily complexes with proteins and minerals, thereby forming the complexes of protein/mineral-phytic acid. These complexes are not only nondigestible but also render proteins and minerals non-available for metabolic processes (Hossain and Jauncey 1989). In addition, tannin and phytates reduce lipid digestibility value and apparent dry matter (Nyina-Wamwiza et al. 2010; Gosh and Mandal 2015).

Aflatoxin refers to toxic metabolites of the fungi *aspergillus flavus* and *Aspergillus parasiticus* and it causes physiological impairment, including death, in animals and human (Han et al. 2013). Aflatoxin M, one of the metabolites of aflatoxin, can even pass-on to milk of the animal fed on aflatoxin-contaminated groundnut oil cake (Sahin et al. 2016). While there are species-level differences in susceptibility to aflatoxin, within a given species young ones are more susceptible than adults. Though adult cattle show more resistance, loss of appetite and reduced milk yield are common problems (Han et al. 2013; Sahin et al. 2016). Castor bean meal has antinutritional factors such as ricin, ricinine, allergen, and chlorogenic acid; and, therefore, they need to be removed prior to livestock feeding (Anandan et al. 2005; Lade et al. 2013a; Sousa et al. 2017). The major problem with sunflower is chlorogenic acid that forms green-colored complexes due to oxidized chlorogenic acid (CGA)–protein interactions (Sahin et al. 2016).

The niger meal was reported to be free from any toxic substance but contains more crude fiber than most oilseed meals. Defatted niger seed and its extracts contain a small amount of chlorogenic acid in the free phenolic fraction (2.6 mg/g) and traces of free sinapic and caffeic acids, which are natural source of antioxidants (Shahidi et al. 2003). Though niger oil cake/meal is free from antinutritional factors, it often poses a problem of aflatoxin B1 due to *Aspergillus niger* mold infestations resulting from prolonged storage of the cake/meal under unfavorable condition (Nasirullah et al. 1982; Dutta et al. 1994; Dange and Jonsson 1997; Szonyi et al. 2015). Aflatoxin B1 is a carcinogenic toxin and it can be found in milk of the animal fed with infested niger cake/meal (Bhagya and Shamanthaka Sastry 2003; Gebremedhin et al. 2009; Szonyi et al. 2015). Aflatoxin problem of niger oil cake/meal can be solved by proper storage conditions (Bhagya and Shamanthaka Sastry 2003; Gebremedhin et al. 2009; Szonyi et al. 2015).

Simarouba glauca, belonging to Simaroubaceae family, is also called by common name aceituno. Being a tree-borne oilseed, all its parts can be utilized in one way or the other. High protein content (50–55%) is reported in deoiled meal cake (Rao et al. 1986; Rath et al. 1987; Chikara et al. 1998). Toxic factors to animals such as bitter principle quassinoids (Severen 1953) and other phytochemicals (Vaughan 1970) are found in the deoiled meal of simarouba.

8.5.1 The Need for Testing Aflatoxin in Cow Milk

The major bottleneck in using groundnut cake/meal as animal feed is that it has inherent and extraneous problems. Inherently, groundnut meal/cake has antinutritional factors such as phytic acid and antitrypsin (Elzupir et al. 2009; Han et al. 2013). Extraneously, groundnut often get contaminated with fungus *Aspergillus flavus* that produces a mycotoxin called aflatoxin (Han et al. 2013; Sahin et al. 2016). Among four different compounds, viz., B_1, B_2, G_1 and G_2, that constitute commonly called mycotoxin "aflatoxin," B_1 and B_2 are metabolized in animal liver into M_1 and M_2, whereas G_1 and G_2 cannot be metabolized. Among them, B_1 is the most toxic and more widely prevalent in nature and therefore pose the risk of presence in cow milk. If lactating cows are fed with aflatoxin-contaminated feed, a metabolite of the mycotoxin M_1 and M_2 together called "aflatoxin M" is passed on even to milk of the cow (Elzupir et al. 2009; Sahin et al. 2016). Therefore, it is necessary to test aflatoxin B_1, independently, in addition to testing only total aflatoxin (M) to meet the regulatory requirements, for the resason that B_1 constitutes 70–95% of the aflatoxin M leading to a situation where aflatoxin M is below the regulatory limit but B_1 may be above the limit (Elzupir et al. 2009; Sahin et al. 2016).

8.6 Utility of Oil Cake/Meals

A bird eye-view of ways and means of utilizing oil cake/meal is given in Fig. 8.3. Oil cake/meal can be edible or non-edible depending upon the biological nature of the oilseed. While edible oil cake/meal is entirely or significantly free from antinutritional, allergenic, and poisonous factors, non-edible oil cake/meal contains them at significant levels. While edible oils cakes/meals can be readily used as food or feed, non-edible ones require to be subjected to an appropriate processing method. Nonetheless, non-edible oil cakes/meals can be directly used as soil amendments or organic fertilizers.

8.6.1 Human Nutrition

Groundnut oil cake (GOC) is obtained as byproduct after oil is extracted from groundnut seed. Approximately, 80% of the groundnut produced globally is utilized for oil extraction. Therefore, huge quantity of GOC is produced every year. Traditionally, GOC is used as manure or cattle feed due to its high content of protein (Desai et al. 1999).

It is prudent to explore the possibility of deploying protein in meal or defatted meal in human diet and thereby attempting to secure nutrition for people suffering from malnutrition in many developing countries. Groundnut cake can be converted into flour that has good blending compatibility with other flours such as that of wheat, rice, ragi, etc. Therefore, protein and other nutritive properties of cereals can be enhanced by blending groundnut cake. Venkataraghavan (1998) and Gopala-

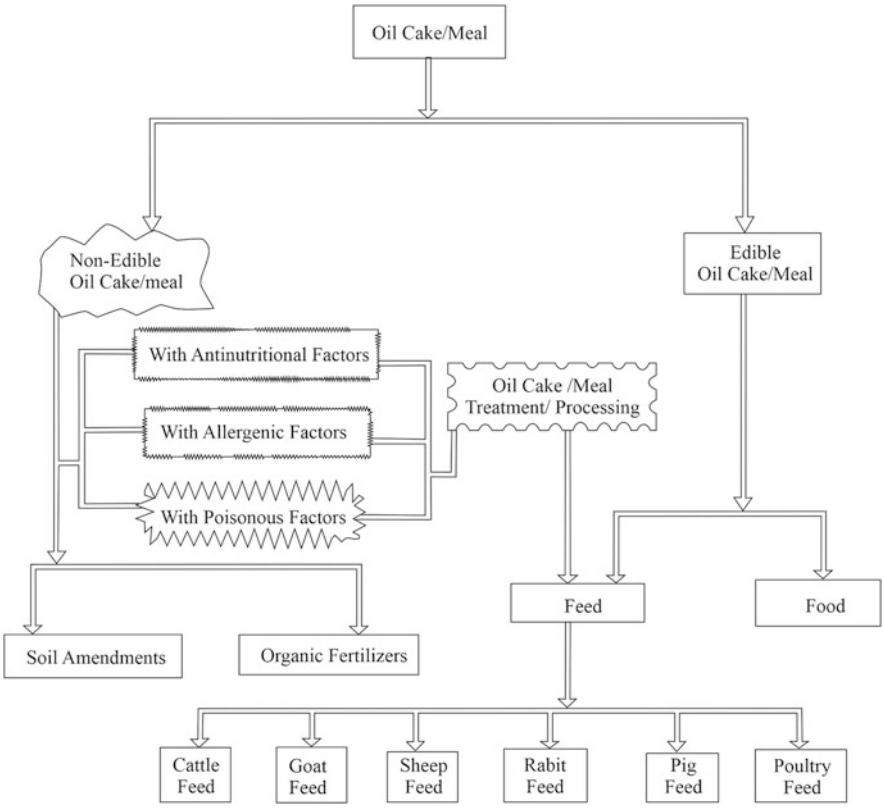

Fig. 8.3 Various ways and means for utilizing oil cake/meal

Krishna (2007) recommended the possibility of using groundnut flour in various food preparations, viz., extruded (fabricated) food, multipurpose supplement, breakfast cereal flakes, infant and weaning foods, snack foods, bakery products, low fat groundnut concentrate, composite flour.

Some of these products are practically developed by Central Food Technological Research Institute (CFTRI), Mysore, India (Parpia 1988). The major impediment in utilizing the defatted groundnut is the unhygienic conditions that may prevail in oilseeds processing factories/units that render the cake/meal unfit for dietary usage for human consumption. Purohit and Rajyalakshmi (2011) developed a technique to hygienically extract defatted oil cake. Further, Gosh and Mandal (2015) have developed thermal and bioprocessing of GOC to enhance its nutritive value and to remove its antinutritional factors (ANFs). Recently, deoiled cake has been utilized to make several value-added food products of Indian recipe (Srivastava et al. 2018).

8.6.2 Livestock Nutrition

In case any particular ingredient of animal feed is used, the following criteria determine the suitability (acceptable nutritional value) of the ingredient in the diet:

1. No adverse effect on the growth and development of the animal in question.
2. Efficient feed utilization.
3. Unaltered body composition.
4. Cost effectiveness.

One of the major protein meals used in the livestock diet, particularly for ruminants, poultry, and pigs is the sunflower meal (Dominguez et al. 1995). It is considered to be a safe feed for all species as it contains no antinutritional factors and has satisfactory mineral content (Demeter et al. 1980; Adesina 2018). However, its only limitation is its fiber content (6%), for the greater part is contributed by the seed husk. Efforts were made to reduce the depressive effect of husk residues by alkaline treatment and by a second grinding action (Demeter et al. 1980) a final solution could be achieved by modifying the crushing technology to attain a meal free of husks.

Another factor as to whether the seed subjected to oil extraction is decorticated or undecorticated introduces two variants: decorticated and undecorticated oil cakes. In case of groundnut, fiber content is very high in undecorticated cakes compared to the decorticated ones (Alhassan et al. 2017). However, it is deficient in lysine, methionine, cystine, tryptophan; and, also low in calcium, carotene, and vitamin D. Groundnut cake can be used as a source of protein in formulating livestock feeds (Ali 1995). Depending upon the method used for the oil extraction, groundnut meal/cake is available in three broad types: traditional (*ghani*) pressed, solvent extracted, and expeller pressed cakes (Jain et al. 2015).

There are oil cakes suitable for particular purposes as feed in livestock production. For instance, olive cake has high nutritional value particularly in rearing rabbits (Dorbane et al. 2016). Similarly, reproductive (Silva et al. 2015) as well as meat production (Diniz et al. 2010; Oliveira et al. 2010) can be enhanced in ruminants (e.g., goats, Silva et al. 2015) with nutrition management intervention using castor meal as an alternative protein source.

8.6.3 Poultry Nutrition

Niger oil cake is a ready-to-use feed for any type of livestock that is capable of digesting fibrous feed, and it is a rich source of protein and minerals, apart from being devoid of toxic substances. In Western countries, niger seeds are important components of bird seed mixtures or bird feed (Marini et al. 2003; Shahidi et al. 2003) and also are used as green manure (Bhagya and Shamanthaka Sastry 2003). There are particular oil cakes/meals such as those of sesamum (Yasothai 2014), flax, and niger (Gueguen and Pascal 2016; Sharma et al. 2014) are most suited for making

feed formulations, for the poultry industry, for feeding layers, broilers, and chicks. A limited number of vegtable oil cakes have been used in poultry feed due to the digestability issues. However, with the intervention of biotechnological applications such as fermentation techonology (Madeira Jr. et al. 2011; Fernandes et al. 2012; Gosh and Mandal 2015), genetic engineering tools (Natália et al. 2017; Chan 2018), and CRISPR/Cas-mediated genome editing approaches (Lee et al. 2020; Si et al. 2020), it is possible to make it compatible the otherwise incompatible oil cake/meals for making poultry feeds.

8.6.4 Fishery Nutrition

High level of plant-derived protein in the diet is detrimental to growth of the carps (Kumar et al. 2011). This effect has been observed with the addition of higher quantity of linseed, raw mustard (Hossain and Jauncey 1989), copra meal (Mukhopadhyay and Ray 1999b), and sesame seed meals (Mukhopadhyay and Ray 1999a; Roy et al. 2014; Das and Ghosh 2015) in carp diets. Addition of fermented groundnut oil cake (GOC) up to 45% of the fish meal is economical in fish diet formulation (Gosh and Mandal 2015). However, the effect of levels of its addition on fish nutrition and health needs to be ascertained before its commercial application. This is due to antinutritional factors, high fiber, and low palatability. However, such problems can be tided over through biotechnological interventions. For instance, through the application of modern tools of biotechnology such as genetic engineering (Natália et al. 2017; Chan 2018) and genome editing (Lee et al. 2020; Si et al. 2020), it is possible to genetically engineer vegetable oil crops to make their oil meal/cake suitable for the fish meal formulation. In addition, fermentation techonology (Madeira Jr. et al. 2011; Fernandes et al. 2012; Gosh and Mandal 2015) holds key to convert otherwise unsuitable oil cakes/meals into those suitable for feeding fish. Extracellular enzymes, viz., protease, amylase, cellulase, lipase, and tannase, can be produced through solid-state fermentation. In solid-state fermentation, microbial cells are immobilized on to solid phase and the nutrients required for growth of the microorganism in question are supplied in the liquid phase. The microorganism produces and releases the extracellular enzymes that are available in the liquid phase. These act as the digestive enzymes to increase the nutritional value of the substrate used in the fermentation. When fermented vegetable oil cakes are used for formulating fish meal, these enzymes help enhance digestibility in the fish gut (Madeira Jr. et al. 2011; Fernandes et al. 2012; Gosh and Mandal 2015).

8.6.5 Plant Nutrition and Soil Health

After oil is extracted from oilseeds, the remaining solid portion is dried as cake which can be used as manure (Reddy 2005; Nagaraj 2009; Lewis et al. 2019). There are two types of oil cakes:

Table 8.5 Average plant nutrient content of non-edible and edible oil cakes (modified from Reddy 2005; Nagaraj 2009)

Oil cakes	Nutrient content (%)		
	Nitrogen (N)	Phosphorus (P_2O_5)	Potassium (K_2O)
Edible oil cakes			
Coconut cake	3.0	1.9	1.8
Cotton seed cake (decorticated)	6.4	2.9	2.2
Groundnut cake	7.3	1.5	1.3
Linseed cake	4.9	1.4	1.3
Niger cake	4.7	1.8	1.3
Rapeseed cake	5.2	1.8	1.2
Safflower cake (decorticated)	7.9	2.2	1.9
Sesamum cake	6.2	2.0	1.2
Non-edible oil cakes			
Castor cake	4.3	1.8	1.3
Cotton seed cake (undecorticated)	3.9	1.8	1.6
Karanja cake	3.9	0.9	1.2
Mahua cake	2.5	0.8	1.2
Safflower cake (undecorticated)	4.9	1.4	1.2

1. Edible oil cakes: These can be used as food for human nutrition or as feed for livestock and aquatic animals, e.g., coconut cake, groundnut cake, etc.
2. Non-edible cakes: These cakes are not nutritionally fit for use as food or feed, e.g., Mahua cake, castor cake, jatropa cake, neem cake, etc.

While both edible and non-edible oil cakes can be used as manures, it is more economical to use non-edible oil cakes as manures and edible as food and/or feed (Rothlisberger et al. 2012; Lewis et al. 2019). Before applying as manure, it is important to make it thoroughly powdered so that it is amenable for mineralization, a quintessential process in making nutrients available in the soil for uptake by plant roots. Plant nutrient content on average basis of oil cakes is given in Table 8.5.

Oil cakes can be effectively utilized as sources of nitrogen (N), phosphorus (P), and potassium (K) to manage plant nutrition and soil health bolstering sustainable agricultural production in the regime of organic farming (Reddy 2005; Nagaraj 2009; Rothlisberger et al. 2012). Along with bone meal and fish manure, oil cakes constitute a class of organic manures called concentrated organic manures, for they contain higher concentration of nutrients compared to bulky organic manures (Reddy 2005; Nagaraj 2009; Rothlisberger et al. 2012). The organic nitrogen present in complex form is first, through bacterial action in the soil, microbially converted into ammonical and nitrate form of nitrogen through a process called mineralization. It is only in ammonical and nitrate form that nitrogen can be readily absorbed by plants. These concentrated organic manures, also called organic nitrogen fertilizers, have advantage over inorganic nitrogen fertilizers, for they slowly release nitrogen

and make it available to plant over a prolonged period of time (Rothlisberger et al. 2012; Lewis et al. 2019).

Mustard oil cake (MOC), for instance, has many advantages in terms of concentration of lignin (5.3%), carbon (44.6%), and nitrogen (5.5%) when compared to other sources of organic manure, namely cow dung, sugarcane trash, and press mud. In addition, the MOC has the lowest C/N ratio (8:1) and is the best suited manure for improving the fertility and health of the soil (Lewis et al. 2019). However, there are some biocides in MOC that adversely affects the growth of the crop plants (Rothlisberger et al. 2012) and this negative effect can be reduced by incubating for 56 days after incorporating in the soil before cultivating crop (Lewis et al. 2019).

8.7 Approaches for Valorization of Vegetable Oil Cakes/Meals

Various strategies and methods used for removing, inactivating, or nullifying antinutritional, allergic, and/or poisonous factors that render oil cakes/ meals non-edibility are schematically represented in Fig. 8.4.

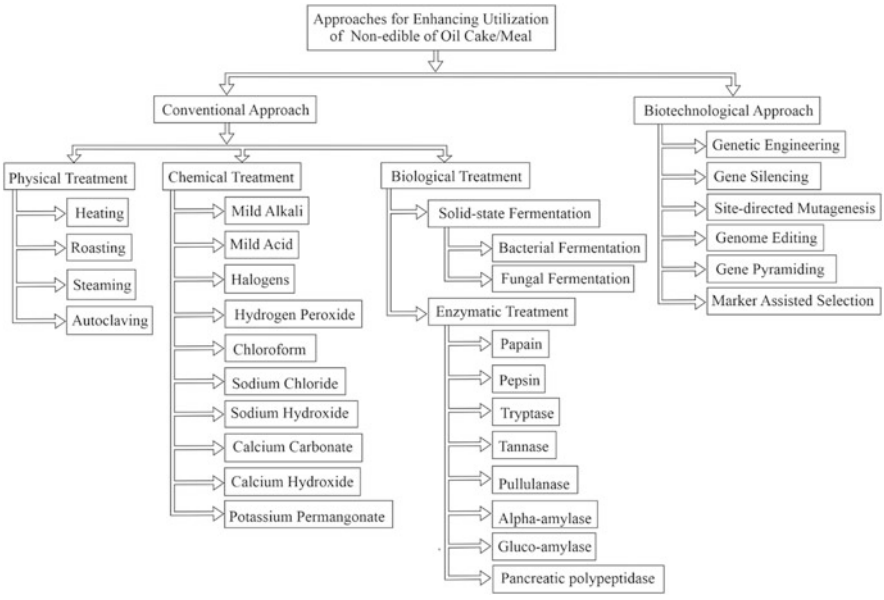

Fig. 8.4 Overview of various approaches/methods for ameliorating antinutritional and poisonous factors for enhancing the utilization of non-edible oil cake/meal

8.7.1 Conventional Approaches for Improving Utilization of Oil Cakes/Meals

8.7.1.1 Physical Treatments

Gosh and Mandal (2015) have developed a method to subject GOC to heat treatment followed by solid-state fermentation. Behura et al. (2010) devised a method for partial detoxification of simaruba oil cake (SOC) by treating it with ammonia followed by roasting. Steam treatment of expeller extraction cake of castor bean helps reducing ricin poison from 0.8–1.0% to <10 ppm.

Protein rich castor oil cake/meal carrying ricin toxin propelled the researchers across the globe to devise suitable methods to detoxify it in order to use the cake as animal feed. Several physical methods were employed: autoclaving (Kodras et al. 1949; Rao et al. 1988; Anandan et al. 2005), boiling (Perrone et al. 1966; Petrosyan and Ponomorov 1937), steam treatment (Kodras et al. 1949; Punj 1988), heating (Tangl 1939), and use of ultraviolet rays (Balint 1972, 1973).

Autoclaving with 15 psi for 60 min and lime treatment (40 g/kg) (Anandan et al. 2005) or dry-autoclaving for 15 min at 125 °C (Kodras et al. 1949) was reported to have had completely detoxified the cake, whereas autoclaving at 20 psi for 60 min was sufficient for incorporation of cake in sheep diets (Rao et al. 1988). Steam treatment at 5 kg/cm^2 for 15–30 min (Kodras et al. 1949; Punj 1988) or use of vapor at 150–300 psi followed by abrupt decompression (Ckiego 1950) was sufficient to detoxify the cake for using as 10% concentrate mixtures for sheep feed. Perrone et al. (1966) deployed repeated boiling for short periods of time by mixing ground press cake and water, with changing the water after each boiling, followed by filtering the resultant material, which is then washed with water and finally dried, whereas just boiling for 1–2 h was sufficient for feeding pigs (Petrosyan and Ponomorov 1937) or just heating to 140 °C for 60–90 min was enough for using the cake to feed sheep (Tangl 1939).

Incubation of the meal in the presence of a mild alkali or acid followed by neutralization or mild oxidation with hydrogen peroxide (Kodras et al. 1949) or extracting the press cake with halogens and alkalis followed by autoclaving (Massart and Massart 1942) was effective in detoxifying the cake. Further, use of ultraviolet rays to uncouple the two chains of the ricin protein significantly reduced its toxic effect (Balint 1972, 1973).

8.7.1.2 Chemical Treatments

Apart from physical treatment, several chemical (Rudolph 1942, 1943; Le Breton and Moule 1947; Ambekar and Dole 1957; Melo et al. 2008; Lade et al. 2013a) and enzymatic (Le Breton and Moule 1947; Melo et al. 2008) treatments are also suggested by various researchers. Toxins in castor meal can be effectively removed by treating it with sodium chloride, sodium hydroxide, calcium hydroxide, and calcium hydroxide (Ambekar and Dole 1957) or by treating the press cake with hot water and chloroform (Rudolph 1942, 1943). Twenty-four-hour water soaking along with NaCl (2%) and $Ca(OH)_2$ (0.25%) reduced ricin content in castor bean meal (Lade et al. 2013a).

Process of hydrolysis using acids (Melo et al. 2008) as well as using enzymes (Le Breton and Moule 1947) is found to be effective in reducing the toxicity of castor cake/meal. Acid hydrolysis process using 0.25 mol L^{-1} of H_2SO_4, at 120 °C for 30 min (Melo et al. 2008) contributed to significant reduction of toxicity in castor meal. Also, ricin can be detoxicated by treatment with sodium ricinoleate, potassium permanganate, hydrogen peroxide, or halogens (Carmichael 1927, 1929). A new processing technology called reactive seed crushing (RSC) combines the seed crushing, solvent extraction, oil refining, transesterification, and meal detoxification into a single step process. Upon subjecting castor bean to RSC, it resulted in detoxified meal and a castor oil methyl ester of the quality acceptable for the downstream processing (Dubois et al. 2013).

Castor ricin treated with papain, pepsin, tryptase, and pancreatic polypeptidase showed a reduction of toxicity (Le Breton and Moule 1947). The optimized enzymatic hydrolysis having the following parameters, per gram of castor oil cake/meal: 200 μL of a-amylase, at 90 °C; 200 μL of glucoamylase and 100 μL of pullulanase, both at 60 °C; yielded 75 g L^{-1} of total reducing sugars corresponding to 91.4% of hydrolysis efficiency. These sugars can be subsequently converted to 34.5 g L^{-1} of ethanol (Melo et al. 2008).

8.7.2 Biotechnological Approaches for Valorizing Vegetable Oil Cakes/Meals

8.7.2.1 Genotype-Based Approach

Mutation Breeding

Antinutritional factors reduce the nutritional value of oil cakes/meals by interfering with the digestion, absorption, and availability of nutrients (Clarke and Wiseman 2000; Nega and Woldes 2018). Through mutation breeding such antinutritional factors can be reduced or nullified in mutant lines and such lines can be released as varieties (Clarke and Wiseman 2000). If different mutant lines are developed for different antinutritional factors, such lines can be further utilized for gene pyramiding (Sect. "Gene Pyramiding"). Soybean mutant lines with low (Gillman et al. 2015; Yu et al. 2019) and ultra-low (Patent no. US20120317675A1) levels of trypsin inhibitors have been developed. In addition to intrinsic antinutritional factors of the oil cake/meal, there exist some peculiar situations as in case of groundnut, for instance, *aspergillus* infects seeds during storage (Adithya 2016). In addition, groundnut is easily infested by *Aspergillus flavus* at different stages of production, storage, and handling. Therefore, dealing with aflatoxin problem in groundnut oil cake gains a prime importance. Since aflatoxins are relatively stable to heat, destruction by heat treatment is not a viable option. One of the possibilities is breeding *aspergillus* resistant groundnut genotypes through mutation approaches (Adithya 2016).

In Vitro Culturing

When plants are subjected to tissue culture and are regenerated, there is a potentiality to induce soma clonal variations due to oxidative stress damage (Cassells and Curry 2001; Tanurdzic et al. 2008; Nivas Ravindra et al. 2012). The following are the genetic and molecular basis of soma clonal variations (Krishna et al. 2016; Moniruzzaman et al. 2016): (1) chromatid breakage and rearrangement (Czene and Harms-Ringdahl 1995; Alvarez et al. 2010); (2) changes in chromosome number (Mujib et al. 2007; Leva et al. 2012); (3) somatic crossing over and sister chromatid exchange (Duncan 1997; Bairu et al. 2011); (4) point mutations (D'Amato 1985; Ngezahayo et al. 2007); (5) changes in organellar DNA (Cassells and Curry 2001; Bartoszewski et al. 2007), somatic gene rearrangement, and DNA amplification (Karp 1995; Tiwari et al. 2013); (6) transposable elements (Hirochika et al. 1996; Gupta 1998; Sato et al. 2011); (7) RNA interference (Miguel and Marum 2011); (8) DNA methylation (Guo et al. 2007; Linacero et al. 2011), histone modification and other epigenetic variations (Kaeppler et al. 2000; Guo et al. 2006; Smulders and de Klerk 2011); and, (9) segregation of pre-existing chimeral tissue (Brar and Jain 1998; Vázquez 2001; Ravindra et al. 2012; Nwauzoma and Jaja 2013). Once genetic variability is created, it is possible to screen and identify the genotype having significantly or completely reduced antinutritional and/or toxic (poisonous) factors in seed. Some useful soma clonal variants have been successfully obtained in various crops: enhanced lysine content, for instance, in rice (Sharpe and Schaeffer 1993), darker and stable skin color in sweet potato (Moyer and Collins 1983), neurotoxin-free *Lathyrus sativus* (Yadav and Mehta 1995), and fruits with fewer seeds in bell pepper (Bell sweet, Evans 1989).

Gene Pyramiding

Gene pyramiding is one of the advanced breeding strategies to remove antinutritional factors (Hameed et al. 2018). Through gene pyramiding approach, it is possible to reduce or nullify antinutritional and/or toxic factors in oil meals/cakes by accumulating the favorable loci as it has been done for other traits in soybean (Anderson et al. 2019), brassica (Mei et al. 2020), rapeseed (Zhou et al. 2018), sunflower (Qi and Ma 2020), safflower (Anjani et al. 2018), groundnut (Janila et al. 2016), sesame (Dossa et al. 2019), linseed (Prabha et al. 2017), castor (Singh et al. 2011), palm oil (Zhang et al. 2018), and coconut (Lantican et al. 2019). However, this strategy would take a very long time in tree species.

Genetic Engineering

Genetic engineering offers an immense potential to alter the antinutritional and/or toxic factors in the vegetable oil cake/meal (Kajla et al. 2017; Petersen et al. 2018). The use of genetic engineering to knock out or silence the expression of genes related to allergens and ricin could be highly productive. The genes that produce both types of proteins are highly expressed during seed development, but the gene expression could be suppressed up to 10,000-fold with the proper choice of promoter and application of gene-silencing techniques (Chen et al. 2004, 2005). Ribonucleic acid interference (RNAi)-mediated silencing of ricin genes has been achieved at

laboratory scale (Natália et al. 2017). Ricin content being a relatively simply inherited trait and with knowledge of candidate genes governing the trait, efforts need to be made to map these candidate genes as well as to identify complete set of genes governing a particular phenotype (Chan et al. 2010; Natália et al. 2017). In view of the fact that there are more than two dozen ricin homolog genes and putative pseudogenes (Chan et al. 2010), currently available knowledge on the genome and target genes needs to be utilized in strategizing biotechnological approaches for developing plants with no toxin (Rivarola et al. 2011; Chan 2018). For instance, inactivation of candidate genes through transgenic approaches or mutagenesis (Ostergaard and Yanofsky 2004; Zhang et al. 2010; Chong and Stinchcombe 2019), including the deployment of CRISPR/Cas9 for genome editing (Lee et al. 2020; Si et al. 2020) and pyramiding of mutant alleles (Malav et al. 2016; Vigano et al. 2018; Chukwu et al. 2019) via molecular marker-empowered breeding approaches.

Though limited, attempts are under way in this regard. In castor, using the promoter of native ricin gene (Ashfaq et al. 2009, 2010), a set of gene-silencing constructs have been developed utilizing ihpRNAi, transitive RNAi, and artificial microRNA approaches to target the DNA segments common to ricin and *Ricinus communis* agglutinin (RCA) genes (Sai-Kumar et al. 2009; Soma-Sekhar et al. 2009, 2010). These constructs have been validated using tobacco (Soma-Sekhar et al. 2010) and it may provide insights to genetically transform castor bean (Ashfaq et al. 2018).

In soybean, antinutritional factor phytic acid has been removed by expressing phytase enzymes through genetic engineering (Clarke and Wiseman 2000). Seed sinapine (Kajla et al. 2017) and glucosinolate (Petersen et al. 2018) contents have been successfully altered in brassica through genetic engineering.

Genome Editing

Genome editing offers capability to design crops from the ground up (Bao et al. 2020; Young et al. 2019). The most applied crop improvement tool of the twenty-first century will be genome editing with the first wave of its application being evident in soybean (Bao et al. 2020) and maize (Young et al. 2019). Genome editing can be accomplished by means of four technologies: zinc finger nuclease (ZFNs, Urnov et al. 2005; Baltes et al. 2014), transcription activator-like effector nucleases (TALENs, Christian et al. 2010; Haun et al. 2014), clustered regularly interspaced short palindromic repeats/CRISPR associated protein (CRISPR/Cas, Barrangou et al. 2007; Jansen et al. 2002; Zhang et al. 2016), and base editing system where nucleotide deaminase is fused with a Cas9-D10A nickase (nCas9, Chen et al. 2017; Li et al. 2017; Qin et al. 2020; Zong et al. 2017).

Using ZFN, successful genetic mutations have been achieved in tobacco (Townsend et al. 2009), maize (Shukla et al. 2009), *Arabidopsis* (Lloyd et al. 2005), and soybean (Curtin et al. 2011). TALENs editing tool has been utilized in rice (Li et al. 2012), tobacco (Zhang et al. 2013), wheat (*Triticum aestivum*, Wang et al. 2014), and soybean (Haun et al. 2014).

Primarily CRISPR/Cas9 system requires desired sgRNA/Cas9 ribonucleoproteins (RNPs) to be introduced to the interior of the cell (Barrangou et al. 2007; Jansen et al. 2002). This can be achieved in two ways: stable expression (Ma et al. 2015) and transient expression (Zhang et al. 2016). Aside from CRISPR/Cas9, another system by name "CRISPR/Cas12a," particularly LbCas12a and AsCas12a have been deployed in editing the genomes of various plant species: LbCas12a and AsCas12a for Arabidopsis (Tang et al. 2017), LbCas12a in cotton (Li et al. 2019a), soybean (Kim et al. 2017), *Chlamydomonas reinhardtii* (Ferenczi et al. 2017), and rice (Li et al. 2019b). The stable RNP systems have been successfully applied to edit apple (Malnoy et al. 2016), Petunia (Subburaj et al. 2016), lettuce (Woo et al. 2015), maize (Svitashev et al. 2016), and wheat (Zhang et al. 2016). Third generation base editor (BE3) system is another variant that has been successfully used for the conversion of C:G to T:A in rice, cotton, *Arabidopsis*, maize, and *Triticum aestivum* (Chen et al. 2017; Li et al. 2017; Qin et al. 2020; Zong et al. 2017).

8.7.2.2 Fermentation Process-Based Approach

By deploying suitable microorganism in fermentation process using oil cake/meal as substrate, desired results can be achieved (Taiwo et al. 2012; Gupta et al. 2018; Chebaibi et al. 2019). By this method either the composition of the oil cake/meal can be altered (Taiwo et al. 2012; Gupta et al. 2018; Chebaibi et al. 2019) or its digestibility can be enhanced (Gosh and Mandal 2015).

Altering Composition of Oil Cake/Meal

In order to remove or deactivate antinutritional factors (ANFs) vegetable oil cake/meal can be subjected to a suitable fermentation and/or bioprocessing (Gosh and Mandal 2015; Phulia et al. 2018).

Through fermentation, lectin, lignin, oxalate, and phytic acid contents were reduced in castor oil cake (Taiwo et al. 2012). In oil cake, phenolic compounds, tannin and flavonoids were reduced and protein content was enhanced when subjected to fermentation by *Fusarium flocciferum* and *Rhizodiscina cf. lignyota* (Chebaibi et al. 2019). Gupta et al. (2018) deployed *Aspergillus niger* and *Paecilomyces variotii* to reduce cellulose content in jatropha oil cake by producing cellulase enzymes (Phulia et al. 2018).

Kifle et al. (2017) developed a method of treating groundnut seeds with *Trichoderma harzianum* strain kd (Tkd) to control preharvest infection of *Aspergillus flavus* in groundnut field. Buddhivant et al. (2016) utilized the groundnut oil cake (GOC) to produce phytase under solid phase fermentation by *Aspergillus niger* and demonstrated the application of GOC in the animal feedstock.

Fermentation with microorganisms, viz., *Paecilomyces variotii* and *Aspergillus niger*, was able to detoxify castor bean residue during the process of biodiesel production (Madeira Jr. et al. 2011; Fernandes et al. 2012). *Aspergillus niger* was grown using a castor cake as a substrate in a solid-state fermentation followed by treatment with calcium oxide or calcium hydroxide at 4 or 8% which not only

reduced the toxicity but also abolished its allergenic properties (Fernandes et al. 2012).

Enhancing Digestibility of Oil Cake/Meal

By isolating the gut flora of the animals that can easily digest vegetable oil/cake and using them for bioprocessing of the cake/meal, bioprocessed cake/meal can be fed to those livestock species that otherwise cannot digest (Jackson et al. 1982). Gut flora, namely tannase-producing yeast *Pichia kudriavzevii* can be isolated from the gut of the target animal and can be utilized for fermenting groundnut oil cake (GOC) (Gosh and Mandal 2015). The bioprocessed GOC serves as an alternate plant-derived protein source with reduced phytic acid, trypsin inhibitor, tannin, and fiber contents, and abundant availability of free fatty acids and free amino acids (Gosh and Mandal 2015).

Alternatively, microbial enzymes can be commercially produced with solid-state fermentation (SSF) wherein microorganisms are immobilized on to a solid surface such as membranes, and nutrients are supplied in liquid phase. When metabolic activity is triggered in the immobilized microbial cells, extracellular enzymes are released into the liquid phase of the fermenter. These enzymes are separated and purified for their commercial application. Subsequently, the enzymes catalyze the biochemical reactions that breakdown the substrates of respective enzymes in the vegetable oil cake/meal into products that are biochemically different from their respective precursors.

8.8 Future Prospects

Wherever meals or cakes are rich in minerals, they need be encouraged to be used as concentrated organic manure. If they are rich in protein they should be used as food and/or feedstock. If some of the antinutritional factors interfere with their utility as food and/or feed, they have to be appropriately tackled using modern biotechnological approaches such as fermentation and bioprocessing (Madeira Jr. et al. 2011; Fernandes et al. 2012; Gosh and Mandal 2015), genetic engineering (Natália et al. 2017; Chan 2018), CRISPR/Cas-mediated genome editing (Lee et al. 2020; Si et al. 2020), etc. Necessary strains from the gut of appropriate animals can be isolated and characterized for their deployment in bioprocessing of oil meal or cakes (Madeira Jr. et al. 2011; Fernandes et al. 2012; Gosh and Mandal 2015). Further research in this regard is required to be focused on inventing aflatoxin detection kits so as to prevent aflatoxin-contaminated groundnut cake from being included in animal feed (Kifle et al. 2017).

Deploying genetic engineering tools for the improvement of microbial strains and/or oil plants (Natália et al. 2017; Chan 2018), reduction or nullifying of non-palatable, toxic, and antinutritional factors needs to be undertaken in a greater measure so as to efficiently and effectively utilize wastes and/or byproducts of vegetable oil industry in meeting nutritional and/or food/feed security at global

level in the scenario of never receding human population and ever-changing climatic conditions (Kumaraswamy et al. 2019; Lee et al. 2020; Si et al. 2020).

8.9 Conclusion

The vegetable oil cake/meal which is a rich source of protein and minerals has been indigenously used as cattle feed or manure. Utilization of meal or defatted meal for incorporating into food and/or feed products could be an excellent means of serving protein in the diets of malnourished people in industrialized, developing, and underdeveloped countries: either directly in form of food or indirectly through the livestock feed. However, there are bottlenecks and hurdles that impediment the way in which the vegetable oil cake/meals can be utilized to meet the nutritional goals. While antinutritional components are the bottle-necks, poisonous components are the major hurdles. Even though there are conventional and traditional approaches for valorizing oil cakes/meals, they have several limitations that are germane to the methods per se. The advent of new approaches such as application of biotechnological, genomic, and genetic engineering tools holds immense potentials to valorize the otherwise limited food and/or feed-value of nutrient-rich vegetable oil cake/meal. Such possibility is already evident from the research results, though carried out in limited number of laboratories. More efforts and funding for research in this direction are the need of the hour for developing biotechnological process and/or methods required for enhancing the utilization of the byproducts of vegetable oil industry for securing nutrition for humans and/or livestock. It is prodigiously imminent particularly in the face of emerging production and productivity challenges and constraints in agriculture and allied sectors on the one hand and ever-increasing demand for the human and livestock nutrition on the other hand.

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Omics Tools: Approaches for Microbiomes Analysis to Enhance Bioenergy Production

9

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Abstract

Exponentially increasing energy demand and decreasing fossil fuel resources signal alarming conditions in the coming future. Need of renewable, sustainable and eco-friendly energy sources thus becomes obligatory. Biological energy sources and means of production show high potential to meet the scarcity. Biofuels like bioalcohols, biodiesel and biogas are efficiently replacing and supplementing fossil fuel energy. Microorganisms play vital role in biofuel production by catabolizing biomass through in-built pathways or itself can be used as energy source. Till date, complex microbiomes are prevalent for large scale production, resulting in biomass consumption even by less efficient or inhibitory microbes, hence decreasing process efficiency and energy yield. Omics tools comprising transcriptomics, genomics, proteomics and metabolomics have provided ultimate techniques for microbial strain development or improvement to deal such limitations. Henceforth, this chapter focuses on conventional as well as improved microbial systems developed by contribution of omics tools, to meet the current global energy demand.

Keywords

Bioenergy · Biofuels · Microbiomes · Omics tools · Strain improvement

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9.1 Introduction

Accelerated increase in global energy demand by 2.3% in 2018 compared to 1.1% in 2016 yells for increase in energy sources. Utilization of oil, coal and gas accounts for 32%, 26% and 23%, respectively, of total energy sources, according to Enerdata reports for year 2018 (Global energy statistical yearbook (2019) World Energy Statistics, Enerdata). Such a high utilization of fossil fuels and the increasing demand, point towards a highly alarming situation in concern to environmental pollution. Also, the increased global consumption of fossil fuels indicates the scarcity prone future in context to energy resources. Although there is 14% increase in fossil fuel production in year 2018 but this increase will not be enough to meet the highly rapid pace of consumption in near future. Along with this the pollution related issues will transform into great hurdle in achieving sustainable existence (Global energy statistical yearbook 2019 World Energy Statistics, Enerdata). Scientific society is intensely engaged in finding alternatives for the present date fossil fuels. Renewable and environment friendly sources of energy are being developed and used to cure the upcoming adverse situations. Bioenergy is the form of chemical energy comprised in biomass which either acts as direct energy source like wood or is processed to provide energy such as biogas and biofuels. Bioenergy is a highly potential solution to the abovementioned problems being eco-friendly, renewable, efficient and cheap (Behera et al. 2015). Till 2017 the consumption of biomass as energy source was 10%, increase in the bioenergy sources and production of conveniently used bioenergy forms can be a boon for the society (Global energy statistical yearbook 2018). Presently, major bioenergy forms are biofuels like bioalcohols, biodiesel and biogas, which are produced by processing of biomass by the action of microbiomes in controlled conditions especially anaerobic atmosphere (Luque et al. 2008). Either improving the variety of biomass or the composition of microbiomes can enhance the bioenergy production by encouraging the action of efficient species and strains. In current scenario, a large variety of biomass is being used for bioenergy production, thus improvement in microbiome and its subjection to such huge substrate variety can give highly positive outcomes (Luo et al. 2011; Mudhoo et al. 2018).

Omics tools comprise tools which assist in genomic, transcriptomic, proteomic and metabolomic analysis. These tools provide huge amount of information from the very small RNA or DNA to the highly complicated biochemical pathways (Horgan and Kenny 2011). Implementation of omics tools for the analysis of microbiomes is issuing great results, thus encouraging their frequent usage for the purpose (Kirtipal and Shanker 2020). Omics analysis of microalgal cultures to enhance lipid production, for conversion into fuel, has provided insight regarding the high yielding strains (Anand et al. 2019). Warnecke et al. in their metagenomic microbiome analysis reported the role of gut bacteria present in wood-feeding termite (*Nasutitermes* species) in degradation of lignocellulosic biomass and showed great scope in biofuel production by implicating such microbiome (Warnecke et al. 2007). Genomic and transcriptomic analysis of microbiomes provided information regarding cellulase producing strains like *Trichoderma reesei, Clostridium cellulovorans* and

Acidothermus cellulolyticus and the genes responsible for the translation of enzymes of cellulase system, respectively (Maki et al. 2009; Ivanova et al. 2017). Jung et al. implied this knowledge of genes for the preparation of recombinant organisms like *Zymomonas mobilis*, *Escherichia coli* and plant itself for their use in cellulose degradation to produce biofuel (Jung et al. 2012). All these studies along with many more prove the importance as well as need of omics tools for enhancement of bioenergy production. Hence, this chapter illustrates different forms of bioenergy and microbiomes used for their production, along with great emphasis on omics tools and their application for developing efficient biological systems to uplift the production process. In addition, contribution of bioinformatics in analysing huge omics tools data has also been discussed briefly.

9.2 Biofuel Types and Microbes for Production.

The term bioenergy signifies energy obtained from biological source. The ultimate and sole source of natural energy is Sun. The energy received from this ultimate source is converted into biomolecules by photosynthetic organisms mainly plants and algae. They fix carbon dioxide from air and water to convert it into biomolecules or biomass. This biomass can then either be used directly or be processed to work as fuel or energy source. The various forms of biomass which can be used directly for energy production by burning are wood, dried plants, bagasse and organic waste produced from animal husbandry, human settlements and industries. The production of processed high efficiency biofuels is done either by microbial or chemical route. The major types of processed bioenergy sources or biofuels are bioalcohols, biodiesel and biogases (Ness and Moghtaderi 2007; Rojo 2008; Mondal et al. 2017).

9.2.1 Bioalcohols

Bioethanol, chemically called ethyl alcohol (C_2H_5OH), is a most prevalent biologically produced fuel used worldwide. It is produced from different feedstocks like starch, sucrose, lignocellulosic biomass and algal biomass by microbial fermentation. The production process consists of three steps: pre-treatment, hydrolysis and fermentation. The factors affecting the bioethanol production are sugar concentration, temperature, pH, rate of agitation, time of fermentation and size of inoculum. It is either used as ethanol directly or as blend of ethanol and gasoline called as gasohol. It can also be utilized to improve gasoline or to enhance octane and in blends of diesel with bioethanol to decrease the exhaust emission. Bioethanol is advantageous in many aspects than gasoline, its octane number is high, that is, 108, flame speed is high, flammability limits are broader, have high vaporization heat, low toxic effects, biodegradability is high and causes low air pollution (Waldron 2010; Zabed et al. 2017). Yeast, mainly *Saccharomyces cerevisiae*, is the oldest and most essential player in fermentation process of sugars especially hexose sugars, to bioethanol. It has high yield (greater than 90%), is tolerant to

ethanol at concentration more than 40 g/L, and its production rate is greater than 1 g per litre per hour. Also, yeast is simple to grow, utilizes less expensive media, undiluted broth with inhibitor resistance, and slows down the growth of contaminants. The shortcomings of yeast fermentations which can act as inhibitor to production of ethanol are inability of yeast to break pentose sugar, intolerance to very high ethanol concentration and high temperature (Sánchez and Montoya 2013). But some strains of yeast like *Pachysolen tannophilus*, *Pichia stipitis*, *Candida shehatae* and *Kluyveromyces marxianus* possess the capability to catabolize pentose sugar. Some yeast species capable for xylose fermentation are *Clavispora, Candida sp.*, *Debaryomyces sp.*, *Brettanomyces* and *Schizosaccharomyces*. Bacteria also contribute for ethanol production in anaerobic conditions. *Bacillus polymyxa, Bacillus macerans, Clostridium acetobutylicum, Klebsiella pneumoniae, Aerobacter sp., Aeromonas hydrophila, Erwinia sp., Lactobacillus sp. and Leuconostoc sp.* are well known to break xylose into ethanol and by-products like CO_2, lactic acid, acetic acid, 2,3-butanediol (Schneider and Jeffries 1989). Soleimani et al., in their study reported *Lactobacillus plantarum* M24 to be the most efficient bacterium for bioethanol production from corncob, corn, pinecones and paper (Soleimani et al. 2017). Ire et al., in their bioethanol production study using xylanolytic and cellulolytic bacterial co-cultures in simultaneous saccharification and co-fermentation of bagasse which was exploded by steam showed *Bacillus cereus* (GBPS9) and *Bacillus thuringiensis* (serovar kurstaki HD1) to be the most potent bacterial strains (Ire et al. 2016). Pandey et al. produced bioethanol from lignocellulosic azolla, a cheap substrate, by employing *Klebsiella oxytoca* and *Saccharomyces cerevisiae* (Pandey et al. 2014).

Butanol is recently gaining importance as biofuel because of its high energy density, high compatibility with engines of cars, low hygroscopic nature, etc. It is produced by solventogenic bacteria mainly of genus *Clostridium*. The most commonly used species are *C. acetobutylicum* and *C. beijerinckii* that produce acetone, butanol, ethanol in 3:6:1 ratio via ABE (acetone–butanol–ethanol) fermentation process. Though ABE fermentation is applied for large scale production, but the economy of the process suffers due to acetone as undesirable by-product which can corrode rubber and plastic parts of engines and reduce yield of butanol. The major feedstock used for the production of biobutanol comprises of corn, sugarcane and molasses which are not only used as fuel source but also as food source so high demand of fuel will result in food security issues (Sauer 2016; Zhang et al. 2016). Abundant and renewable lignocellulosic waste is now procuring great attention by the scientific society for biobutanol production. But the composition and difficult to degrade nature of lignocellulose pose demand for pre-treatment (Cao et al. 2016). Many studies have been conducted for butanol production by using different fermentation types, microorganisms and feedstocks. *C. saccharoperbutylacetonicum N1–4* employed for separate hydrolysis and fermentation of sago starch resulted in production concentration of 10 g/L. *C. beijerinckii* in simultaneous saccharification and separate hydrolysis and fermentation of wheat straw gave butanol concentration of 7.4 and 8.09 g/L, respectively (Alalayah et al. 2009). To enhance the yield many genomic and metabolomic based studies and integrated fermentation techniques

have been implied which will be discussed in upcoming sections (Lillington et al. 2020).

Biopropanol is not as good as an option to be used as biofuel as its energy density is lower than butanol and biodiesel and is not significantly higher than ethanol. Although its octane number is high and is less corrosive, pure propanol as biofuel is not suitable but can contribute a significant fraction in biofuel mixtures obtained from isopropanol–butanol and ethanol fermentation or algal protein fermentation (Choi et al. 2012; Dusséaux et al. 2013; George et al. 1983; Huo et al. 2011; Lee et al. 2012).

9.2.2 Biogas

Biogas is mixture of gases produced by anaerobic digestion of biomass especially organic waste. It is composed of mainly carbon dioxide and methane and some trace gasses. Complex microbiomes act on organic waste to convert it to final product (Nasir et al. 2012). Usage of food waste was found to enhance stability, efficiency, and economy of process by improving the methane production but as digestion of food waste alone, for biogas production is an unstable process, it is not so suitable for the purpose (Kuo and Dow 2017). Many researches have proved usage of mixture of feedstocks to be highly beneficial for enhancement of biogas production, improved rates of degradation and capacity of digester. The positive effects of such co-digestion lie in balanced micro- and macronutrient availability for microbiomes, ideal moisture level and buffering capacity, reduction in concentration of toxic or inhibitory compounds and improved process kinetics (Zamanzadeh et al. 2017). Past research on food and manure waste co-digestion showed enhancement in hydrolysis rates in bio-methane potential assays. This cooperative effect was due to high nutritive balance, dilution of inhibitors and toxic compounds, availability of trace metals for formate and syntrophic acetate oxidation (Ebner et al. 2016). Zamanzadeh et al. in their co-digestion study investigated the effect of mesophilic and thermophilic temperatures on microbial ecology. At mesophilic temperature in digestors, food waste and food waste with manure biogas production, bacteria *Chloroflexi* and *Firmicutes* dominated with 54% and 60%, respectively. In mesophilic reactors, Methanosaeta dominated in mesophilic digesters, whereas *Methanosaeta* and *Methanobacterium* had equal percentage of abundance in mesophilic co-digesters. Thermophilic digesters showed abundance of *Firmicutes* and *Synergistetes* in both food waste and food waste with manure using digesters. In concerns to archaea, *Methanothermobacter* dominated in both thermophilic digesters (Zamanzadeh et al. 2017). Fernandez et al. in their biogas production study by using algae as feedstock proposed that anaerobically digesting *Durvillaea Antarctica* and *Macrocystis pyrifera* and blend of these two in ratio of 1:1 in two phase system resulted in production of methane in biogas (Fernandez et al. 2008). Hence, they proposed production of methane by two phase system of digestion to be suitable with algal species.

Consolidated or combined bioprocessing of lignocellulosic substrate to produce hydrogen provides cost-effective and highly efficient process compared to one solely dedicated to cellulose production. Many studies used *Clostridium thermocellum*, a cellulolytic thermophilic bacterium, and *Caldicellulosiruptor saccharolyticus*, an extreme thermophilic and cellulolytic bacterium, for consolidated bioprocessing. A recent study investigated that cellulolytic and moderately thermophilic *Thermoanaerobacterium* genus alone is effective for both hydrogen generation and cellulose degradation. Their isolation results showed a novel strain of *Thermoanaerobacterium* genus, namely *Thermoanaerobacterium thermosaccharolyticum M18* capable of growing at 60 °C to have high potential for both high cellulose degrading activity and hydrogen production (Zhao et al. 2014).

9.2.3 Biodiesel

Biodiesel is a product of transesterification reaction of fats and alcohol obtained from biological sources. In this reaction esters of plants and animal fats are converted into mixtures of fatty acids esters by using alcohols. The leading sources of fats are plants seeds like rapeseed, soybean, palm, coconut, olive, jatropha, line seed, cotton seed, tung, safflower, sunflower, etc. The alcohols used are usually short chain alcohols especially those produced from biological sources like bioethanol and biomethanol. Although these sources are highly efficient but use of plant sources result in high cost of production, land area usage and food insecurity as majority of the abovementioned fats are used for human consumption (Zahan and Kano 2018). Algae has recently gained great importance for biodiesel production as it contains 20–80% of lipids which can be easily extracted and converted. It is economical and will also retain food security. The lipid composition of microalgae ranges from 30% to 40% but in some it may even reach to 80%. *Botryococcus braunii* contains around 40% lipids which can be easily extracted. Screening of *Spirulina maxima*, *Chlorella vulgaris*, *Scenedesmus obliquus*, *Dunaliella tertiolecta*, *Neochloris oleoabundans* and *Nannochloropsis sp.* was performed to evaluate best quantitative and qualitative source for biofuel production (Khan et al. 2017). Out of them *Nannochloropsis sp.* and *Neochloris oleoabundans* were reported to give high oil yield of 28.7% and 29%, respectively. Under nitrogen deficient conditions a significant increase in oil quantity was found, which made the above percentages to greater than 50%. In concerns to focus on biodiesel production *Scenedesmus obliquus* provides most suitable type of fatty acid composition consisting of linolenic acid and some other polyunsaturated ones. The others like *Neochloris oleoabundans*, *Dunaliella tertiolecta* and *Nannochloropsis* can be used for biodiesel production in combination with other algal and vegetable oils (Gouveia and Oliveira 2009). Comparative analysis between *Spirogyra* and *Oedogonium* for biodiesel production resulted in high biomass after extraction of oil in *Spirogyra* compared to *Oedogonium*, which also produced higher amount of sediments like water, pigments and glycerine. The oil content extracted was higher in *Oedogonium* than *Spirogyra*, hence *Oedogonium*

was found more suitable for the purpose (Sharif et al. 2008). Another advantage associated with algal biodiesel production is, large scale cultivation of algae for biodiesel production on waste water will help in bioremediation thus will promote eradication of toxic compounds from waste water. Though the major drawback with algal source is use of fossil energy during production and large water quantity, a unified renewable energy park concept for production of biofuel and synergistic energy with null carbon emission has been proposed (Subhadra and Edwards 2010). Fatty acid methyl esters produced by algae *Chlorella* and *Nannochloropsis* meet the standards published by EN14214 but because of higher unsaturation shows impaired stability (Kleinováa et al. 2012). Hence many studies have been done to promote and enhance the algal oil content and stability. Genomics and metabolomics have made great contribution for such enhancement which is discussed later in detail.

9.3 Global Biofuel Production and Usage

Global biofuel production over the years has increased at nearly a mild rate due to lack of sustainable production, standard process parameters and microorganisms, high cost of production and adoption by aviation and marine transport. Thus, there is a great need to develop technologies related to production and utilization of biofuels to reduce environmental pollution by fossil fuel use (Mataa et al. 2010). The global biofuel production was 59 million tonnes in 2015 which had grown to 81 million tonnes in 2017 and is expected to reach 284 million tonnes by 2030 according to International Energy Agency. Production of conventional biofuels mainly used for transport consisting of starch and sugar based ethanol, crop oil biodiesel and hydro-treated oils from vegetables grew up to 4% till 2017 with an average annual growth of production of about 2.5%, also expected in upcoming 5 years (Biofuels for transport (2017) Tracking clean energy progress, International Energy Agency. https://www.iea.org/tcep/transport/biofuels/. Accessed 24 Apr 2019). Non-OECD (Organization for Economic Co-operation and Development) Asian countries and Latin America are being expected to be the leading producers. Biofuel demand and production in brazil are expected to strengthen due to the new RenovaBio policy which may enhance investments in production capacity of biofuels (Oliveira and Coelho 2018). China aims to even out 10% of bioethanol blends worldwide in gasoline which will increase the production requirement by 6 times and have promoted investments in ethanol production (Dyk et al. 2016). Although the requirement of biofuels is somewhat decreasing in Europe and the USA due to development of high efficiency engines in vehicles, the increase in production is sustained (Araújo et al. 2017).

Biodiesel, the renewable hydro-treated vegetable oil and hydro-processed fatty acids and esters, is anticipated to increase in near future due to its potential to be used in unblended form without the requirement of engine modifications and supply infrastructure for fuel (Shereena and Thangaraj 2009). Europe, the USA and Singapore are primary producers of biodiesel from residue and waste feedstock. Although the production output is expected to grow but the biodiesel production

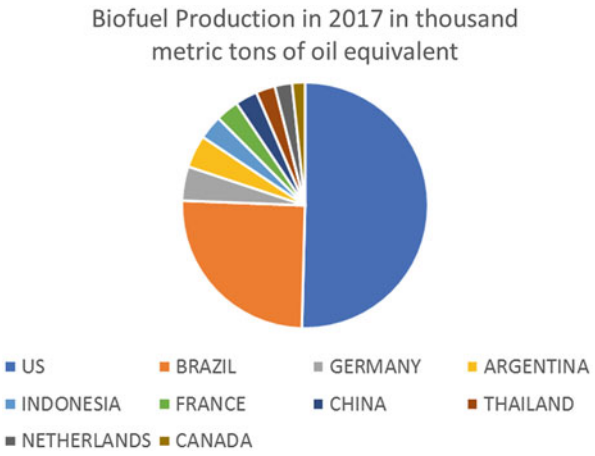

Fig. 9.1 Leading biofuel producing countries of 2017 in thousand metric tons of oil equivalent (Source: WBA Global Bioenergy Statistics 2017)

from wasted oil and fats from animal accounts for only 6–8% of total biofuel production (Biofuel Supply Chain 2011; Whitacre 2011). Thus, new techniques are required to enhance the production for both waste utilization and bioenergy production.

Biogas is produced from organic matter by anaerobically digesting it. It is composed of mainly methane CO_2 and some trace gasses. The feedstock required for its production contains sewage and manure, organic waste of household and residual waste from agriculture (Paritosh et al. 2017). This type of biofuel can work directly in transportation or be used after burning into energy and heat. The global production of biogas has enhanced swiftly from 2000 to 2014 with an average production growth of 11.2%. By applying an energy average density factor of 21.6 MJoule/Nm^3 World Bioenergy Association (WBA) evaluation stated that the biogas production of 2014 was 1.27EJ which increased to 1.31 EJ in 2016. The major contributing country for this increase was Europe which enhanced its production from 49.8% in 2014 to 54% in 2016 (WBA Global Bioenergy Statistics 2017). The country wise production profile of biofuels is shown as a pie chart in Fig. 9.1.

9.4 Omics Tools

Omics tools illustrate deep knowledge about all the cell's biological processes. From the core to the surface all the changes occurring in a living cell can be elaborated by the application of omics tools. The omics approaches lying behind these tools consist of genomics, transcriptomics, proteomics and metabolomics which investigate information lying in deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins and metabolites, respectively. Using single layer approach can limit the information, which can be effectively incurred by integrating the different approaches of omics (Chakravorty et al. 2018).

Along with this, the important information from resulting data obtained from these omics approaches is efficiently extracted, annotated and analysed by bioinformatics. Several tools can integrate, visualize and model omics data from different approaches. Mixed omics type data is integrated and analysed by applying clustering methods and support vector machine (SMV) based on Bayesian algorithm (Mirza et al. 2019). Integrated networks can be visualized by using software like Cytoscape. Homogeneous networks involving protein to protein and heterogenous networks including intraspecies protein to protein interactions and interspecies protein to DNA interactions are integrated by these tools based on approaches which are network-based and implicate graph theory (Schneider and Orchard 2011).

9.4.1 Genomics

Genomics is study of total genome along with various aspects of genetics. It deals with rDNA (recombinant DNA) technology, sequencing of genome and its bioinformatic analysis, to illustrate the functional and structural information connected to genome (Pareek et al. 2011; Verma et al. 2018). The difference between genomics and genetics lies in the fact that genetics consider single gene or its product at a time span, whereas genomics take into consideration the full genome of organism at one go (Ombrello et al. 2014). The various genetics features considered in genomics are interactions of alleles and loci present in the genome and other genetic interactions like pleiotropy, epistasis and heterosis (Luo et al. 2017). Fred Sanger and his group kept the foundation of genomics by inventing procedures and techniques for genome sequencing, mapping, storage of data and analysis by bioinformatic. These advancements initiated the Human Genome Project (HGP) in the 1990s and with the collaboration of different research groups around the world got accomplished in 2003 (Merrill and Mazza 2006). Today, techniques have been developed for high throughput sequencing like Next-Gen Sequencing (NGS), Whole Exome Sequencing (WES), Whole Genome Sequencing (WES), Real time PCR (RT-PCR) and Single Nucleotide Polymorphism (SNP), which are highly efficient. The sequencing data received from such sequencing techniques is arranged and analysed by bioinformatics tools to annotate structure and function of genome (Gasperskaja and Kučinskas 2017).

9.4.2 Transcriptomics

Transcriptomics reveals the information embedded in the DNA of an organism. It provides deep insight of coding mRNA and non-coding rRNA, tRNA, miRNA, siRNA, etc. These RNA pools vary in their abundance with varying conditions and requirements of cell and display the regions of DNA which are actively transcribed at an instance (Lowe et al. 2017). Thus, comparative analysis of transcriptome provides information regarding genes which are expressed differentially in different populations of cells or in same group of cells at different times or under different

experimental conditions (Wang et al. 2019). The standard analysis protocol for transcriptomics includes extraction of RNA, reverse transcription (RNA to cDNA), amplification of cDNA by PCR (quantitative reverse transcription polymerase chain reaction), microarray hybridization, then construction of library and RNA sequencing (Boone et al. 2018). But due to the drawbacks of high cost and low sequence coverage this procedure is not effective for full transcriptome characterization in eukaryotic (multicellular) organisms (Hrdlickova et al. 2016). Expressed sequence tag (EST)-based techniques and Serial Analysis of Gene Expression techniques (SAGE technique), which have been developed recently, can overcome the above limitations and allow the full transcriptome analysis in an efficient manner. EST are single strand sequence from either 5′ or 3′ end of complementary DNA used to recognize the expressed gene. These are short tags and do not consider full cDNA. SAGE generates tags of short sequences from 3′side of mRNA which are sequenced and estimated quantitatively to measure the transcript expression (Wang et al. 2009). NGS platforms developed so far provide immense increase in speed and amount of RNA sequence data generated which amplifies the abilities regarding identification of alternative splicing of sequence, aberrations in sequence and non-coding RNAs (Kukurba and Montgomery 2015).

9.4.3 Proteomics

Proteomics refers to analysis of whole protein pools of a species, system, organ or organism. This proteome or protein pool differs from one cell to another and it changes over the course of time (Zhang et al. 2013). In addition, the activity of protein, analysed by rate of reaction by protein in the concerned process, often gets modulated by expression of other proteins. Hence, proteomics investigate time and position at which protein is expressed, its production and degradation rates, abundance at steady state, protein modification, subcellular localization, protein related metabolic pathways and nature of interaction with other proteins (Mandad et al. 2018). All such analysis considered under proteomics has been benefitted by enhancement of databases of protein and DNA sequences, Tandem Mass Spectrophotometry (MS), Differential In-Gel technology (DIGE) and improvement of database searching algorithms (Mesri 2014). Leading databases which provide great support to analyst to compare their results with the existing ones and infer important knowledge are IntAct, UniProtKB, PRIDE and Reactome. Other structure analysis-based proteomics tools are Nuclear Magnetic Resonance (NMR) Spectroscopy and X-Ray Crystallography (Snyder et al. 2005).

9.4.4 Metabolomics

Metabolomics deals with wide range study of low weight (50–1500 Dalton) organic molecules of cell called metabolites present in biofluids, cells, tissues, organs or whole organisms (Riekeberg and Powers 2017). The composition of low weight

molecules and how they interact with the concerned living system comprise the metabolome. These small molecules are synthesized, absorbed and degraded inside and between the systems and along with environment (Khan et al. 2018a). More precisely, it is the study of metabolic products and substrates and the effect of genetic factors and environment on them. The constitution of metabolome displays the current conditions of organism and changes in its composition reflect the biochemical changes which are occurring during the course of time (Kosmides et al. 2013). There are two approaches employed in metabolomic analysis: targeted and untargeted. The approach focused determines the experiment design, sample preparation and the techniques used. The untargeted approach or global approach quantitates all possible metabolites from a wide sample range, on the contrary, the targeted approach focuses on a particular set of metabolites and their quantification and characterization. The latter is used to study drug metabolism and effect of modification in genes or therapeutics on enzymes. Various analytical techniques for both untargeted and targeted studies used are NMR and MS conjugated with other techniques of separation like chromatography (Commisso et al. 2013). In a recent study on microphytobenthic biofilms, an untargeted metabolomic techniques for analysis of non-polar and polar metabolites fraction have been proposed by utilizing GC-MS and LC-MS techniques (Gaubert-Boussarie et al. 2020). Multilayer omics or integration of different omics approaches encourages understanding of combined effect of biological activities or processes and reveals compiled information from molecules at different levels, thus is highly beneficial (Manzoni et al. 2016).

9.5 Contribution of Omics Tools in Microbiome Analysis for Bioenergy Production Enhancement

9.5.1 Genomics and Transcriptomics

Application of omics tools for analysis of microbiome can provide deep insight about the composition of microbiome at genomic, transcriptomic, proteomic and metabolomic levels (Aguiar-Pulido et al. 2016). Such huge amount of information by these tools can allow us to manipulate systems very easily at these levels for the enhancement of bioenergy source production (Fig. 9.2). Till date many studies have been done by applying omics tools analysis of microbiomes to achieve high yields. Results of a large number of studies have showed high potentials of these techniques and tools to work for the purpose (Henry et al. 2014). All four omics approaches consisting of transcriptomics, genomics, proteomics and metabolomics have contributed greatly to increase the biofuel or bioenergy production by providing huge informational support for making modifications at various levels especially genetic level (Radakovits et al. 2010; Jagadevan et al. 2018). Single level omics is not as effective and efficient as multi-level omics which compiles information from more than one omic approach and provide a complete extract of knowledge which can be used to understand and develop new or modified systems (Blum et al. 2018).

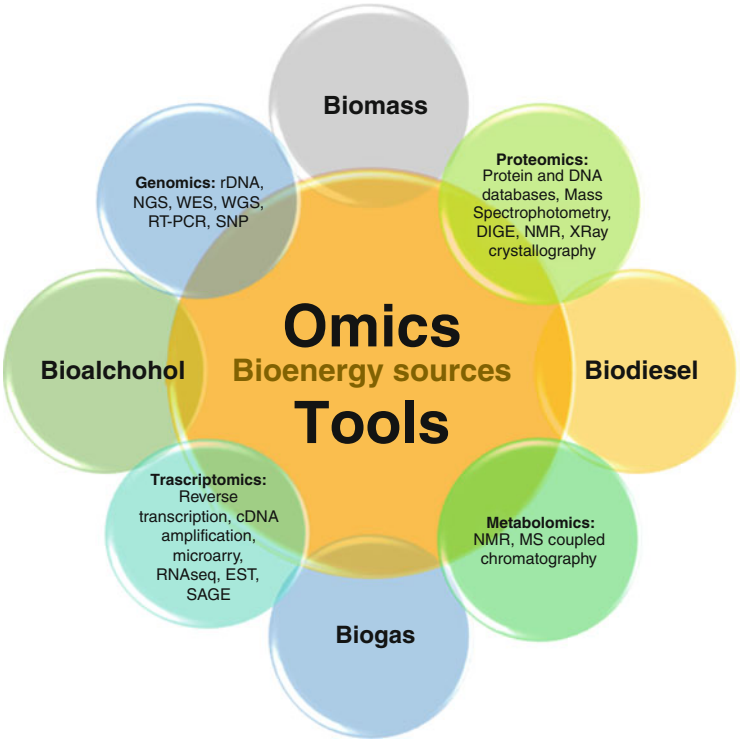

Fig. 9.2 Omics approaches and tools which can be applied to enhance production of different bioenergy sources

Bioethanol production is based on the catabolizing capability of yeast in relation to catabolism of 6-C molecule (glucose) into 2-C molecule (ethanol), without the production of carbon dioxide. *Saccharomyces cerevisiae* is a crab-tree positive yeast and has high ethanol accumulation capacity in aerobic conditions whereas *Candida albicans* leads to breakdown of sugars (glucose) into carbon dioxide under similar conditions and is crab-tree negative yeast. In crab-tree positive organism, removal of 6-C molecule shifts catabolism to ethanol oxidation into carbon dioxide, this is called diauxic shift (Zabed et al. 2014). This process of production of bioethanol through fermentative glucose metabolism and conversion of produced ethanol to carbon dioxide is affected by alcohol dehydrogenase enzyme (coded by locus ADH1). Alcohol dehydrogenase catalyses acetaldehyde reduction to ethanol as well as reverse of it but with low efficiency. *S. cerevisiae* possess two genes coding for ADH out of which ADH1 is expressed continuously but ADH2 gets induced by lower levels of intracellular glucose concentration and its substrate is ethanol. ADH2 gene expression is regulated by various factors affecting its transcription, genomic and transcriptomic analysis has disclosed the DNA binding regions and structure of these factors. Based on this knowledge synthetic biology approach has been

developed to modify ADH gene to enhance specificity for substrate and catalytic activity along with genome engineering of *S. cerevisiae* with coding region of protein to increase the tolerance to ethanol wide carbon source catalysis. Researches are in continuum to find out ADHs encoding new genes by employing metagenomics and many such variant genes have been identified in due course of time (Azhar et al. 2017).

Genomic analysis has revealed many genes capable of utilizing pentose sugar for ethanol production. Genetically engineered organisms that transform pentose sugars to ethanol have been derived but have not yet been utilized at commercial level. Application of genetically engineered organisms which can convert both cellulosic and pentose (xylose) sugar to bioethanol can increase the yield to great extent along with reduction in cost of production (Moysés et al. 2016). In 1995 Zang et al. in their metabolic engineering study prepared a genetic construct for assimilation of xylose in *Z. mobilis* to procure effective yield of ethanol. They widened the substrate range from hexose only to hexose plus pentose. The operons synthesizing enzymes of pentose phosphate pathway and for assimilation of xylose were developed and inserted into *Z. mobilis* to produce a strain capable of growing on xylose as well as glucose and efficiently producing ethanol. This strain was effective for usage with lignocellulosic biomass to produce bioethanol (Zhang et al. 1995). In another study recombinant *E. coli* strain was developed from genes of *Z. mobilis* for converting pyruvate molecule to bioethanol (Dien et al. 2004). Ho et al. transformed *S. cerevisiae* with a recombinant construct containing genes of xylulokinase and xylitol dehydrogenase and xylose reductase of *P. stipites* to produce strain capable of catabolizing both glucose and xylose to ethanol in co-fermentation (Ho et al. 1998; Jin et al. 2003). Extensive work had been done to develop recombinant efficient strains of *E. coli* for the production of ethanol which removed the dependence on alcohol dehydrogenase producing host. This recombinant strain was transformed by plasmid containing *pdc* and *adh B* gene obtained from *Z. mobilis* (Beall et al. 1991; Ingram and Conway 1988; Ingram et al. 1987). Ulaganathan et al. sequenced whole genome of *S. cerevisiae* NCIM3107 strain by Illumina MiSeq platform and analysed the strain for bioethanol yield and found moderate yield compared to other strains. The genome size obtained was of 11.8 Mb containing 5435 sequences of protein coding genes. Many variations in genetic makeup were found which can promote identification of highly potent genes for the purpose and hence they proposed that sequencing of other strains also can be of great benefit (Ulaganathan et al. 2015).

C. beijerinckii and *C. acetobutylicum* have been widely studied for production of ethanol, butanol and acetone in ratio 1:6:3 in acetone–butanol–ethanol fermentation process. But the production of butanol is not economically suitable in comparison to ethanol production from yeast, as acetone produced cannot be utilized, because of its corrosive nature, as fuel. Also, acetone production along with butanol reduces the yield of butanol. Hence, much efforts have been made to eliminate or reduce the production of acetone by blocking genes of pathways responsible for its production. But a limitation associated is that, with reduction of acetone, gene blocking also reduces the butanol yield due to butyrate and acetate (volatile acids) accumulation. An alternative approach of converting acetone to isopropanol, which has high energy

density, widely used as fuel, chemical and solvent, has been taken into consideration by introducing genes responsible for isopropanol synthesis. The gene introduced was responsible for synthesis of alcohol dehydrogenase having dependence on NADPH. It shifted ABE fermentation in *Clostridium* to IBE (isopropanol–butanol–ethanol) fermentation and converted acetone to isopropanol (Millat and Winzer 2017; Wang et al. 2018; Kolesinska et al. 2019).

16 sRNA and phylogenetic analysis was done to identify a new *Clostridium strain NJP7* which can degrade hemicellulose to produce isopropanol and butanol by acetone, isopropanol and butanol (AIB) pathway. They found this strain to produce butanol directly from hemicellulose in consolidated bioprocessing. The presence of *sADH* gene responsible for acetone to isopropanol conversion was also identified and efforts were made to enhance the production of butanol and isopropanol by using reducing agents and factors, exogenous acids and biodiesel-based extraction in situ. Also, characterization of xylanases, the hemicellulose degrading enzymes was done to moreover enhance the butanol production (Xin et al. 2017).

In a meta-analysis study of fermentative bacteria with capability to convert lignocellulose to biofuels like hydrogen and ethanol researchers did comparative analysis of genes responsible for pyruvate metabolism and involved in pathways of end product synthesis. They also investigated corresponding end products produced to get major biomarkers for accessing microbe potential of production of ethanol and hydrogen. They considered organisms whose genomic sequences are already known, members of *Euryarchaeota*, *Firmicutes* and *Thermotoga* species. In their analysis of whole genome by using bioinformatics tools they concluded that absence of acetaldehyde dehydrogenase and bifunctional enzyme alcohol or acetaldehyde dehydrogenase genes, is closely associated with high hydrogen, but low ethanol yields in *Pyrococcus*, *Thermococcus*, *Thermotoga* and *Caldicellulosiruptor*. *Closterium* species, *Copelatus subterraneus*, subspecies *tengcongensis* and *Ethanoligenens harbinense* contain genes both for hydrogen and ethanol synthesis and had uniform and mixed patterns of product formation. *Bacillus* and *Geobacillus* species in hydrogenase genes absence, showed high lactate yield instead of high yield of ethanol. Presence of genes of proteins which enhance production of NADH in *Thermoanaerobacter pseudethanolicus* produced low hydrogen and high ethanol yield. All these results can provide significant support to develop engineered strains with desirable product forming capabilities (Carere et al. 2012).

Varied sources can provide potential microbiomes for biofuel development. Bacterial community residing in paunch of hindgut of a woof eating protozoa of *Nasutitermes* species was subjected to metagenomic analysis and it was revealed that the bacterial microbiome contained many sets of genes for the hydrolysis of xylan and cellulose, and this can be potentially employed for biofuel production. Especially *fibrobacter* and *spirochete* were found to possess enzymes for degrading lignocellulose. Thus, metagenomic analysis has great potential to increase the source organism for various biofuel production (Warnecke et al. 2007).

Microbiome of ruminant animal's rumen supports the plant fibre digestion. Metagenomic analysis of microbiome of camel rumen provided many species actively responsible for degradation of lignocellulose and fermentation of volatile

fatty acids (having short chain). Gene density of glycoside hydrolases was very high compared to other ruminant animals. Presence of significant levels of sequences coding for dockerins, scaffoldins and cohesins proved high potential of lignocellulose degradation mediated by cellulosome. Evaluation of metagenome assembled leads to formation of 65 bins of genomes showing presence of highly diverse enzyme range for degradation of lignocellulose. *Bacteroidetes* associated species displayed high gene proportion of genes responsible for enzymes taking part in oligosaccharide degradation and debranching. Loci for polysaccharide utilization were also observed in genomes of *Bacteroidetes* showing high specificity for substrates and degradation of carbohydrates. Species of *Fibrobacteres* and *Firmicutes* showed high hemicellulase and cellulase genes, thus proving themselves the key player in degradation of lignocellulose. Volatile fatty acid pathway analysis demonstrated presence of genes essential for acetate synthesis in many species excluding *Euryarchaeota* and *Elusimicrobia*. Propionate production by succinate pathway became evident in species of phyla *Firmicutes*, *Bacteroidetes*, *Fibrobacteres* and *Bacteroidetes*. Butyrate via butyryl CoA and acetate CoA transferase pathway was generated by *Lentisphaerae* and *Bacteroides* species but in *Firmicutes* species it was generated by pathway of butyrate kinase. Above findings by using metagenomics showed camel rumen microbiome as a highly potential source of efficient microbes which can be employed for biofuel production (Gharechahi and Salekdeh 2018).

Biogas production is anaerobic digestion of organic waste by complex microbiome, where different species play different roles and form a collective organization. Interpretation of microbial species involved in the process reveals the network of interaction and the application of this knowledge to select the beneficial ones. Dissection of microbiome taking part in anaerobic process of biogas production was performed by a research group using high output sequencing by Illumina platform which displayed a million genes and extracted the sequence of 106 genomes of microbes. This was made possible by applying a novel strategy which combines two binning protocols. Phylogenetic and taxonomic predictions by using more than 400 proteins put forward that this microbial community contains many new species. They also identified by performing functional analysis the important genomes involved in significant metabolic pathways like degradation of fatty acids, utilization of carbohydrates, fermentation of amino acids and oxidation of acetate. In addition, this analysis leads to identification of new archaeon which possesses the methylotrophic type of methanogenic pathway. Such extensive study provided wide knowledge about genomes actively taking part in biogas production and will greatly support to develop further processes for biogas production (Campanaro et al. 2016).

Microbiomes often contain many unwanted species which may create hindrance in production process of biogas. Development of management systems for microbial species can improve the situation by focussing on specific species of microbes which actively participate in production process. A biogas metagenomic hybrid assembly database (BioMETHA) is a sequence database from the biogas microbiomes which was prepared by applying assembling strategy to metagenomic datasets from

different sources. In this database, long sequence reads analysed by nanopore sequencing and short sequence reads from second gen-sequencing were combined. The database contains 231 bins of genomes which represent different taxonomic units. 13,190 genes which were nonredundant taking into consideration 207 k coding reads were identified by annotation based on function. The mapping of metagenomic DNA from different sources of biogas production enhanced to 73%. Along with this database a collection of reference sequences of enzyme commission was made which contained genes having importance in biogas production processes. The entries of enzyme commission in this collection were 235 arranged in 52 modules of metabolism. Meta-transcriptomic data of enzyme commission reference sequence was also mapped, and the coverage was 93%. All this data collection and organization can act as reference to study various parameters and problems which may encounter in production of biogas. Also, it will provide information about specific microbial species and sequences important for biogas plant development and a reference management system based on functional annotation (Grohmann et al. 2018). Many such genetic resources have been developed for production of biofuels which can be of great support to optimize the production process to get the best yield results.

Biofuel production by microalgae can be an efficient alternative to fulfil the increasing energy demand but with microalgae biofuel, yield to production cost ratio is quite low as compared to petroleum. Hence, there is a great need to enhance the lipid production in microalgae which will improve biofuel yield. Advancement in genomics and metabolomics has provided opportunity to investigate the biofuel producing genes and metabolic pathways which can be focused to develop genetically modified or engineered algal strains to get high lipid production. Knowledge of large number of genome sequences of microalgae further provides wide opportunities to explore lipid metabolism pathways and genes of concerns for development of genetically engineered algal strains for biofuel production by applying omics tools. As the genome sequencing of all the microalgae was not done in context to lipid metabolism so the specific information in this concern is lacking (Radakovits et al. 2010; Khan et al. 2018b). Recently, transcriptome analysis of some microalgal (oleaginous) species which were unsequenced yet has provided better understanding of lipid metabolism. This proved transcriptomics an effective approach for quantification of mRNAs in cell colony or in isolation. It is a more exhaustive approach to search for the differentially expressed genes in different environments and can be used to look for potential genic targets which can be subjected to engineering to increase the lipid production in microalgae (Rismani-Yazdi et al. 2012). First transcriptomics analysis study on *D. tertiolecta* disclosed a sequence of enzymes responsible for taking forward the pathways of synthesis and catabolism of TAG (triacylglycerol), fatty acids and starch. Further analysis showed that the metabolic pathways that were reconstructed in this microalgae strain were having similarity with that of *C. reinhardtii* and of plants. It also demonstrated genetic inherent ability of connecting metabolism of starch with fermentation of ethanol through glycolysis. Similar transcriptomic approach was also employed to reveal the TAG synthesis enzymes in *C. variabilis* strain *UTEX395* in conditions

with complete presence of nitrogen and absence of nitrogen. Transcriptomic data of *C. reinhardtii* under different stress conditions is present which provide great support for the same. Whole genome expression profile analysis for the exploration of mechanisms involved in TAG accumulation induction in *C. reinhardtii* was performed and identification of 3 vital genes of acetyltransferase (*DGATT1*, *DGAT1* and *PDAT1*) actively taking part in accumulation of TAG was done. Nitrogen deprivation in cells of *Chlamydomonas* showed metabolism switching from conversion of acetate to glucose, to a direct conversion of acetate to fatty acids with means of downregulating gluconeogenesis and activity of glyoxylate cycle. Similarly, the activity of *PDAT* and *NRR1* genes involvement in accumulation of TAG and assimilation of nitrogen in *C. reinhardtii* was assured. Later, it also became apparent by omics analysis that profile of fatty acid modified for increase in oleic acid and stearic acid is vital for enhancing the properties of biofuel produced from algae (Misra et al. 2013).

Next-gen pyrosequencing and transcriptomic assemblage of *D. tertiolecta* produced 1,363,336 reads of high quality with length average of 400 bases. These were trimmed based on size and quality and nearly 45% reads were collected into isotigs (33,307) with coverage of 31-fold and singletons (376,482). Blast analysis of resulting sequences and their annotation by using Kyoto Encyclopedia of Genes and Genomes orthology identifiers and gene ontology identifiers gave information regarding many starch and lipid biosynthesis pathways and catabolism pathways in *D. tertiolecta* (Rismani-Yazdi et al. 2011).

9.5.2 Proteomics

Proteomics studies have played an eminent role in understanding the biological pathways related to bioenergy production. Both crops and microorganisms have been analysed by proteomics tools to identify and characterize important protein expressions in varying biological systems. Abundance of protein, interaction between proteins, subcellular location, turnover of protein can be analysed by proteomics tools for dynamic understanding of biological pathways. Leading enzymes used for bioenergy production are alpha-amylase, cellulase, protease, lipase and glucoamylase. For biodiesel production lipase is an important enzyme, whereas for cellulosic fuel production enzymes like exoglucanase, endoglucanase and beta-glucosidase are required. Lignin breakdown needs enzymes like manganese peroxidase, laccase and lignin peroxidase. Microorganisms like *Aspergillus niger*, *Penicillium restrictum*, *Trichoderma sp.*, *Phanerochaete chrysosporium*, *Clostridium thermocellum*, *Erwinia chrysanthemi*, etc. are good source of the abovementioned enzymes for lignocellulose and lipid conversion to biofuels (Dashtban et al. 2009). Studies on improvement of these strains have been conducted by genetic engineering and identification of new species has benefited the biofuel industries. *C. acetobutylicum* is a native source organism for butanol production. Proteomic tools were employed to produce a thorough reference map of proteome of this species. For improved understanding of relation between butanol tolerance and

its yield a comparative proteomic analysis of *wild type* (*DSM1731*) and a *mutant* (*Rh8*) was done and 102 proteins which were differentially expressed were identified (Mao et al. 2010). Proteomic analysis of microalgal species like *SynechocystisPCC6803* and *C. reinhardtii* provided information of proteins present in subcellular locations and under conditions of stress. Microalgal analysis was done by using SDS PAGE after protein extraction using organic solvent and purification by ethanol phenol method, chloroform phenol method desalting, etc. (Anand et al. 2017). Proteomic analysis for analysing hexane resistance (used for extraction of lipids from microalgae) in *Synechocystis* microalgal species was done to prove it an efficient lipid producing organism for biodiesel production. Isobaric tags for absolute and relative quantification or iTRAQ were used to analyse overall metabolic response to a treatment of 0.8% hexane. 50% of growth inhibition in cells resulted from analysis, suggested hexane resistance which is a required trait for biofuel production from algae. 164 proteins were found to be upregulated and 77 were noted to be downregulated by keeping a cut-off expression change of 1.5-fold. Functional annotation of these deferentially expressed proteins by comparing with databases proved their role as transporters, membrane proteins, sulphur relay proteins and some proteins responsible for protection against hexane. These protecting proteins can be of great importance for effective biofuel production and can be employed to produce engineered species (Liu et al. 2012).

Proteomics study for n-butanol toxicity analysis in engineered *E. coli* strain was done by using iTRAQ labelling technique, HPLC and ESI-Q-TOF. This analysis revealed that the stress due to n-butanol has same components as other types of stresses convey including respiratory function imbalance, heat shock along with stress on cell envelope, oxidative stress and metabolic biosynthesis and transport related problems. Some n-butanol toxicity associated genes were revealed like *nlpD*, *degP* and *phoU* and they were found to be upregulated. Analysis of effect of carbon storage regulators and biofuel flux increase by proteomics employed 5600 triple time of flight mass spectrophotometer. Protein associated with energy metabolism especially citric acid cycle was found to be increased accompanied with upregulation of 20 proteins which were new in this regard (Rutherford 2011).

C. acetobutylicum has ability to produce high value biofuel compounds (acetone, ethanol, butanol and hydrogen) by degrading lignocellulose but presence of lignin provides inhibition in the production process and thus pre-treatment and process optimization become an essentiality. Analysis was done to evaluate the effect of lignin on cell growth of *C. acetobutylicum*. This was done by providing two types of growth medium: one containing only cellobiose and the other contained both lignin and cellobiose. Proteomic techniques like 8plex iTRAQ based quantification of proteins, gas chromatography and microscopy were used to study lignin effect on proteome, process of fermentation and morphology of cell, respectively. Reduced biofuel production was revealed in presence of lignin and accumulation of butyric and acetic acid was made evident. Proteome analysis resulted in identification of 583 proteins, out of which quantification of 328 proteins denoted presence of at least two unique peptides. Comparative studies for up- and downregulation of proteins showed pathways like glycolysis and fermentation were found to be downregulated.

DNA repair, GTP/ATP based activities, translation or transcription were also affected with the lignin presence and altered the morphology of cell. All such proteomic analysis will help in developing targeted strategies of metabolic engineering for enhanced biofuel production and hence will overcome energy crisis (Raut et al. 2016).

9.5.3 Metabolomics

Metabolomics constitute an important place in systems biology. Complete analysis of metabolites in a cell and monitoring their interactions and outcomes during developmental stages and different stress conditions provide a precise picture of the cell's physiological state. Advancement in recent technologies has provided developments in post-genomic analysis and hence in metabolomics. Development of different chromatography analysis, mass spectrophotometry and tools for computational analysis has provided great scope for accurate quantification of intracellular composition of metabolites and their fluxes. Metabolomic investigations can lead to pathway optimization through engineering of the pathways having low efficiency for the purpose. Metabolism of carbon is an essential step to be characterized and modelled by metabolomics to provide accurate picture of energy, redox and carbon sources. Data from metabolomics can be used to make models of flux of carbon through the study of metabolic networks which will help in developing metabolic engineering strategies for new and potent strains (Tang 2011; Martien and Amador-Noguez 2017).

Models of metabolism of species like *Thermus thermophilus*, *Geobacillus sp*. and *C. acetobutylicum* were made by $_{13}$C metabolic flux analysis (MFA) method which is based on isotopic labelling of data at steady state. These models verified the gene annotations, previous characterizations of noncanonical pathways and major sinks and sources of reduced cofactors and energy (Cordova et al. 2017; Au et al. 2014). $_{13}$C MFA method was employed for quantification of impact of enhanced production of fatty acids on the *E. coli* pathways of central metabolism and it was revealed that citric acid cycle and pentose phosphate pathways were nodes of high flexibility. The flexibility in flux across these pathways was ensured by carbon accommodation and demand of redox from overproduction of fatty acids. Though Entner–Doudoroff (ED) glycolysis was found to be rigid as flux through it did not changed despite of increased acetylCoA and NADPH supply for synthesis of fatty acids. Thus, upregulation of ED can be a potent strategy for enhancing the production of fatty acids by *E. coli* (He et al. 2014).

Reduced fuel production requires appropriate reduced cofactor (NAD(P)H) supply. Quantity of these cofactors can be estimated by combining catabolic and synthesis pathways activities in both secondary and primary metabolism. Metabolic analysis at system level provides information regarding regulation of redox. Also, metabolic regulation of cell responses for enhanced NAD(P)H demand has been done (Metallo and Heiden 2013).

Metabolite quantification at system level was employed to investigate the mechanism through which phosphoglucomutase and transaldolase are expressed constitutively in upper glycolysis and pentose phosphate pathway, respectively. Such expression in *Fusarium oxysporum* results in high production of ethanol and also improves regeneration of NADPH causing carbon flux shift toward production of ethanol and away from acetate. $_{13}$C MFA showed flux via oxidative pentose phosphate pathway and turnover of NADH into NADPH by enzyme transhydrogenase enhanced to provide support for NADPH requirement for overproduction of fatty acid (Anasontzis et al. 2014). Another analysis proved that while mevalonate (requires low reducing species than fatty acid) overproduction improved transhydrogenase activity sufficiently which supported NADPH requirement but the flux through pentose phosphate pathway did not increase. All together these analyses disclosed the improvement of production of biofuel by opting for manipulation of pentose phosphate pathway activity and reactions of transhydrogenase (Hollinshead et al. 2014).

Metabolomics also have potential of identification of competing pathways which results in process inefficiencies. $_{13}$C isotope labelling in steady state was performed to investigate competing reactions for the production of 1–4 butanediol in *E. coli* engineered strain. Results showed that succinate semialdehyde (first intermediate) was getting side-tracked to succinate and being used in citric acid cycle. This knowledge encouraged analysis by transcriptomics and resulted in two succinate semialdehyde dehydrogenase identification which were being highly expressed, and their knockdown brought up significant enhancement in butane-di-ol yield (Martin et al. 2015).

Commercial biofuel production requires few microbial processes from different microorganisms present in mixed culture microbiomes. Many microbes present in such microbiomes inhibit the production process or may have no worth in this concern and must be simply consuming the substrate. Metabolic engineering of microbes can integrate or consolidate all these processes in single step in a specific microbe which will increase the efficiency of process to a great extent. In turn to increase the biofuel yield metabolic engineering mainly focuses on directing metabolic fluxes towards maximum yield, enabling employment of low-cost feedstock for biofuel production and increasing tolerance to stress especially by inhibitors (Chen et al. 2020). Also, by using the metabolomic analysis of microbiomes as reference many advancements for biofuel yield increment can be made by focussing specific pathways for specific type of biofuel (Carere et al. 2008). Metabolically engineered yeast capable of displaying hydrolytic enzymes on its surface have showed high potential for commercial bioethanol production from lignocellulosic waste, starch and polysaccharides (Shigechi et al. 2004; Busci et al. 2018). *Z. mobilis* bacterium engineered metabolically for production of bioethanol from pentose sugar is already in commercial use (Yang et al. 2016). Bacterial strains like *Bacillus subtilis* and *E. coli* have been used extensively for development of strains for ethanol production because of high familiarity to their molecular biology and have wide range of substrate utilization. *E. coli* was modified by introduction of foreign genes as well as disruption of competing product pathways. It was transformed to a new

strain called *E. coli KO11* for higher production of ethanol. Addition of *Z. mobilis* genes of alcohol dehydrogenase and pyruvate decarboxylase and deletion of fumarate reductase resulted in the production of this strain. This strain was adapted for a production of 95% yield in complex medium and had high tolerance to ethanol compared to original organism. A limitation associated with this strain was it was not able to multiply at 3.5% concentration of ethanol and needed complex supplements, thus enhancing the cost of production (Hasona et al. 2002).

Butanol production by *C. acetobutylicum* has two phases: first is acidogenesis and the other is solventogenesis. In first phase, it grows and forms butyric and acetic acid from substrate and due to these acids, the pH decreases below 5. In second phase, the produced organic acid gets converted to butanol, acetone and ethanol in ratio of 6:3:1. The by-products ethanol and acetone are unwanted and are inevitable. Metabolic engineering of the concerned strain by using plasmid capable of getting integrated with host genome has been done. It resulted in inactivation of genes of unwanted products. Along with this, efforts to reduce the toxicity of n-butanol to the host have been made through metabolic engineering and approaches of mutagenesis (Millat and Winzer 2017).

Dark fermentation has high biohydrogen production potential compared to photofermentation. Many species like *Enterobacter aerogenes*, *Clostridium butyricum* and *E. coli* are capable to perform dark fermentation but due to presence of some repressors and negative regulator their yield efficiency is less. Genetically engineered *E. coli* having inactivated, repressor (*hycA*) of formate hydrogen lyase (*FHL*) and regulator (negative) was made by metabolic engineering. The converted strain showed high hydrogen production (about 300 litre hydrogen gas per litre per hour) by using formate as substrate. Further modification of organism was done by disrupting genes of fumarate dehydrogenase and lactate dehydrogenase for the improvement of hydrogen yield (Vardar-Schara et al. 2008).

Metabolic engineering for production of higher alcohols like propanol, butanol, isobutanol, etc. has made great contribution in increment of biofuel yield. *C. acetobutylicum ATCC824* was engineered to produce high butanol titre by knocking down four genes (*ack*, *pta*, *buk* and *ptb*) responsible for small chain fatty acids production. A mutant having double knockout of *pta* and *buk* genes produced maximum titre of 16 g/lit. Further modification of *adhE1* gene overexpression which consumes NADPH cofactor made the results much better as to 18.9 g/lit in batch reactor (Dusséaux et al. 2013).

Considering the abovementioned and many other contributions of different omics approaches in enhancement of biofuel or bioenergy production, it becomes evident to conclude that omics tools play a very significant role for the purpose and can be of great importance in meeting the increasing energy demand around the globe.

9.6 Conclusion

Alarmingly increasing global energy requirement put forward an urgent need to look for renewable, eco-friendly sources of energy. Bioenergy via biofuels is a great solution to the exponentially growing scarcity and environmental damage related to fossil fuels. Microorganisms play a very important role in production of biofuels. Selection of effective and efficient microbiomes can provide an exceptionally high yield of biofuels. For significant selection and development of suitable microorganism it is required to have an overall knowledge of all the process parameters from genomic level to metabolomic level. Omics tools are best suitable for providing detailed information about the pathways, substrates and the microbes responsible for production of biofuels. Genomics, transcriptomics, proteomics and metabolomics together perform highly exhaustive analysis and give out large amount of data regarding the purpose. Application of such analysis as reference for finding the optimized microbial consortium for the purpose of developing effective strains by genetic and metabolic engineering has shown outstanding results in till date researches. Many have been commercialized and many are on the way. Hence omics tools can be of great benefit in coming future for analysis and development of microbiomes for enhanced production of bioenergy.

9.7 Prospects

Exhaustive works by using omics approaches have been done for analysing microbiomes for enhanced bioenergy production. A large increment in production yield has been observed at laboratory level but only a few have passed the commercial tests. But the high improvements at laboratory levels provide incredible support to the fact that after further analysis and refinements many new strains can be commercialized in coming future and can fulfil the ever-increasing need of energy by giving high biofuel production. Model organisms like *E. coli*, *Z. mobilis*, *Chlamydomonas*, etc. are highly studied species about which almost whole information from DNA to RNA to protein to metabolites is present and thus they can be modified and engineered very easily taking reference from omics analysis. A lot number of studies and high production improvements by omics tools prove them a strong sole contender in coming future for development of biofuel technology. This chapter is a mild effort to disclose the power of highly potent omics tools and encourage their application in further research for enhancement of biofuel production profile around the globe.

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Omics (Genomics, Proteomics, Metabolomics, Etc.) Tools to Study the Environmental Microbiome and Bioremediation

10

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Abstract

The term microbiome refers to the collection of microorganisms or their hereditary material from a specific biological system. Microbiomes are widespread and inescapable and contemporary in the soil, water, air, and in/on other living beings. Changes in the microbiome can conflict the wellbeing of the natural specialty where they occupy. In order to collect more comprehensive knowledge about these microbial communities, various measures have been pursued. Exploration of natural microbiome can be of a specific interest for revelation of novel creatures or novel gene or new microbial metabolites. Technologies that explore

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the roles, relationships, or characteristics of biomolecules of cells, such as DNA, RNA, proteins, or small metabolites, named by conjoin suffix—"omics", as in genomics, transcriptomics, proteomics, or metabolomics, respectively. Genomics and transcriptomics research has elevated because of advances in microarray technology. Fast advancement in "omics"—metagenomics, metatranscriptomics, metaproteomics, metabolomics leading to a greater understanding of the patterns, processes, and mechanisms governing the structure and dynamics of microbiomes. Albeit each "omics" technology gives a valuable data independently yet when they use in consolidated structure, they delineate an increasingly extensive picture. The omics technologies have critical commitment to comprehend ecological bioremediation mechanisms. Omics advancements help in discovering of genes involved in biodegradation, to find out the functions of missing genes and to explore the metabolic pathways of bioremediation. Genomics has been used to study pure cultures with regard to bioremediation. Proteomics-based examinations have been helpful in elaborating changes in the structure and function of proteins as well as in the identification of key proteins associated with the physiological reaction of microorganisms when presented to anthropogenic contaminations. Transcriptomic or metatranscriptomics apparatuses are utilized to increase utilitarian bits of knowledge into the exercises of ecological microbial networks by considering their mRNA transcriptional profiles.

Keywords

Genomics · Proteomics · Metabolomics · Metagenomics · Metatranscriptomics · Bioremediation

10.1 Introduction

Technologies that measure some properties of enormous group of cell biomolecules, for example, genes, proteins, or cellular metabolites, have been named by adding the suffix—"omics", as in "genomics". Omics refers to the technologies which used to investigate the roles, connections, and activities of the different kinds of particles that make up the cells of a living being (Lay et al. 2006). These technologies include: Genomics, the study of genes and their function (Pandey and Mann 2000); Proteomics, the study of proteins (Pandey and Mann 2000); Metabolomics involves the investigation of cell metabolites associated with cell digestion (Rubakhin et al. 2011); Transcriptomics, the analysis of the mRNA profiles (Wang et al. 2009); Glycomics, the investigation of cell sugars (Horlacher and Seeberger 2006); Lipomics, lipids profiles analysis (German et al. 2007).

Omics technology gives the tools expected to take a gander at the distinctions in DNA, RNA, proteins, and other cell particles among species and individuals of a species. Only 1% microorganisms is cultivable in lab condition, remaining cannot be isolated on agar plate. Multi-omics technologies play the important role in identification of these unknown (uncultivated) microbes and also give the information of

their metabolic potential as well as activity of complex microbial communities (Gutleben et al. 2018). These kinds of molecular profiles can shift with cell or tissue introduction to synthetic concoctions or medications and in this way have potential use in toxicological evaluations. Omics investigations can frequently be directed in high-throughput measures that produce huge measures of information on the useful as well as auxiliary adjustments inside the cell. "These new techniques have just encouraged huge advances in our comprehension of the molecular reactions to cell and tissue harm, and of annoyances in useful cell frameworks" (Aardema and MacGregor 2003). The omics technologies will keep on adding to our comprehension of natural bioremediation mechanisms. Regulators are keen on these new innovations, however, are as yet dealing with how to fuse the new data and advances in regulatory decision-making.

Microorganisms have the crucial role in nutrient cycles, interact with every living being, and structure bedrock of maintainable environments. For execution of bioremediation methodologies it is fundamental that we see how the earth (oxygen, water, nutrients, temperature, and pH) balances microbial exercises. Microbial exercises like oxidation, reduction, adsorption, physical binding, immobilization, vitalization, or transformation are major microbial activities involved in bioremediation (Plewniak et al. 2018). Just couple of proteins by their particular capacity are engaged with bioremediation. Nonetheless, there are numerous catalysts which by their particular genes are associated with cell metabolic capacities yet under pressure conditions instigated by anthropogens, for example, hydrocarbons, colours, fragrant, and xenobiotic mixes they perform alternate works in metabolic pathways engaged with biodegradation. The total genome sequencing has turned into an ordinary wonder and there is a huge augmentation in microbial genome databases. Thus, it is conceivable to estimate the contribution of genes associated with bioremediation. Transcriptome and proteome profiles are essential tools to find out the genes associated with bioremediation. Cellular expression of proteins and metabolites changes with the environmental condition. These differentially expressed biomolecules give the missing connections in the degradation pathways. Park et al. (2019) reported the genomic and transcriptomic basis of PAH bioremediation by a potent fungi *Dentipellis* sp. KUC8613. Inventive achievements in advancements of sequencing, fingerprinting methods, microarray, and mass spectrometry alongside bioinformatics instruments have driven a change in outlook in describing microbial exercises at sub-atomic level. Simultaneously the utilization of molecular techniques has prompted acknowledgment that microbial decent variety is a few folds higher than at any other time foreseen. Any one specific microorganism is unequipped for handling all the metabolic responses to degrade recalcitrant compounds; however, a group of organisms collectively process all the reactions for bioremediation. Developing fields like metagenomics, metatranscriptomics, metaproteomics, and metabolomics have explained and are tackling the intricate biodegradation pathways.

This chapter describes the molecular profiles of microbial activities and current situation and future uses of omics technologies in bioremediation systems. A significant accomplishment in the field of microbial biology was the finding of the

16S rRNA gene and its quirk. The genes comprise highly conserved, universe, and stable sequences that are available in all microorganisms and are considered as a "gold standard" for describing phylogenetic affiliations of microbes. Ongoing advances in atomic methods, including high-throughput methodologies, for example, microarrays and metagenomics, have opened up new viewpoints and pointed towards new open doors in contamination reduction and environmental management. The present capability of microarrays and metagenomics is competent to examine the hereditary decent variety of ecologically applicable smaller scale living beings and recognize new utilitarian genes associated with the catabolism of xenobiotics (Eyers et al. 2004). The computational-based comment and similar genomic investigations of DNA sequences have given data in regard to gene function, genome structures, biological pathways, metabolic and regulatory systems, and advancement of microbial genomes, which has extraordinarily upgraded our comprehension of microbial metabolism (Schoolnik 2001; Ward and Fraser 2005; Sharan and Ideker 2006; Cardenas and Tiedje 2008; da Rocha 2008; Zhang et al. 2010).

10.2 Microbiome

A group of microorganisms, (for example, virus, bacteria, and fungi) in a specific environment is called microbiome. The microbiome facilitated by all people, different creatures, and plants are significant in wellbeing, sickness, and nourishment. Environments such as soils, seas, and structures have different microbiomes. We additionally use microbiomes in a wide scope of modern procedures, for example, biofuel and sustenance generation and water treatment. Microbiome can be intricate, and frequently contain particular sub-communities inside a bigger entirety. They are not static because of environmental variables (Kembel et al. 2012). The human microbiome involves all the small-scale life forms related with an individual; however, every parts of the body has its own particular microbial network—the microbiome of the skin is altogether different from that of the gut, which is distinctive again from that in the vagina. The group hereditary assorted variety of all the various microorganisms in a microbiome can be huge, and many may not be cultivable.

Researchers concentrating these minuscule environments examine the various microorganisms living in an environment (sometimes referred to as the microbiota), their consolidated group hereditary material in every creature's genome (also called a metagenome), the particular biological, chemical, and physical conditions in which they live and with which they collaborate, and how they change after some time. By examining the decent variety and capacity of microorganisms in their own surroundings and the factors that influence them, researchers aim to reveal the role they play in human, animal, and plant wellbeing, and in the environment. Microbiomes are an imperative part of the environmental biodiversity. Microbiomes are major to key geochemical forms—such as the nitrogen cycle, on which life on earth depends—so understanding the microbiome will be urgent to comprehension and meeting the

challenges of natural change (Yadav et al. 2017). Researchers are starting to explore how to oversee microbiomes for our benefit. Interdisciplinary approaches consolidating genomics, microbiology, and logical displaying have enabled the improvement of prescient models to recognize biomarkers and diagnostics that help us to manage microbiomes. However, with continued speculation and viable multidisciplinary work, microbiome research can possibly convey crucially significant advances in different zones fundamental to our general public and economy.

10.2.1 Human and Microbiome

Microbiome research is changing our impression of human science—where already we would have thought of this regarding how our genome cooperates with the earth, we presently should incorporate the metagenome of the microorganisms with which we have coevolved. There is expanding proof that gut microbiomes specifically are central to the wellbeing and sustenance of people and creatures. A large number of the basic metabolites in the human circulatory system on which our cells depend are made by microorganisms in the gut (Althani et al. 2016).

There is still much research to be done to comprehend the utilitarian connections between the microbiome and malady, yet this work is opening up an immense scope of chances in human wellbeing, for example, developing biomarkers from the oral, lung, or gut microbiome to distinguish early hazard factors and analyse illnesses, such as constant obstructive aspiratory infection and type 2 diabetes; using faecal microbiota transplants (FMTs) to rebalance the gut microbiome, a procedure officially demonstrated to be amazingly effective in handling *Clostridium difficile* diseases; developing probiotics and prebiotics to re-establish the parity of microbiomes and treat conditions, for example, urinary tract contaminations, diarrhoea, chronic liver illness and possibly even obesity and understanding how the gut microbiome impacts our ability to react to numerous medications (King 2014; Schloissnig et al. 2013).

10.2.2 Environment and Microbiome

Microbiomes in the soil and seas assume principal roles in various ecosystem processes which are basic for all types of life on Earth; for instance, cycling of nutrients (carbon and nitrogen cycle). Man-made environments like—structures and vehicles have their own microbiomes with significant impacts on disease infection and human wellbeing. Human activities and natural changes are affecting the diversity and function of microbiomes, with genuine ramifications (Spor et al. 2011). By better understanding the effects of ecological factors on microbiomes and their versatility to change, researchers may make significant contribution to re-establishing or overseeing microbiome functions. For instance, researchers are examining: How environmental change influences ecological microbiomes in soils, seas, and ice sheets and whether this will accelerate their contribution to ozone

depleting substance emanations; the role of the soil microbiome in transmitting pathogens and the spread of antimicrobial opposition; how the microbiomes of surfaces in medical clinic support pathogen transmission, with the end goal of improving disease control; and the potential exploitation of marine microbiomes for biotechnological application (Claus et al. 2016; McLellan et al. 2015; Cai et al. 2014).

10.2.3 Biotechnology and Microbiome

Microbiome research is additionally a chance to help build up the bioeconomy. For a considerable length of time, microbes have been tackled for a wide scope of industrial, pharmaceutical, and natural biotechnologies, including the generation of medicines, food, drink, and biofuels, water treatment and bioremediation of waste and contaminations (Pylro et al. 2014; Singh and Trivedi 2017). Researchers are currently utilizing new tools to better understand, monitor, and endeavour microbiomes to optimize existing processes to improve quality, efficiency, and safety and to develop completely new processes. For instance, UK and Irish analysts are examining how microbial communities could be utilized more successfully to change over waste into biofuel and bioenergy, and treat waste water.

Microbiomes and the related metagenomes possibly offer undiscovered assets for the disclosure of new product of potential industrial, agricultural, or medical use. Researchers and industry are bioprospecting microbiomes, including human and animal guts, soils and seas, and related databases of genomic and protein with the point of distinguishing novel microorganisms, substances, and catalysts of potential use for a wide assortment of applications. Research opportunities at present being explored include: Using microbial enzymes for industrial procedures, fine synthetic creation, and natural tidy up applications; developing bio-treatment facilities for the creation and recuperation of synthetic substances, pharmaceuticals, materials, biodegradable plastics, nutrients, and composts from practical sources; improving anaerobic assimilation forms effectively utilized for the treatment and reusing of water, sewage, and industrial waste, and progressively productive utilization of methane as a fuel; optimizing processes for production of biofuels; and identifying new genes for engineered science applications (Singh and Trivedi 2017; Treu et al. 2016; Spirito et al. 2014; Carney et al. 2014).

10.3 Omics Technologies in Ecological Bioremediation

Bioremediation is the usage of living beings or subsidiaries from life forms to degrade environmental hazardous substances or pollutants. The genetics and biochemistry of biodegradation mechanism opened the scope for investigation of collaborating parts associated with the system biology. Major elements of system biology are biodegradation network, computational biology, and omics tools like metagenomics, genomics, transcriptomics, proteomics, and metabolomics (Jaiswal

Table 10.1 Role of microbes in bioremediation of environmental pollutants

Microorganisms	Function	Environmental pollutant	References
Alcanivorax borkumensis	Biodegradation	Petrol and oil	Lee et al. (2017)
Pseudomonas putida	Biodegradation and biotransformation	Industrial cyanide-containing wastes, or the 2,4,6-trinitrotoluene	Wibberg et al. (2016)
Stenotrophomonas maltophilia strain AJH1	Biodegradation	Anthracene, phenanthrene, naphthalene, fluorine, pyrene	Arulazhagan et al. (2017)
Pseudomonas sp. (strain CPSB21)	Redox reaction	Cr^{6+}	Gupta et al. (2018)
Pseudomonas sp.	Biodegradation	BTEX (benzene, toluene, ethylbenzene, and xylene)	Hassan and Aly (2018)
Bacillus licheniformis M2–7	Biotransformation	Benzo-pyrene	Guevara-Luna et al. (2018)
Rhodococcus erythropolis strains	Bioaugmentation	Petroleum product	Pacwa-Płociniczak et al. (2019)
Bacillus sorensis strain.	Biodegradation	Hydrocarbon	Oualha et al. (2019)
Planococcus sp. S5 strain	Immobilization	Naproxen	Dzionek et al. (2018)
Halorientalis hydrocarbonoclasticus	Biodegradation	Hexadecane, PAHs (naphthalene, anthracene, phenanthrene, pyrene, and benzo[a]anthracene)	Zhao et al. (2017)
Acinetobacter radioresistens APH1	Biodegradation	Phenol	Liu et al. (2020)
Rhodococcus spp.	Biodegradation	Wide range of organic pollutants, including nitriles, halogenated hydrocarbons, and numerous aromatic compounds	DeLorenzo et al. (2018)

et al. 2019) (Table 10.1). Major advantage of bioremediation is its lower cost compared with traditional techniques and this is also stable and eco-friendly process (Azubuike et al. 2020; Kuiper et al. 2004). Enzymes have colossal catabolic potential for remediating environmental wastes; however, the interactions between microscopic organisms and pollutants are complex and appropriate remediation does not always happen. Also, many synthetic compounds lack biological catalyst therefore biodegradation has not been investigated (Wackett and Hershberger 2001). Subsequently, the field remains a rich territory for the utilization of new biotechnological strategies to encourage bioremediation, for example, metabolic engineering, metabolomics, proteomics, genomics, and transcriptomics (Table 10.2).

Metabolic engineering includes diverting the cell's metabolism to accomplish a specific objective utilizing recombinant engineering (Bailey 1991). One of the first

Table 10.2 Omics technologies used for study of bioremediation

Omics technology	Role in bioremediation	References
Genomics	It has been to control the phylogenetic position of the microorganisms involved in bioremediation process and also for modulating genetic engineer—*Rhodococcus ruber* for a wide range of hydrocarbon degradation	Liang et al. (2020)
Metagenomics	This has been used to discover the abundance of microorganisms and their genes that are mostly active and functional during degradation of environmental pollutants	Bharagava et al. (2019)
	This also has been used for identification of major environmental pollutants	Williams et al. (2014)
Transcriptomics	This has been used for evaluating the physiology of mixed and pure microbial cultures isolated from the environment	Park and Choi (2020)
	This is also effective in gene expression analysis using RNA-Seq	Yuan et al. (2020)
Proteomics	This has been used to monitor the changes in proteomes of microbes in response to contaminants	Kucharzyk et al. (2020)
Metaproteomics	This has been used for analysing the microbial catabolic enzymes known to play a role in biodegradation pathways Metaproteomics helps in soil protein identification which can give information about the soil biogeochemical potential and pollutant degradation and be an indicator of soil quality and regeneration	Heyer et al. (2017), Bastida et al. (2019)
Metabolomics	This omics technique provides the huge amount of information regarding the metabolites produced by microbes in contaminated environment	Draghi et al. (2017), Gieg and Toth (2019), Zhao et al. (2019)

and best instances of this methodology in bioremediation was the metabolic engineering of *Pseudomonas* sp. B13; five diverse catabolic pathways from three distinct microscopic organisms were joined to allow biodegradation of methyl-phenols and methyl-benzoates in an individual microbe (Rojo et al. 1987). Ju and Parales (2009) revealed first time microscopic organisms to use chloronitrobenzenes for development without the expansion of co-substrates and make the principal strain that develops on 3-chloronitrobenzene; chloronitrobenzenes are fabricated for pesticides, fungicides, colours, and polymers. Fernández et al. (2009) examined the biodegradation of nitroaromatic compounds such as 2,4,6-trinitrotoluene by model bacterium *Pseudomonas putida* KT2440. With the help of DNA microarrays and mutants, the researchers (Fernández et al. 2009) found the microorganism responds to the

dangerous compound through the initiation of a progression of detoxification capacities including nitroreductase, isoquinoline oxidoreductase, dehydrogenase, and chaperones to counteract or fix cell harm. The researchers additionally demonstrated that multidrug efflux pump genes (*mexEF/oprN*) are induced to lessen intracellular trinitrotoluene concentration. This work is weighty in that not many gatherings have connected transcriptomics to bioremediation, and this procedure guarantees to help unwind unexpected regulatory bottlenecks identified with effective remediation.

The Ramos group also has been used whole transcriptome profiling to decide mutualistic collaborations in the rhizosphere for strains pertinent for bioremediation; for instance, 90 rhizosphere upregulated genes were recognized for *Pseudomonas putida* developing on corn roots (Matilla et al. 2007). In spite of the fact that *P. putida* is a significant bacterium in the rhizosphere, numerous other key species for biodegradation exist, which are not as easily manageable to genetic engineering. One of these is *Rhodococci*, Gram positive microbes which assume a significant role in biodegradation of (hetero-) aromatic substances. Tomás-Gallardo et al. (2009) investigated the tetralin degradation pathway in *Rhodococcus* by using a combination of proteomics, genetic engineering, and heterologous expression.

Significantly less in the open personality is the danger of subsurface pollution, a sort of tainting which is additionally the least comprehended. Parisi et al. (2009) attempted metabolomics investigations of samples taken from oil defiled springs. Their discoveries proposed that contaminants of regulatory concern (COC), for example, benzene, are generally hard-headed to degradation. Rather, the autochthonous microbial communities specially utilized other non-COC hydrocarbons even when animated with nutrient amendments. These discoveries uncover that a conceivable hindrance for successful remediation under anaerobic conditions could be the nonattendance of reactant microorganisms. Further in the line of subsurface tainting, Scheibe et al. (2009) present a genome-based metabolic model of the metabolism of *Geobacter sulfurreducens*, and coupled this to a hydrological transport model, so as to anticipate in situ uranium bioremediation. As *Geobacter* metabolic activities to decrease U (VI) underground is fundamentally subject to the accessibility of acetic acid derivation as an electron contributor and Fe (III) as an electron acceptor and ammonium as a key nutrient. The model precisely anticipated the behaviour of *Geobacter* in a field trial with uranium bioremediation, showing the intensity of coupling genome-scale metabolic models with hydrological models for field-scale behaviour. In the domain of pesticide remediation, Govantes et al. (2009) reported the microbial degradation pathways of herbicide atrazine.

Microorganisms, the main living organism to be inescapable in condition, have basic capacities in every biological cycle. Microbial activities are affected by different environmental factors like temperature, moisture, pH, oxygen, and nutrients. Genes and relating encoded compounds are vital for microbial activity but environmental conditions also have a significant role in their regulation. Soil organic matter has significant impact on biological degradation of hydrocarbon compounds. Microbial degradation of polycyclic aromatic hydrocarbons is generally slow in organic matter deficient soil (Yang et al. 2011).

Microorganisms have sets of catabolic genes, fit for handling different metabolic pathways, which are coordinated in such a way that xenobiotic mixes are changed over to intermediates which can enter in central metabolic pathways, (for example, Kreb's cycle, glycolysis, and others). The monstrous capability of microorganisms does not depend exclusively on the abundance of catabolic proteins that they have, but additionally upon their ability for versatile change (Houghton and Shanley 1994). Single microorganisms do not possess all metabolic genes to degrade toxic pollutants; however, microbial consortia may be alternate option to degrade the pollutant. Another methodology is to build up a recombinant strain, harbouring differing metabolic pathways, utilizing novel molecular and omics technologies.

To characterize the catabolic potential for biodegradation it is important to think about the wide assorted variety of catabolic routes advanced by microorganisms and furthermore the decent variety of enzymes of a given gene family or even between gene families. The present capacities to evaluate and foresee catabolic capability of natural locales include gene fingerprinting, catabolome clusters, metagenomics and corresponding "omics" technologies (Vilchez-Vargas et al. 2010). Literature suggested that over the last few years, high-throughput technologies have been widely used in environmental microbiology and biotechnology to find out the appropriate catabolic pathway of biodegradation of environmental pollutant for bioremediation (Stenuit et al. 2008; Carmona et al. 2009; Trigo et al. 2009; Singh et al. 2008). Some important omics technologies like genomics, proteomics, transcriptomics and metagenomics, metabolomics and metaproteomics are described here in terms of environmental microbiome and bioremediation.

10.3.1 Genomics

Genomics is an interdisciplinary field of science concentrating on the structure, function, evolution, mapping, and altering of genomes. A complete set of DNA of a living organism is called genome. Rather than genetics, which alludes to the investigation of individual genes and their function in inheritance, genomics goes for the collective characterization and quantification of genes, their interrelations and impact on organism (WHO 2002; WHA 2004). Genes may coordinate the generation of proteins with the help of enzymes and messenger molecules. Whereas protein is the building block of the body and controls the different biochemical reactions in body and communicates the signals between cells. Genomics also includes the sequencing and investigation of genomes through high-throughput DNA sequencing and bioinformatics to determine the function of whole genomes (Culver and Labow 2002). Advances in genomics have set off an upheaval in revelation based research and science to encourage comprehension of even the most intricate natural frameworks (Kadakkuzha and Puthanveettil 2013). The field of genomic also includes the investigations of intragenomic phenomenon such as epistasis, pleiotropy, heterosis, and different collaborations among loci and alleles inside the genome (Pevsner 2009).

Next generation sequencing (NGS) has a crucial role in grasping of physiological and genomic characteristics of microorganisms pertinent to bioremediation (Zhou et al. 2018). The contemplations of researcher have been changed after the utilization of bioremediation to the pushed sciences like genomics which furnished different responses. For example, molecular examinations have shown that *Geobacter* species are critical in the bioremediation of natural and metal contaminants in subsurface conditions. The sequencing of a few genomes of the genus *Geobacter*, as well as firmly related microorganisms, has altogether altered how *Geobacter* species work in sullied subsurface circumstances. For instance, before the sequencing of the *Geobacter* genomes, *Geobacter* species were considered to be non-motile; however, genes encoding flagella were found in the *Geobacter* genomes (Childers et al. 2002). Further assessments revealed that *Geobacter metallireducens* unequivocally makes flagella exactly when it is growing on insoluble Fe(ra) or Mn(IV) oxides. Genes for chemotaxis were furthermore clear in the *Geobacter* genomes, and exploratory assessments have revealed that *G. metallireducens* has a novel chemotaxis to Fe (II), which could help direct it to Fe (III) oxides under anaerobic conditions.

It has been revealed that pili genes are also available in *Geobacter* species. These genes are expressed during the growth on insoluble oxides (Childers et al. 2002). Inherited assessments have indicated that the role of the pili is to help in association with Fe(III) oxides. This energy dependent mechanism for finding and reducing Fe (ra) oxides in *Geobacter* species appears differently in relation to the procedures for Fe (III) reduction in other well-studied organisms, for example, *Shewanella* and *Geothrix* species. Exploration of hidden physiological attributes of *Geobacter* species is important in the control of conditions in subsurface circumstances to upgrade the limit of *Geobacter* species to oust characteristic and metal contaminants from defiled groundwater. Thus genomics discovered the function of unknown genes that is important in bioremediation of polluted environment.

10.3.2 Transcriptomics

Transcriptomics is the investigation of the transcriptome—the total arrangement of RNA transcripts that are delivered by the genome, under explicit conditions or in a particular cell—utilizing high-throughput strategies, for example, microarray examination. Correlation of transcriptomes permits the distinguishing proof of genes that are differentially expressed in distinct microbial cell, or because of various treatments. RNA profile analysis technique is known as transcriptomics which is a powerful association between the genome, the proteome, and phenotype. Gene expression regulation is a key procedure to adapt in changing environment. DNA microarrays are a staggeringly astonishing stage in transcriptomics that enable affirmation of the mRNA expression level of all desired genes of a living being (Schena et al. 1998; Golyshin et al. 2003; Diaz 2004). The most testing issue in microarray trials is explanation of information (Dharmadi and Gonzalez 2004).

Generally, several genes might be up- and down-regulated in a specific stress condition. In this specific circumstance, a few factual issues become more complex

and show systematic error. Transcriptomics or metatranscriptomics instruments are utilized to increase utilitarian bits of knowledge into the exercises of natural microbial communities by contemplating their mRNA transcriptional profiles. Jennings et al. (2009) performed transcriptomics investigation on a cis-dichloroethene (cDCE)-acclimatizing *Polaromonas* sp. JS666 strain so as to recognize the genes upregulated by cDCE utilizing DNA microarrays.

10.3.3 Proteomics

Protein profiles analysis technique is known as proteomics; it includes the investigation of proteins of an organism (Anderson and Anderson 1998). The terms "proteomics" and "proteome" were presented in 1995 (Wasinger et al. 1995), which is a key post genomic include that rose up out of the development of huge and complex genome sequencing datasets. Proteins are indispensable pieces of living creatures, with numerous capacities. The term proteomics was began in 1997, in relationship to genomics, the investigation of the genome (James 1997). The proteome is the whole arrangement of proteins that is delivered or altered by a life form or framework. Proteomics has empowered the identification of consistently expanding quantities of protein. This fluctuates with time and unmistakable prerequisites, or stresses, that a cell or life form experiences (Anderson et al. 2016). Proteomics is an interdisciplinary area that has profited significantly from the hereditary data of different genome ventures, including the Human Genome Project (Hood and Rowen 2013). It covers the investigation of proteomes from the overall degree of protein composition, structure, and function. It is a significant segment of utilitarian genomics. Proteomics reveals to exploratory investigation of proteins and proteomes, but frequently is utilized explicitly to refer protein refinement and mass spectrometry.

Proteomics-based examinations have been helpful in deciding changes in the organization and plenitude of proteins, just as in the distinguishing proof of key proteins associated with the physiological reaction of microorganisms when presented to anthropogenic contaminations. Proteomic investigation is especially fundamental in light of the fact that the watched phenotype is an immediate after effect of the activity of the proteins instead of the genome grouping.

Sikkema et al. (1995) reported that proteome have crucial role in bioremediation of (polycyclic aromatic hydrocarbon) PAH. The updates in 2-DE for use in compartmental proteomics have been made by exhibiting an elective procedure for multidimensional protein identification technology (MudPIT) (Paoletti et al. 2004). Mass spectroscopy has modified the ecological proteomics towards the bioremediation (Aebersold and Mann 2003). Matrix assisted laser desorption/ionization time-of-flight MS (MALDI-TOF-MS) is the most widely used mass spectroscopy approach to determine the proteins of extracted 2-DE gels. (Aebersold and Mann 2003; Aitken and Learmonth 2002; Landry et al. 2000). Surface-upgraded laser-desorption-ionization MS (SELDI-TOF-MS) technique is another way to determine the proteins associated with polycyclic aromatic hydrocarbon degradation pathways (Merchant and Weinberger 2000; Seibert et al. 2005; Emanuele 2010; Knigge et al.

2004). Joo and Kim (2005) also reported about the fluid chromatography MS (LC-MS) technique for direct location and identification of potential contaminants in water.

10.4 Comparative Analysis of Omics in Bioremediation

In perspective on a general examination of transcriptomics and proteomics, the total assessment of whole genome sequencing is especially helpful to find out the physiology and metabolic pathways of bioremediation-appropriate microorganisms. DNA microarray is also an important genomic technique for identification of unknown microorganisms and gene expression analysis with regard to bioremediation (Gygi et al. 1999). In this way, whole genome clusters are more acknowledged with respect to desired gene expression pattern than proteomic contemplates. Literature supported that both transcriptomics and proteomics are important tools to illustrate the sub-metabolic pathways (Kuhner et al. 2005; Eymann et al. 2002). However, genomics information is essential to supplement the proteomic approach (Hegde et al. 2003). Regardless, proteomics would hold its central position in functional transcriptomics or potentially genomics. Kuhner et al. (2005) suggested that analysis of protein particles is an important way to determine the in situ mineralization by microbes. Protein profile analysis not only gives the information of the individual organism but also provides the data on the destiny and goal of protein molecules inside and outside the cell that must be found by means of a joint transcriptomics, proteomics, and interactomics approach.

10.5 The Most Current Omics

10.5.1 Metagenomics

The 16S rDNA sequencing was the first genomic tool to explore the bioremediation, isolation of microbes and their identification by 16S rDNA technology is the most significant step in the investigation of biodegradation. A metagenomics approach can likewise be attempted in mass identification of environmental samples associated with xenobiotics degradation. At that point extra approach is genomic investigation to recognize the enzyme engaged with bioremediation. Genomic examination demonstrated how different microbial strains are able to synthesize enzyme that have crucial role in degradation of polycyclic aromatic hydrocarbon or polychlorinated hydrocarbon or heavy metal take-up by turning the genes on and off as the microorganism distinguishes something inviting. Next further step is Metagenomics which plays the vital role to detect the specific genes involved in particular enzymes synthesis in terms of bioremediation or catabolism of environmental pollutant (Shekhar et al. 2020).

Metagenomics methodologies also assume a significant technology in understanding the diversity and density of microbial community and their function in an

environmental lattice. Metagenomics also explores the biodegradation and detoxification of organic and inorganic toxic substances at the polluted site. Metagenomics methodologies might be valuable to discover the potential microbial degrader for the bioremediation of a particular toxin or catabolic gene in terms of the degradation and detoxification of the particular contamination. Metagenomics is additionally utilized for looking at the microbial utilitarian decent variety at various polluted sites affected by a particular toxin. Further, advances in sequencing innovations, for example, NGS additionally enables us to explore further and more profound layers of the microbial communities and is imperative in exhibiting an impartial perspective on phylogenetic arrangement and practical assorted variety of natural microbial communities (Zwolinski 2000). The accessibility of entire genome sequences from several ecological microorganisms relevant to bioremediation has been valuable to decide the genetic stock of chemicals engaged with degradation of anthropogenic contaminant (Galvao et al. 2005).

Metagenomics is a culture independent technology which overcomes the major limitation of cultivation dependent studies as it includes extraction of nucleic acids straightforwardly from environmental samples which designated whole set of microbial community genomes present in a given biological system (Desai et al. 2009). Over the last few years, metagenomics-based techniques have been valuable to decide novel gene families and additionally organisms engaged with bioremediation of xenobiotics. Currently, DNA microarrays have been used for microbial ecological research and have been valuable in monitoring microbial communities and their efficiency in terms of bioremediation (Bae and Park 2006). The utilization of these "omics" methods in bioremediation research has altogether helped in describing or monitoring contaminant biodegrading microbes and recognizable proof of novel biodegradation pathways. Numerous investigations have revealed the development and screening of metagenomics libraries to distinguish genes engaged with bioremediation. Martin et al. (2006) designed metagenomics libraries to unravel environmental and metabolic function of microbial communities associated phosphorus removing microbial communities.

Two correlative methodologies can be utilized to distinguish contaminant degrading biocatalysts in microbial networks. One is governed by nucleic acid sequence examination and functional annotation, in light of the content of accessible sequence databases (Cardenas and Tiedje 2008). The other is guided by the perception of toxin degrading phenotypes, harboured by recombinant metagenomics clones. In the above both cases, just the cloning of the targeted metagenomic sequence, permits the gene function.

Sequence based metagenomics depends on extraction of entire DNA from environmental sample thereafter high-throughput or shotgun sequencing. In the course of recent years many sequencing based metagenomics projects have focused on intensely contaminated biological systems to think about the structure of microbial population (Chouari et al. 2003; Guermazi et al. 2008), interaction of microbial species, metabolic pathways, and the genes engaged with species survival in such conditions (Yamada et al. 2012). Functional metagenomics has given new bits of knowledge into the environmental role of individual species from these

communities, with ferrous iron oxidation assuming a key role in the microbial and geochemical process in AMD biological systems. Kim et al. (2006) examined fragrant hydrocarbon catabolic pathways in *Pseudomonas putida* KT 2440 utilizing a joined proteomic approach dependent on 2DE/MS and cleavable isotope-coded affinity label investigation.

Jadeja et al. (2014) use the sequence based metagenomics to retrieve the novel oxygenase sequence from a typical effluent treatment plant. The sequences were contrasted with catalysts recognized in past metagenomes from various wastewater structures and at various areas, including a sewage treatment plant, an upgraded organic phosphorus treatment plant, and a tannery waste treatment plant (Albertsen et al. 2012). Many reports are available in literature which prove that sequences based metagenomics has played a vital role in the study of biodegradation of xenobiotics compounds (Eyers et al. 2004; Lu et al. 2012; Bartossek et al. 2010.

As opposed to sequence based metagenomics approaches, action based metagenomics is very proficient to at the same time screen a microbiome for novel compounds and gain biochemical information with respect to substrate specificity. This innovation depends on the perception of a phenotype, connected to the reaction(s) engaged with breakdown of the targeted toxic substance. It has three requirements: (1) cloning of DNA or cDNA sections in the range of 2 and 200 kbp long into an expression vector (plasmids, cosmids, fosmids), or even bacterial for the making of metagenomics or metatranscriptomics libraries, individually; (2) heterologous expression of cloned genes into a microbial host; (3) plan of delicate phenotypic screens to separate clones of interest with targeted function, likewise called screening "hits". Sequencing endeavours are then focused on the hits and give whole gene sequence coding to useful proteins. Since hit genes are not recovered based on sequence homology with definitely known genes, action based metagenomics is the main realized approach to distinguish new protein families or to ascribe new capacities to definitely known protein families. Two conventional high-throughput systems are generally utilized for screening. One depends on direct recognition of colouration or discolouration, when hued substrates or chromogenic substrates are utilized, or of a decrease in obscurity of the response medium when insoluble substrates are utilized. The other depends on heterologous complementation of an auxotrophic host by foreign genes, which permits growth of microbial host on specific culture media.

10.5.2 Metatranscriptomics

Metatranscriptomics refers to investigation of extraction and examination of metagenomic mRNA (metatranscriptome) that gives the data about the regulation and expression of protein profiles of complex microbial communities present in the natural sample. Metatranscriptomics gives a depiction of the gene expression in a given environmental sample at a given time and under explicit conditions by catching the complete mRNA (Aguiar-Pulido et al. 2016).

At present, in situ metatranscriptomics investigations of microbial communities are uncommon due to challenges related with the processing and handling of RNA samples including the recuperation of mRNA from environmental samples, short half-lives of mRNA species, and purification of mRNA from other RNA species (Simon and Daniel 2011). These constraints can be evaded by use of direct cDNA sequencing utilizing NGS advancements. This gives reasonable access to the metatranscriptomics and permits the entire genome expression profiling of a microbial community. What is more, the immediate measurement of the transcripts is likewise attainable (Simon and Daniel 2011). In this way, the activity and significance of ammonia oxidizing archaea in soil environments have been demonstrated. Currently, Shi et al. (2009) demonstrated the contribution of small RNAs (sRNAs) in numerous ecological procedures, for example, carbon metabolism and nutrient accumulation, by comparison of metatranscriptomics datasets from the Hawaii Sea Time-series station ALOHA.

Metatranscriptomics is the investigation of transcripts of the whole interfacing network to pick up bits of knowledge into the up- or down-regulation of genes under adverse conditions in natural microbial communities. The first regulatory point for effective combination of a protein from a gene is the regulation of gene expression, one of the important processes for surviving in influenced ecological conditions. Regularly expanded mRNA can be in any event subjectively connected with higher rates of contaminant biodegradation (Schneegurt and Kulpa 1998). The greatest concern while contemplating RNA is that it is significantly less steady than different particles and in this way samples must be appropriately obtained, handled, and stored. Additionally, these means can present inclination and consequently legitimate safety measures are important to gain true and precise actualities.

10.5.3 Metabolomics

Beyond genomics, transcriptomics, and proteomics, the examination front lines are presently growing toward the worldwide investigation of the whole collection of cell metabolites inside a microbial cell, this newly presented technique is known as "metabolomics". There are so many reports available in literature which prove that microbial metabolome has important role in degradation of environmental pollutant. Recently, Keum et al. (2009) depicted investigations on relative metabolome analysis of *Sinorhizobium* sp. C4 during the degradation of phenanthrene. Tang et al. (2009) also reported a fluxomics examination on *Shewanella* sp. known to have cometabolic pathways for bioremediation of dangerous metals, radionuclides, and halogenated natural mixes.

Due to the genome wide requirement of functional genomics contemplates we have seen a huge development in systems for worldwide recognition and measurement of cell metabolites during the past few years (Gavaghan et al. 2000; Roessner and Bowne 2000; Soga and Heiger 2000; Soga et al. 2002a, b; Allen et al. 2003; Castrillo et al. 2003; Dunn et al. 2005). In this manner, metabolomics has developed another field in science with the guarantee to accelerate the practical investigation of

genes with obscure capacity (Xia 2011). Thus, Metabolomics has emerged as a new field of science with the aim to accelerate the functional analysis of genes with unknown function (Villas-Bôas et al. 2005).

Today, metabolomics methodologies and tools are applied in different fields like—human and animal nutrition (Whitfield et al. 2004; Gibney et al. 2005; Rist et al. 2006), disease analysis and treatment (Hartmann et al. 2006; Malhi and Guts 2006), biomarker disclosure (Goodacre 2005; Schlotterbeck et al. 2006), toxicology (Robertson 2005), stoutness considers (Hochberg 2006), chemical revelation (Saito et al. 2006), sedate disclosure (Harrigan 2006), transplantation (Wishart 2005), agriculture (Dixon et al. 2006), and bioremediation (Singh 2006).

The systematic procedures created for metabolomics studies permit the screening of several metabolites from complex natural samples with relative high-throughput rate, and the information produced by these strategies give helpful data in regard to the metabolism of living beings related with the samples. Being the intermediates of biochemical responses, metabolites assume a significant role in associating a wide range of pathways that work inside a living cell. In this way, the degree of metabolites in a cell or tissue determines the integrative information of cellular activities and, subsequently, characterizes the phenotype of a cell or tissue because of hereditary or ecological changes (Penuelas and Sardans 2009). Metabolomics can be extremely helpful in bioremediation studies. Metabolome examination covers the distinguishing proof and measurement of all intracellular and extracellular metabolites with molecular mass lower than 1000 Da, utilizing distinctive systematic methods (Villas-Bôas and Bruheim 2007; Johnson et al. 2012). As with the transcriptome and the proteome, the metabolome is context-dependent, the degrees of every metabolite are relying upon the physiological, formative, and obsessive condition of a cell, tissue, or living being. However, a significant distinction is that, dissimilar to with mRNA and proteins, it is troublesome or difficult to set up an immediate connection among genes and metabolites. The tangled idea of cell metabolism, where a similar metabolite can take an interest in a wide range of pathways, confuses the elucidation of metabolite information (Villas-Bôas and Bruheim 2007).

In addition, the genome, transcriptome, and proteome investigations depend on analysis of object made out of four unique nucleotides (genome and transcriptome) or 22 amino acids (proteome). Those compounds are exceedingly comparable chemically, and encourage the advancement of high-throughput explanatory methodologies. In case of metabolome, however, there is an enormous change in chemical structures and properties. The metabolome comprises amazingly different chemical compounds, from ionic inorganic species to hydrophilic starches, unpredictable alcohols and ketones, amino and non-amino natural acids, hydrophobic lipids, and complex common items (Villas-Bôas and Bruheim 2007; Zhao and Hartung 2015). This unpredictability makes it practically difficult to investigate the changes in chemical structure and properties of biomolecules. Besides, the exceptionally quick turnover of metabolites adds to the multifaceted nature of metabolome investigation; numerous metabolites are available in fairly low concentration and there are extremely high transitions through the metabolite pools. It is

accordingly critical to extinguish the metabolism quickly, which calls for proficient techniques for extinguishing and extricating metabolites from living cells. In this manner, metabolite investigation possesses sample preparation, metabolite partition and identification, and data analysis and result interpretation.

In any case, there is no single diagnostic technique for proper examination of all metabolomes of a cell or organism. Various terms are frequently utilized in the field of metabolomics to refer to various scientific methodologies. Two significant terms that are frequently used to portray investigation of a part of the metabolome are metabolite profiling and target examinations. Metabolite profiling is characterized exactly as the set of all metabolites or subsidiary products (distinguished or obscure) identified by dissecting an sample utilizing a specific logical system, together with an estimation of quantity (Seo et al. 2009; Villas-Bôas and Bruheim 2007; Vazquez-Duhalt et al. 2005; Singh 2006). Whereas target examination is characterized as the investigation of a list of metabolites ignoring all non-focused compounds. Whether profiling or target investigation is utilized, what is going on in metabolomics system is the heartiness of the explanatory procedures created, which license the synchronous examination of several compounds utilizing a solitary strategy. Inside this specific circumstance, mass spectrometry and atomic attractive reverberation are the most much of the time utilized strategies for location in the investigation of the metabolome, which all the time is joined with proficient chromatographic methods. Today is all around perceived that metabolome examination is an incredible methodology for finding novel metabolic pathways and describing metabolic systems (Weckwerth and Fiehn 2002; Seo et al. 2009; Villas-Bôas and Bruheim 2007; Vazquez-Duhalt et al. 2005; Singh, 2006). Metabolomics information reveals physiological reactions to formative, hereditary, or natural changes (Weckwerth and Fiehn 2002). However, metabolomics information is still most troublesome and complex to decipher and coordinate with different OMICS-related information. Still, there are diverse systematic procedures accessible that encourage the understanding of metabolomics information towards explanation of metabolic pathways. Two of these are of extraordinary interest for clarifying biodegradation pathways of xenobiotics mixes: isotope distribution analysis of metabolites and molecular availability examination utilizing ultra-high mass accuracy techniques.

10.6 Conclusion

Microorganisms assume a significant role in the degradation and detoxification of hazardous substances and furthermore help in the biogeochemical cycling of minerals in the environment. In this way the information of organisms in contaminated lattice is required to all the more likely comprehend the system of bioremediation of a particular pollutant and discover the key enzyme of catabolic gene included. In précis, the OMICS strategies ought to be created in a way to help finding answers for ecological question with regard to biochemistry, physiology, microbiology, geochemistry, and ecology.

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Microalgae: Omics Approaches for Biofuel Production and Biomedical Research

11

Arti Sharma, Sandeep Shukla, and Rajesh Pratap Singh

Abstract

Rapidly emerging countries like India and China are lifting millions of people out of poverty. This perpendicular growth in the number of people demanding access to reliable and affordable energy will drive energy demand in the decades to come. Much effort has been employed toward optimizing microbes and predominantly microalgae, to resourcefully produce compounds that can be substitute for fossil fuels. Oils acquired from algal feedstock are rich in triacylglycerols and could be converted into biodiesel via transesterification. Apart from the triacylglycerols and carbohydrates which are predominant in microalgae, there are several biomolecules like pigments and vitamins which play crucial role in pharmaceutical industries. There is an urgent need to understand which drives the production of such economic important biofuels or chemicals to improve the sustainability of the process. Integrative omics is a strong technique to know the complete system of microalgae and develop as microbial cell factories. Genomics and transcriptomics of microalgae have provided basic understanding toward lipid biosynthesis. Proteomics and metabolomics are now complementing "microalgal omics" and offer accurate functional insights into the attendant static and dynamic physiological contexts. Current chapter focuses on the application

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of omics approaches which considered powerful tools for a better understanding of algae cells metabolism. Then, the data would be used to develop sustainable strategies for biodiesel and by-products yield and quality improvement and a profitable microalgae industry.

Keywords

Microalgae · Proteomics · Genomics · Transcriptomics · Metabolomics

11.1 Introduction

The biological and biomedical scientific landscape has seen the escalation in the use and applications of "*omics*" technologies in the last decade. These technologies offer approaches that allow for a comprehensive description of nearly all components within the cell (Maghembe et al. 2020). Microalgae not only play a significant ecological role, but also of commercial importance. They have emerged as a promising group in the production of bioproducts and biofuel, as well as for the remediation of effluents. Indigenous inhabitants have used microalgae for centuries and the commercial application of microalgae has been extensively reviewed (Bleakley and Hayes 2017). The productivity of the microalgal production process depends on higher biomass, productivity, and yield and process robustness (Ummalyma 2020). These parameters mainly depend on the host microorganism. Natural screening, mutagenesis, bioprocess development, genetic and metabolic engineering strategies have been implemented to enhance the metabolic capabilities of the host microbes (Barh et al. 2013). The problems such as the accumulation of lethal intermediates or metabolic stress consequential decreased cellular fitness need to be solved. The inadequate knowledge about the regulatory mechanisms of key enzymes and the complex associations between genotype and phenotype are still obstacles to the development of efficient cell factories. The introduction of heterologous genes or deletion genes in specific metabolic pathways does not always consequence in the desired phenotype. Currently, remarkable innovations in platforms for omics-based study and application development have imparted crucial solutions to these problems. A combinatorial approach using numerous omics platforms and the incorporation of their outcomes is now an effective strategy for clarifying the molecular systems that are integral to improving algal productivity (Guarnieri and Pienkos 2015; Rai et al. 2016).

11.2 Proteomics and Molecular Examination of Microalgal Lipid Accumulation

Figure 11.1 shows the diagrammatic representation of identification of potential targets for enhancing the lipid accumulation in microalgae through the proteomic approach. Proteomics based studies are used in several fields such as identification of

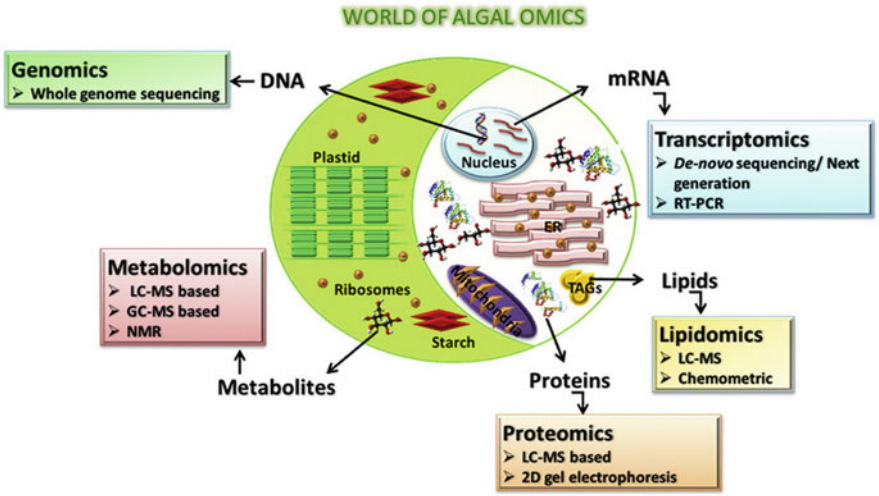

Fig. 11.1 Identification of potential targets for increasing TAG accumulation in microalgae by OMICS approach (Arora et al. 2018)

different diagnostic markers, pathogenicity mechanisms, and biomarkers for vaccine production as well as modification of expression patterns in response to altered signals and interpretation of protein pathways in several diseases (Aslam et al. 2017). Proteomics is basically complex because it contains the analysis as well as the categorization of total protein signatures of a genome. Mass spectrometry along with LC–MS and MALDI-TOF-TOF-MS being frequently used approach, is the main among current proteomics approaches. Though exploitation of proteomics facilities containing the software for equipment and databases as well as the need of skilled personnel substantially raises the costs, hence limit their wider use mainly in the developing world. Additionally, the proteome is an extremely dynamic intricate regulatory system that governs the expression levels of proteins. Microalgae are currently being explored as bio-production platforms for hydrocarbon and lipid-based bioproducts and biofuels. In particular, microalgal triacylglycerides (TAGs) offer a promising feedstock for biodiesel production (Pienkos and Darzins 2009).

However, despite the historical and recently renewed interest in algae-based fuels, our understanding of regulatory mechanisms governing algal lipid metabolism, particularly the regulation of fatty acid and TAG accumulation, remains incomplete. Identification of key regulators of genes, proteins, and metabolites triggering algal lipid biosynthesis and accumulation opens the door for genetic and metabolic engineering strategies targeting increased rates and absolute quantity of lipid accumulation. As such, a molecular examination of microalgal lipid accumulation mechanisms has recently intensified. The nitrogen (N)-deprivation response is perhaps the best-characterized inducer of lipid accumulation in microalgae (Yang et al. 2016). In addition to increased total lipid content, N-deprivation can also induce changes in fatty acid chain length and saturation, with resultant TAGs

more favorable for biofuel conversion (Guarnieri et al. 2011). Transcriptomic profiling has been widely applied to examine this N-deprivation response, yielding characteristic RNA expression signatures associated with deprivation and concurrent lipid accumulation (Radakovits et al. 2012). Such analyses offer powerful insights into the transcriptional regulation of lipid accumulation, but these analyses do not fully define metabolic regulatory control points. This is especially true in algae and higher plants where post-transcriptional and post-translational regulation plays a critical role in protein expression and metabolic regulation (Gillham et al. 1994). Furthermore, mostly employed endpoint analyses (N-replete vs. N-deplete) unsuccessful to completely elucidate the kinetics of expression, which can both illuminate underlying molecular mechanisms governing phenotypic responses and update strain-engineering strategies necessitating induced gene expression. Time-course proteomic analyses provide a means to observe post-transcriptional responses to N-deprivation and concurrent lipid accumulation, which can both complement and further the mechanistic insights gained from transcriptional analyses. However, to date, the accessibility of such proteomic data is relatively limited, in particular for microalgae, compared to that of transcriptomic analyses (Guarnieri et al. 2011; Le Bihan et al. 2011). Microalgae species that have a high lipid content under nitrogen starvation conditions include *P. tricornutum* (KaiXian and Borowitzka 1993), and the green algae *Chlorella spp*. (Illman et al. 2000), *Botryococcus braunii* (Zhila et al. 2005), and *Chlamydomonas reinhardtii* (Miller et al. 2010) and *Dunaliella salina* (Ben-Amotz et al. 1985). The proteome of diatom in nitrogen starvation condition has been compared of nitrogen-replete cells by two-dimensional gel electrophoresis, revealing differences in the responses of central carbon metabolism under nitrogen starvation between diatoms, higher plants, and green algae (Hockin et al. 2012). So far, limited proteomic based studies have been done in microalgae to elucidate the molecular shifts towards lipid accumulation at the proteomic level. *Arthrospira platensis* is a filamentous cyanobacteria whose high protein content suitable as a nutritive supplement for human and animal diets. Furthermore it is easy to grow and tolerate extreme environmental stresses such as pH, heavy metals, and salinity. In this work, changes in the growth, pigments, and proteome of *A. platensis* were studied under two major abiotic stresses limiting productivity iron and salinity. The slight concentrations of stresses showed different effects on fresh weight, pigment constituents, growth rate, and soluble protein of this microalgae. A proteomic analysis was performed using SDS gel electrophoresis first to achieve an overview of gross changes, followed by two-dimensional gel electrophoresis as well as mass spectrometry to identify those proteins whose abundance was affected by these environmental stresses. Eighteen protein spots were differentially expressed under the stress conditions. Out of these, six were obtained with increased abundance responding only to Fe^{2+} stress, and five as a result of NaCl stress alone (Ismaiel et al. 2018). *Prototheca zopfii* is another algae which is associated with bovine mastitis and human protothecosis. By proteomic study, immunodominant proteins were identified of *P. zopfii* for development of vaccine against mastitis and protothecosis. *Prototheca* proteins were separated using 2D-gel electrophoresis. Subsequent immunoblotting with rabbit hyper-immune antiserum revealed 28 immunogenic protein

spots. Mass spectrometry analysis revealed 15 immunogenic proteins, including malate dehydrogenase, elongation factor 1-alpha, and heat shock protein 70, which were formerly reported as immunogenic proteins of other eukaryotic pathogens (Irrgang et al. 2015). These proteins could be potential vaccine candidates against the *P. zopfii*. The current chapter focuses on proteomic analysis of algae and its relevance toward biofuel production and biomedical uses. Without a proteomics study, microalgal omics study is inappropriate. It gives an idea of the particular functional group in both the state (static and dynamic physiological contexts). Targeted proteomics on microalgae has not been reported till now. Proteomics study has used for a better approach for understanding the complexity in the biological world. This can be successfully used for obtaining biodiesel. The motivation behind this proteomic study is to attract attentiveness toward sustainable consumption of microalgae resources for finding indigenous microalgal strains toward exploring their potential role in the production of bioenergy in the country.

11.3 TAG Synthesis Pathways in Algae

By comparison to plant cells, the production of fatty acids in microalgae is commonly thought to take place in the plastid. Following the pass on to the cytosol, fatty acids enter the TAG assembly pathway catalyzed by enzymes associated with the endoplasmic reticulum. In addition, de novo TAG assembly in the chloroplast has been recently proposed to occur in *C. reinhardtii* following N-deprivation of a starch-deficient mutant, raising the question of novel TAG assembly pathways in microalgae not observed in seed plants. Until this hypothesis is further corroborated or refuted, one has to consider that parallel TAG assembly pathways in the plastid envelopes and the ER may exist. In fact, an increasing number of putative plastid-targeted acyltransferases in distinct microalgal genomes provide potentially supporting evidence for a chloroplast TAG assembly pathway (Rastogi et al. 2018). Unlike phospholipids found in biological membranes, TAGs do not perform a structural role in cells, but instead serve as a storage form of energy and carbon. After being synthesized, TAGs are deposited in discrete spherical structures, the LDs, located in the cytoplasm or chloroplast. Whether the latter are distinct from plastoglobuli, carotenoid-rich LDs present in plant chloroplasts remain to be seen. Based on predicted orthology of genes identified in algal genomes, at least two major pathways of TAG synthesis have been proposed to function in microalgae: one is the Kennedy or glycerol phosphate pathway, the other is the monoacylglycerol pathway. In both pathways, TAGs are formed by esterification between an acyl-CoA and a glycerol hydroxyl group. In the Kennedy pathway, the first acyl chain is esterified to glycerol-3-phosphate derived from the glycolytic intermediate dihydroxyacetone phosphate (DHAP). The enzyme is glycerol-3-phosphate-acyltransferase (GPAT) which forms lysophosphatidic acid, the substrate for the second acyltransferase, lysophosphatidic acid acyltransferase (LPAAT). This second reaction leads to the formation of phosphatidic acid, which is a central lipid metabolite giving rise to phospholipids and TAGs. Prior to conversion to TAGs, phosphatidic acid is

dephosphorylated by a phosphatase to form diacylglycerol (DAG) (Amara et al. 2016). The monoacylglycerol pathway starts with 2-monoacylglycerol, which is converted to DAG by a monoacylglycerol acyltransferase. De novo synthesis of monoacylglycerol in plant cells has been previously described and was shown to be catalyzed by GPAT4 and GPAT6 enzymes, which use dicarboxylic and hydroxy acyl-CoA as acyl-donors for the production of monoacylglycerol (Trentacoste et al. 2013). The monoacylglycerols have been considered initially as precursors for the synthesis of waxy polymers; however, their conversion to DAGs has been reported in *Nicotiana bethamina* leaves and recently in fruits of *Myrica pensylvanica*.

11.4 Omics Technology

Omic technologies assume a holistic view of the molecules that make up a cell, tissue, or organism. They are targeted mainly identification of genes (genomics), set of all RNA (transcriptomics) and proteins (proteomics) as well as metabolites (metabolomics) in a specific biological sample in a non-targeted and non-biased manner. This can also be referred to as high-dimensional biology; the integration of these techniques is called systems biology.

11.5 Genomics and Transcriptomics of Microalgae

DNA- and RNA-sequencing studies play a critical role in the quantitative and qualitative improvement of microalgal biomass. The sequence analysis affords useful information about the evolutionary history of the different microalgae groups, thus providing scientific suggestions regarding the role played by particular genes and gene networks. More interestingly, whole-genome sequence information can contribute to our understanding of the molecular mechanisms that microalgae use to adapt to environmental changes, as well as unlocking the potential to develop new and economically important products and technologies (Yao et al. 2017). Genomic and transcriptomic both information can be used to evaluate metabolic pathways and to perform more focused genetic engineering approaches, such as up-regulation or knock out of genes involved in the pathways of interest. Furthermore, a good annotation of the whole genome, alongside to a comparative transcriptomic approach, not only allows to gain insight into the metabolic pathways and their key enzymes, but also to identify regulatory factors and promoters of gene expression. RNA-sequence technology has received a lot of attention nowadays for microalgal worldwide transcriptomic profiling. It is commonly used in transcriptomic analysis of gene expression, predominantly for microalgal strains with potential as biofuel sources. However, inadequate genomic or transcriptomic information for non-model microalgae has limited the understanding of their regulatory mechanisms and hindered genetic improvement to enhance biofuel production (Yao et al. 2017). As such, an optimal microalgal transcriptomic database manufacture is a subject of critical investigation. *Dunaliella tertiolecta*, a non-model

microalgal species, was sequenced by HISEQ 4000 in RNA-Seq studies. The high-quality sequencing data were explored using high-performance computing in a Petascale Data Center. It is subjected to de novo assembly and parallelized mpi BLASTX search with several species. As a result, a transcriptome database was constructed. This enlarged database constructed fueled the RNA-Seq data analysis, which was validated by a nitrogen depletion study that induces lipid production (Yao et al. 2017). Nitrogen depletion (−N) has been used as a technique to stimulate lipid accumulation in many microalgae. *Scenedesmus acutus* is a promising microalga that can be cultivated in wastewater for biodiesel production (Hawrot-Paw et al. 2020). However, the molecular mechanisms limiting *S. acutus* lipid accumulation in response to N-deplete remain unidentified. A physiological study determined that N-deplete reduced cell growth and photosynthetic pigments. On the other hand, it stimulated carbohydrate and lipid accumulation (Sirikhachornkit et al. 2018). The transcriptome exploration revealed that glycolysis and starch breakdown were upregulated; on the contrary, gluconeogenesis, photosynthesis, triacylglycerol breakdown, and starch synthesis were downregulated by N-deplete. Under N-deplete, the carbon flux was moved toward lipid synthesis, and the downregulation of lipase genes may contribute to lipid accumulation. A comparative analysis of the N-deplete transcriptomes of microalgae recognized that the downregulation of many lipase genes was a precise mechanism found only in the N-deplete transcriptome of *S. acutus*. This work unraveled the mechanisms controlling N-deplete induced lipid accumulation in *S. acutus* and provided new viewpoints for the genetic manipulation of biodiesel-producing microalgae. Biodiesel produced by microalgae may overcome many of the sustainability challenges earlier ascribed to petroleum-based fuels and plant-based biofuels. Here, de novo transcriptomic work for the green microalgae *Dunaliella tertiolecta* is being studied and screen those genes which were importance for biofuel production. DNA pyro-sequencing next-generation technology used to *D. tertiolecta* transcripts production. Following size trimming and quality, ~45% of the great quality reads were gathered into isotigs along with 31-fold coverage and singletons. Assembled singletons and sequences were used for BLAST similarity searches and annotated by Gene Ontology (GO) as well as Kyoto Encyclopedia of Genes and Genomes orthology identifiers. These works documented the majority of starch and lipid biosynthesis as well as catabolism pathways in *D. tertiolecta* (Rismani-Yazdi et al. 2011).

11.6 Proteomics in Microalgae

The field of proteomics is complementary to genomics as well as transcriptomics in that it delivers additional information on gene expression and regulation. Proteomics comprises the determination of protein–protein interaction and protein expression studies. Furthermore, proteomics studies aim to recognize post-translational modifications of proteins as well as the organization of proteins in multi-protein complexes and their localization in tissues. Technologies are allowing proteomic

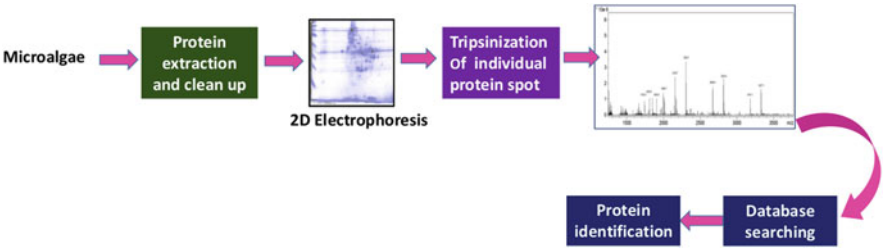

Fig. 11.2 Overview of the proteomic mass spectrometry experimental setup

studies have been in development for numerous decades now (Chandramouli and Qian 2009; Manzoni et al. 2016). A proteomic experiment comprises several steps, which can be taken using altered technological platforms. A critical, primary step is sample preparation and fractionation, which traditionally is being accomplished using two-dimensional gel electrophoresis. Proteins are separated based on their electrical charge in the first dimension (isoelectric focusing) and based on their molecular weight (sodium dodecyl sulfate-polyacrylamide gel electrophoresis,) in the second. The range of 2-DE can be improved by enriching the sample for certain desired proteins. Membrane proteins, for example, can be enriched by a sequential extraction based on the solubilities of proteins in different solutions (Aslam et al. 2017). After sample separation, protein identification is the next step. Proteins of interest can be cut from 2-dimensional gel electrophoresis, trypsinized, and identified by mass spectrometry. Peptide mass data obtained for each sample are queried against a peptide database with known peptide masses of unique proteins (Fig. 11.2). As in transcriptomic methods, proteomics can also be used for the elucidation of certain metabolic pathways. In comparative proteomics, the entire proteome of one sample is compared to that of another sample and kept under altered environmental conditions. Differentially expressed proteins can be identified due to the environmental stimuli and the metabolic pathway they are part of. Compared to the proteomic analysis of specific subcellular compartments or specific protein classes and comparative proteomic studies have so far not been reported. 2-D reference map from *C. reinhardtii* was represented primarily the soluble sub-proteome (Schmidt et al. 2006). They subsequently used this map to explore high light-induced changes to the proteome, at the same time compared the response of both the wild type and a mutant. Likewise, a comparative quantitative proteomic approach was used to study iron deficiency in *C. reinhardtii* and found the stress response proteins, such as peroxiredoxin as well as stress-induced light-harvesting protein (Naumann et al. 2007). In studying the effects of cadmium exposure on microalgae *C. reinhardtii* found a decline in abundance of both large and small subunits of the ribulose-l,5-bisphosphate carboxylase/oxygenase compared to other enzymes involved in photosynthesis (Naumann et al. 2007). Alteration of proteins profile by Cadmium-induced was also investigated in the marine microalga *Nannochloropsis oculata* (Kim et al. 2005). In *Haematococcus pluvialis,* a proteomic approach was used to elucidate the microalga's response to oxidative

stress (Gu et al. 2014) and in the halo-tolerant alga *Dunaliella salina* the molecular basis of salinity tolerance was clarified by proteomics (Kim et al. 2005). Finally, in the perspective of harmful algal bloom prediction, proteome reference maps were built for numerous microalgal species and tested them for their capability for species recognition (Wang et al. 2014). Species specific 2-dimensional gel electrophoresis protein profiles were observed for totally species tested and even distinction between closely related species was possible.

11.7 Proteomics: Lipid Production by the Gamma Irradiation Method

Lipid-producing mutant strain of the microalga *C. reinhardtii* has been developed by gamma irradiation. To induce the mutation, *C. reinhardtii* was gamma-irradiated at a dose of 400 Gy. After irradiation, the living cells were stained with Nile red. The mutant (Cr-4013) accumulating 20% more lipid than the wild type was selected (Fig. 11.3). Thin-layer chromatography revealed the triglyceride and free fatty acid contents to be markedly increased in Cr-4013. The major fatty acids identified were palmitic acid, oleic acid, linoleic acid, and linolenic acid. Random amplified polymeric DNA analysis showed partial genetic modifications in Cr-4013 (Baek et al. 2016). To ascertain the changes of protein expression in the mutant strain,

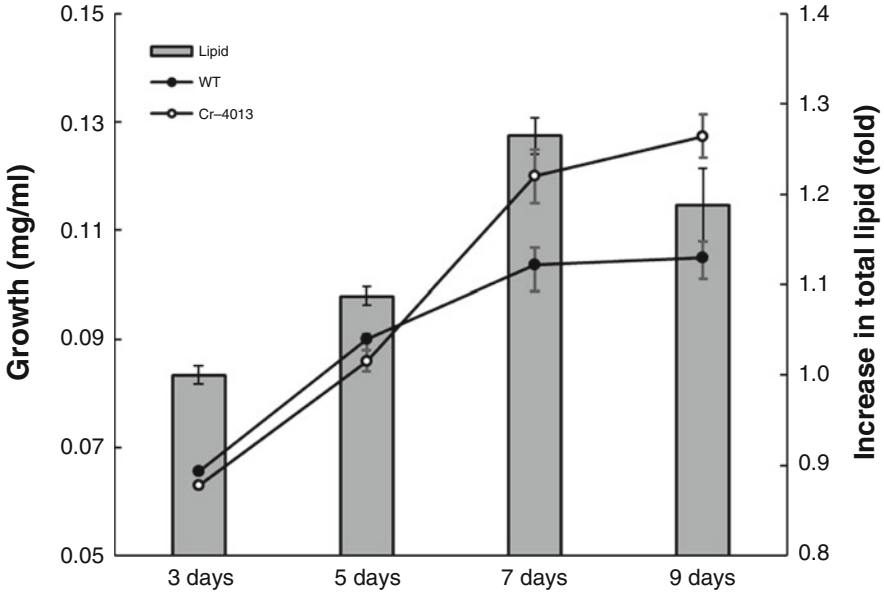

Fig. 11.3 Time profile of cell growth and total lipid changes in a batch culture of *C. reinhardtii* and its mutant Cr-4013. Data shown represent the mean values of growth rate based on cell dry weight. Growth rate of wild type (closed circle line), growth rate of mutant Cr-4013 (open circle line), and increased total lipid level inCr-4013 compared with wild type (gray bar)

two-dimensional electrophoresis was conducted. These results showed that gamma radiation could be used for the development of efficient microalgal strains for lipid production.

11.8 Types of Proteomics

11.8.1 Expression Proteomics

Expression proteomics is used to study the qualitative and quantitative expression of whole proteins in two different conditions. Like the normal cell and a treated cell can be compared to understand the protein that is accountable for the diseased or stress state or the protein that is expressed due to disease (Chernobrovkin et al. 2014). Typically, expression proteomics studies are used for the exploration of the expression of protein patterns in abnormal cells. For example, tumor tissue samples and the normal tissue comparative study can be analyzed by differential protein expression. 2-D gel electrophoresis and mass spectrometry approaches were used to identify the protein expressional changes, which is present and absent in tumor tissue, when compared with normal tissue. Which are upregulated and downregulated can be identified and characterized protein activities, multi-protein complexes and signaling pathways. Identification of these proteins will give important information about the molecular biology of tumor formation and disease-specific manner for use as diagnostic biomarkers/therapeutic targets.

11.8.2 Structural Proteomics

Structural proteomics is used to understand the three-dimensional shape and structural complexities of functional proteins. Structural prediction of a protein when its amino acid sequence is determined directly by sequencing or from the gene with a method called homology modelling. Structural proteomics can give detailed information about the structure and function of protein complexes present in a specific cellular organelle (Bai et al. 2016). It is possible to identify all the proteins present in a complex system such as membranes, ribosomes, and cell organelles and to characterize all the protein interactions that can be possible between these proteins and protein complexes (Manjasetty et al. 2012). Different technologies such as NMR spectroscopy and X-ray crystallography were mainly used for structure determination (Meisburger et al. 2017).

11.8.3 Functional Proteomics

Functional proteomics is used to understand the protein functions as well as molecular mechanisms within the cell, which depend on the identification of the interacting protein partners. The association between unknown protein and partners linking to a

specific protein complex involved in a specific mechanism would in fact be powerfully suggestive of its biological function. Furthermore detailed description of the cellular signaling pathways might greatly benefit from the elucidation of protein–protein interactions in-vivo (Cornett et al. 2018).

11.9 Protein Extraction Methods

Sample preparation is one of the most critical steps in the proteomics study. Good sample preparation can obtain consistent and high-quality results. The efficiency of different protein extraction methods varies depending on the type of samples. Therefore, the protein extraction method needs optimization for different samples, since the amounts and types of non-protein-interfering compounds vary. Algae comprise proteins, carbohydrates, lipids, and nucleic acids in varying proportions and the information is limited on the optimal protein extraction method from algae species. The proteomics investigations of algae are widely used in different fields, mainly including biofuel, biomonitoring, and pollution control. Therefore, the development of an efficient protein extraction method for algae will assist the proteome profiling research in algae (Bleakley and Hayes 2017). Currently, most literature studies focused on the evaluation of protein extraction methods from plant tissues since they contain recalcitrant interferences (polysaccharides, lipids, proteases, oxidative enzymes, and other secondary metabolites) and the presence of cell wall. To date, very few reports investigated protein extraction from algae. Some protein extraction methods can be used for the extraction of proteins from algae (Waghmare et al. 2016).

11.9.1 Direct Lysis Buffer Method

One milliliter lysis buffer (25 mM tetraethylammonium bromide, 8 M urea, 2% Triton, 0.1% SDS, and complete EDTA-free protease inhibitor tablet (Roche Diagnostics) was added to 100 mg algae sample followed by sonication on ice for 30 min (Feist and Hummon 2015). After centrifugation at 18000 × *g* for 60 min, the supernatant was stored at −80 °C until use.

11.9.2 TCA-Acetone Method

In this method, 100 mg of algae sample was resuspended in 1 mL chilled extraction buffer I (10% TCA, 1% polyvinylpyrrolidone, and 2% 2-mercaptoethanol in acetone) and sonicated on ice for 30 min. The mixture was incubated at −20 °C overnight. After centrifugation at 16000 × *g* for 15 min under 4 °C, the supernatant was discarded and the pellet was washed three times with l mL chilled acetone with 0.07% 2-ME. Between each rinse, the mixture was incubated at −20 °C for 60 min (Niu et al. 2018). The pellet was resuspended in appropriate volume of lysis buffer

and incubated at room temperature for 60 min. After centrifugation at 18000 × *g* for 60 min, the supernatant was stored at −80 °C until use.

11.9.3 Phenol Method

Phenol extraction was performed according to Wang et al.'s method with some modification. Briefly, 0.5 mL extraction buffer II (30% sucrose, 2% SDS, 5% 2-ME, and 0.1 M Tris, pH 8) was added to 100 mg algae sample. The mixture was sonicated at 4 °C for 30 min. An equal volume of Tris-buffered phenol solution was added and the mixture was well mixed followed by 5 min incubation. The sample was centrifuged at 16000 × *g* for 5 min under 4 °C and the upper phenolic phase was collected (Awad and Brueck 2020). This procedure was repeated on the residual pellet for two more times and the collection of phenolic phase was combined. Then the solution was precipitated with five volumes of 0.1 M ammonium acetate in 80% methanol at −20 °C overnight. The mixture was centrifuged and the resulting pellet was rinsed with methanol and acetone. The pellet was resuspended in an appropriate volume of lysis buffer and incubated at room temperature for 60 min. After centrifugation at 18000 × *g* for 60 min, the supernatant was stored at −80 °C until use.

11.9.4 Phenol/TCA-Acetone Method

Phenol/TCA-acetone method is a combination of TCA-acetone method and the phenol method and it was based on the work of Wang et al. with some modifications. Briefly, 100 mg algae sample was resuspended in 1 mL prechilled extraction buffer I and sonicated in ice for 30 min. After centrifugation at 16000 × *g* for 5 min under 4 °C, the supernatant was discarded and the pellet was rinsed with 0.1 M ammonium acetate in 80% methanol and acetone with 0.07% 2-ME. Equal volumes of Tris-buffered phenol solution and extraction buffer II were added and the mixture was well mixed followed by 5 min incubation. The sample was centrifuged at 16000 × *g* for 5 min under 4 °C and the upper phenolic phase was collected (Niu et al. 2018). This procedure was repeated on the residual pellet for two more times and the collection of phenolic phase was combined. Then the solution was precipitated with five volumes of 0.1 M ammonium acetate in 80% methanol at −20 °C overnight. The mixture was centrifuged and the resulting pellet was rinsed with methanol and acetone. The pellet was resuspended in appropriate volume of lysis buffer and incubated at room temperature for 60 min. After centrifugation at 18000 × *g* for 60 min, the supernatant was stored at −80 °C until use.

11.10 Technologies of Proteomics

Proteomics mainly used for large-scale experiment. Such type of experiment requires specialized tools, which are developed for the design of the experiment. Three approaches of proteomics technologies have been identified. One is mass spectrophotometer, where the endogenous protein mixtures can be identified along with its analysis (Butterfield and Perluigi 2017). Array-based proteomics, which relates to cDNA microarray and oligonucleotide chips. A third area of proteomic analysis used for localization, metabolism, and physiological parameters of proteins.

11.10.1 Mass Spectrophotometer

Proteomics through the mass spectrophotometer (MS) has improved the analysis of a number of proteins, which means a number of proteins can be identified in a single experiment (Bruderer et al. 2017). The analysis of protein through MS mainly depends on the breakdown of the protein sample into its constituents with the use of specific enzymes such as sequence protease. The protein as a whole itself is of high molecular weight, which enables the analysis, as the protein cannot be eluted from the gel. For example, *C. reinhardtii* has been subjected to proteomics studies, in which around 240 proteins have been identified by providing heat shock. *Synechocystis* 6803 went through the compositional analysis of membrane protein complexes in different growth conditions.

11.10.2 Array-Based Proteomics

Proteomic array-based analysis used for the identification of a large set of proteins (Betzen et al. 2015). In this method, purified ligands are separated independently. It may be from protein, carbohydrates, or peptide from small molecules such as antibodies or antigens. It can be used for analysis of protein and its expression at the level of protein profiling. Different types of protein microarray formats such as tissue array, reverse-phase array, capture arrays, and lectin arrays have been identified, which are achievement importance in recent years. These tools are used in protein–protein interaction studies, immunological profiling, biomarker discoveries, and vaccine development. Such tools are providing meaningful biological insights into the modern biology of microalgae.

11.10.3 Next-Generation Proteomic Tools

Two-dimensional gel electrophoresis used for the separation of a large number of protein along with the MALDI-TOF-MS-based; however, they are time consuming and quantitative ability (Altelaar et al. 2013). Nano-liquid mass chromatography techniques have enhanced the quantitative analysis of the protein. Additionally,

isobaric tags for relative and absolute quantification method have the capability to identify the serum biomarkers and tissue biomarkers as well as drug resistance markers. Stable isotope labeling by amino acids in cell culture method is one more type of mass spectrometry, which is more proficient toward the cell culture system.

11.10.4 Quantification Methods

Selected reaction monitoring and are the best tools in the area of biomarker identification. Multiple reaction monitoring based analysis has been used broadly and has grown much significance in the proteomics study, as these methods have substituted some of the expensive approaches of quantification such as antibody-based study like immunoblotting and ELISA. Multiple reaction monitoring achieved on QTOFs as well as Orbit raps are called pseudo-multiple reaction monitoring as well as parallel reaction monitoring (Rauniyar 2015). In comparison, between multiple reaction monitoring and parallel reaction monitoring, the precise fragment ions acquired during acquisition are not possible through parallel reaction monitoring.

11.11 Post-Translation Modification

Post-translation modifications (PTMs) are used for the cellular processes and the cellular control. Protein activity is restricted by covalent modifiers like phosphate groups or proteolytic cleavage or by ubiquitin moieties. Protein turnover and localization as well as binding interactions can be affected through PTMs. Phosphorylation, methylation, acetylation, lipid modification, glycosylation, and ubiquitination are some of the PTMs which can affect the cellular processes. They directly disturb the protein structure subsequent in the change in the task of the given protein. As such modifications result in the difference in the molecular mass of the amino acid, these differences are significant for being studied in detail. Mass spectrometry, great mass exactness and skill to deal with intricate mixtures are some of the opportunities for describing PTM. A wide explanation of protein stability in the stroma of microalgae, their post-translation modifications and the linking between the two were established. It comprised 2D-gel electrophoresis for the sequestration of the stromal proteins, their arrangement and the description of the PTMs by mass spectrometry joint with bioinformatics action of the data (Mnatsakanyan et al. 2018).

11.12 Metabolomics Approaches in Microalgae

Metabolites are the end products of cellular regulatory processes. Their levels can be regarded as the ultimate response of biological systems to genetic or environmental changes. Similar to the “genome,” “transcriptome,” and “proteome,” the metabolome denotes to the set of low molecular weight metabolites present in a cell (Deidda et al. 2015). The size of the metabolome differs greatly depending on

the organism studied. The metabolic network of *S. cerevisiae* was formed using genomic, biochemical, and physiological information (Lopes and Rocha 2017). Even within the metabolome of one organism, many different kinds of metabolites exist, with different chemical and physical properties. Moreover, the levels at which various metabolites are present within a cell can cover several magnitudes of concentration. The vastness of the metabolome and its diversity make it technologically impossible to analyze all an organism's metabolites in a single analysis. Different strategies and methodologies have, therefore, been developed. Metabolite target analysis is restricted to one or a few metabolites related to a specific metabolic reaction and as such does not constitute a high-throughput approach. This strategy is mainly used for screening purposes (Bingol 2018). To elucidate the function of a metabolic pathway, the metabolite profiling approach identifies and quantifies a selected number of predefined metabolites, which belong to a certain class of compounds or to a specific pathway. Metabolic profiling is often used in the context of drug research to study drug candidates (Trifonova et al. 2013). Two approaches have been picked for metabolome analysis, namely metabolic fingerprinting and metabolic profiling. Metabolic fingerprinting is an untargeted analysis of a sample, allowing comparison of metabolite patterns. Metabolomics profiling represents targeted analysis of specific metabolite classes, providing quantitative data for physiological interpretations. Finally, in metabolic fingerprinting, a rapid, global analysis is performed for sample classification according to origin or biological relevance. Quantification and metabolic identification are generally not necessary, allowing for a higher throughput of samples. In algae, most metabolic analyses have so far been focused on the quantification and identification of secondary metabolites with economical value in food science, pharmaceutical industry, and public health, among others. Fatty acids, steroids, carotenoids, polysaccharides, lectins, polyketides, and algal toxins are among the algal products being studied. Environmental metabolomic studies, in contrast—as the application of metabolomics to characterize the metabolic response of an organism to environmental stimuli or stressors—have to date only rarely been carried out in algae. They describe a procedure for cell preparation and metabolite extraction. In extract chromatograms of standardly grown algae, more than 800 metabolites could be detected, with Ala, pyruvate, Glu, glycerolphosphate, and adenosine 5-′-monophosphate being among the most prominent peaks. When cells were grown under nutrient deficient conditions (depletion of nitrogen, phosphorus, sulfur, or iron), highly distinct metabolic phenotypes were observed. Multidimensional "omics" technologies provide a platform to enhance our understanding of complex biological systems by describing nearly all biomolecules ranging from DNA to metabolites which is being shown in Table 11.1.

Table 11.1 Omics-based investigations in algae

Stress conditions	Study	Organism	References
Transcriptomics			
Light	Intense	*Chlamydomonas reinhardtii*	Stoffels et al. (2019), Xu et al. (2019), Sasso et al. (2018)
Nutrient deprivation	Sulfur Phosphorus Nitrogen	*C. reinhardtii* *C. reinhardtii*	Sasso et al. (2018)
Oxidative stress	Single oxygen Dark anoxia	*Thalassiosira pseudonana* *Neochloris oleoabundans*	Schaum (2019), Rashidi et al. (2019), Zainul Azlan et al. (2019)
Salinity	Transcriptomics and metabolomics	*Chlorella vulgaris* *C. reinhardtii* *C. reinhardtii* *Ectocarpus sp.*	Xu et al. (2019), Xu et al. (2019), Montecinos et al. (2017)
Proteomics			
Light	Intense High light and inoculum size	*C. reinhardtii* *Chlorella sorokiniana*	Stoffels et al. (2019), Reen et al. (2019)
Temperature	Low High	*C. reinhardtii*	Xu et al. (2019)
Nutrient deprivation	Nitrogen Waste water cultivation	*C. reinhardtii* *T. Pseudonana*	Xu et al. (2019), Kageyama et al. (2018)
Oxidative stress	Induced by addition of acetate and Fe2+, and exposure to excess light intensity	*Haematococcus pluvialis*	Niizawa et al. (2018)
Herbicide	Paraquat and Glufosinate	*C. reinhardtii*	Sasso et al. (2018)
Metabolomics			
Nutrient deprivation	Nitrogen	*C. reinhardtii*	Stoffels et al. (2019)
Toxicity	Effect of salinity, light, and nitrogen on differential lipid production	*Nannochloropsis sp.*	Rashidi et al. (2019)
Various stresses	Copper	*C. reinhardtii*	Xu et al. (2019)

11.13 Metabolic Engineering in Algae

Genomic manipulation remains restricted to a few select microalgal laboratory models (e.g., *C. reinhardtii* and *Phaeodactylum tricornutum*). The increasing curiosity in the area of microalgal biofuels will probably lead to the development of new techniques in other micro-organisms and the establishment of novel model systems. Microalgal trans-genics has been formerly reviewed (Radakovits et al. 2010);

nevertheless, the "molecular toolkit" has since expanded due to latest seminal studies. Noteworthy advances contain: (a) the effective expression of transgenes; (b) a new mechanism for gene regulation in microalgae using riboswitches technology; (c) luciferase reporter genes and inducible nuclear promoters (Brogan et al. 2012); and (d) inducible chloroplast gene expression (Doron et al. 2016). Genetic and molecular tool information are mandatory along with metabolic pathways. The sequence analysis will contain the information on metabolomics which opens the part for the metabolic flux examination and the development of metabolic networks. Microalgae having a single cell which are simply cultivable can be concentrated toward metabolic engineering. Moreover, these microalgae perform photosynthesis which aids in understanding the carbon assimilation; this will enhance the research toward bioenergy manufacture. In a recent study, it is deciphered that an microalgae *Volvox carteri* has been transformed genetically (Umen and Olson 2012). Similarly, trophic conversion of green microalgae *C. reinhardtii* and *P. tricornutum* was also described which is a good step in the direction of establishing single gene change. Thus, engineered light-harvesting strains have higher photo-damage resistivity as well as amplified light penetration capacity. The time span from primary transformation to manufacture level is less compared to mammalian-based platforms. It is well recognized that algae is the main source for the manufacture of diverse metabolites and proteins. In numerous studies, *C. reinhardtii* has been reported as a good source for the manufacture of fatty acids.

11.14 Future Needs: Integrating "Omics" in Systems Biology

Thanks to the availability of complete genome sequences and the advent of high data content measurement techniques for transcripts, proteins and their interactions and metabolites, a new level of understanding of cells and organisms has become possible. As already mentioned in the introduction, in systems biology, the main goal is to develop a comprehensive and consistent knowledge of a biological system by investigating the behavior of and interaction between its individual components. One of the key steps in this process involves modeling. Once the structure of the system is unraveled, mathematical algorithms allow its dynamics to be modeled. Mathematical models describe the system, but also allow the prediction of the system's response to perturbations. A framework for systems biology studies was formulated, involving several distinct steps. In a first step, understanding of the structure of the system is required (Lauritano et al. 2019). This involves the identification of the elements of the system, such as gene networks, protein interactions, and metabolic pathways. This knowledge is used to construct an initial model of its behavior (Cooper and Smith 2015). Depending on how much is known about the different components of the system the modeling can be carried out at several levels. Steady-state studies can be done even when knowledge on certain parameters is lacking. Using the available knowledge on the system's structure theoretical upper and lower limits are calculated, as well as an optimal operation point of the system in steady-state. Other types of analyses allow for an

understanding of the dynamic properties of a system, but require knowledge of certain parameters. Secondly, the system is perturbed (genetically or using environmental stimuli) and corresponding responses are measured using high-throughput measurement tools. Data observed at different levels of biological organization are integrated with each other and with the current model of the system. Followingly, the model is adapted in such a way that the experimentally observed phenomena correspond best with the model's predictions. A new set of perturbations is selected and applied to the model, to distinguish between multiple model hypotheses. These steps are continually repeated, thereby expanding and refining the model until the model's predictions reflect biological reality. It is obvious that a systems biology analysis requires collective efforts from multiple research areas, such as molecular biology, computer science, and control theory and is therefore not easy to accomplish. Recent and ongoing studies putting the above systems biology framework into practice have focused on bacterial chemotaxis inf. Coli, sugar metabolism in *S. cerevisiae*, and embryo development in the sea urchin, among others. For algae, no systems biology studies with extensive computational modeling efforts have been reported so far to our knowledge. However, more and more knowledge of the components of algae has become available and the first results on the integration of observations on different levels of biological organization are being published. Metabolite, genomic, and transcriptomic data were used to provide genome-wide insights into the regulation of the metabolic networks utilized by *Chlamydomonas reinhardtii* under anaerobic conditions associated with H2 production. During acclimation to anoxic conditions the hydroge-nase activity, photosynthesis, cellular respiration, and organic acid accumulation of algal cells were monitored. In conjunction with the formation of fermentation products the levels of transcripts encoding proteins associated with the various fermentation pathways were analyzed using real-time PCR. After establishing that the algae were acclimated to the anoxic conditions microarray analyses were carried out to gain insight in the effects of dark anaerobiosis on transcript abundance in a genome-wide context. Results showed that congruent with elevated H2 production following exposure of the algae to anaerobic conditions was the accumulation of various fermentation products. These findings on metabolome level were augmented with real-time PCR analysis. Results obtained by microarray analysis were in agreement with the real-time PCR data. In addition, genes encoding proteins involved in other metabolic pathways as well as encoding transcription/translation regulatory factors were differentially transcribed (Salama et al. 2019). Although this study cannot be considered a true systems biology approach yet—rather than relating individual elements quantitatively to each other and to the covering system, a qualitative analysis is made—here we see the onset of the integration of data gathered on different levels of biological organization to improve our understanding of (a metabolic network of) a biological system.

11.15 Applications of Omics Approaches

Omics is a widely used technique in biological fields, mainly applied in Oncology (Tumor biology), Bio-medicine, Agriculture, and Food Microbiology.

11.15.1 Oncology

Oncology is the study of tumor cell. Tumor metastasis, is the process spread of cancer from one organ to another non-adjacent organ cause death in patients. The main challenge in medicine to describe the molecular and cellular mechanisms of tumor metastasis. Protein expressions analysis and metastatic process help to understand the mechanism of metastasis and facilitate the development of strategies for the therapeutic interventions and clinical management of cancer (Yu and Snyder 2016). Proteomics is a systematic research, the main aim of this research is to characterize the protein expressions, functions of tumor cells and is widely used in biomarker discovery.

11.15.2 Biomedical Applications

Interactions of microbial pathogens with their hosts is called "infectomics." It is a very interesting area in proteomics. It deals with the fundamentals of the infections origin and their effect on organs. The key aim of this research is to stop or cure disease at starting level. Advanced diagnostic issues associated to emerging infections, increasing of fastidious bacteria, and generation of patient-tailored phenotypes (Vladareanu et al. 2016).

11.15.3 Agricultural Applications

The applications of plant proteomics scientific research is still in promising stage. Plant proteomics is also used to know plant–insect interactions that help identify candidate genes involved in the defensive response of plants to herbivore (Van Emon 2016). Population growth and effect of global climate changes imposing severe limits on the sustainability of agricultural crop production.

11.15.4 Food Microbiology

The use of omics technology in food technology is presented mainly for characterization and standardization of raw materials, process development, and detection of batch-to-batch variations and quality control of the ending product. Further attention is paid to the aspects of food safety, especially regarding biological and microbial safety and the use of genetically modified foods (Walsh et al. 2017).

11.16 Conclusions

Despite the growing number of completed microalgae genome sequences, only a few examples of genetic engineering of the metabolism for the production of by-products have been reported. Nevertheless, metabolic engineering in model strains of microalgae will likely provide important leads for proof-of-principle studies in the future. Furthermore, the complete genome sequences provide a valuable framework for the huge amounts of marine metagenome projects. Comparative genomics is a powerful tool to unravel previously unknown gene functions. Each algal genome project provides enough genome sequence data to allow comparative analysis of each genome. It is based on small genome repertoires, particularly for unicellular algae; algal genome information should accelerate studies on, for example, the establishment of cellular components, and it will allow us to elucidate cellular and molecular properties that they have in common with land plants or other eukaryotes. Principally omic projects should aim at different microalgae features such as evolution, adaptation, and divergence compared with other species, gene, protein, and metabolite information, and their interaction. This would facilitate an understanding of the biology of microalgae in detail and the application of these concepts in the production of valuable products.

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Part III

Industrial Waste Management

Waste Utilization and Minimization in Food Industry

12

Soumya Rathore and Anand Kumar Pandey

Abstract

Industrial waste is causing huge damage to the environment by contributing towards pollution. Food industry is also an active participant in promoting pollution. By using food industry waste as fuel, feed or food we can not only minimize the waste but can also reduce the cost of food products. This ensures environmental advantages and great value of money. Food waste can also be utilized for manufacturing of biodiesel, ethanol, methanol, hydrogen gas and methane gas. Although this is not the end of the list of products which can be synthesized by this means, many other value-added products can be produced by utilizing food industry waste. This chapter will give detailed account for the manufacturing of the above products and deals with the prospects related to waste utilization and minimization in food industry.

Keywords

Food waste · Waste utilization · Waste management · Food waste to energy generation

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12.1 Introduction

What is food waste? Any edible and inedible parts of food, both precooked and leftover, can be recovered or reused for consumption. Food waste is generated from different sources like food supply chain, processing industries, hospitality sector like restaurants, household practices and traditional occasions like parties, festivals and marriages (Parfitt et al. 2010). According to the Food and Agriculture Organization (FAO), 'Food waste refers to the discarded or alternative (non-food) use of food that is safe and nutritious for human consumption'. Wastage of food should be avoided as much as possible but sometimes it is unavoidable in nature. 'Avoidable food waste' is defined as food or drink that was at some point prior to disposal, edible (e.g. slices of bread, apples, meat), while 'unavoidable food waste' is waste arising from food and drink preparation that is/was not edible under normal circumstances (peels of fruits and vegetables, bran of cereals, etc.) Though the nutritional value of unavoidable food waste is as good as normal food but it needs some additional treatments and processing to make it usable for consumption (WRAP 2009). From past few years, food waste has been considered one of the major global environmental issues. It not only has environmental impact but it also affects the ethical values, as many people around the world are suffering from hunger and food insecurity. In food processing sector, many by-products are obtained after the processing and contribute to food waste as they are discarded as such but they have the similar nutritional value as the food from which they have originated. The examples of such type of by-products are—whey obtained from dairy industry; bran, husk and germ obtained from milling industry; seeds, skin and peels obtained from fruits and vegetables processing industry (Stuart 2009). When we refer to food waste or food surplus, we often get confused with other related proper nouns. Food surplus or food waste has different definitions when considering different aspects. Common terms relating to food waste and food surplus are food surplus, food loss, and food waste. According to the FAO, food waste in developed countries (such as Germany, Italy, the USA, Japan, Taiwan and India) is generally estimated at the retail and consumption stages. Due to improvement in living standards, enterprises either adjust product size to cater to customer psychology or will remove large amounts of edible parts in pursuit of high-quality products. Therefore, huge amount of vegetables, fruits, and other food are wasted due to their shape or expiry date, as well as other similar reasons. Hence in present scenario, food waste issues are of great importance and are a matter of growing concern for many countries worldwide. Considerable research is already being done on investigating food material waste from origin to retailers, and also restaurant management system has implied the zero-food waste strategy to combat this situation to some extent (Huang et al. 2020).

India has high cultural diversity and food occupies a special part in every festival or cultural event. After all these events, a huge amount of leftover food is thrown away as waste. This leads to wastage of money, resources and hard work of our farmers and contributes to pollution due to disposal of this wasted food. Food wastage is a crucial situation in India as according to a report the wastage of food

in India is nearly equivalent to the total consumption of United Kingdom. Our streets and garbage bins, landfills are enough proof to prove it (Choudhury 2006). In fact, according to the agriculture ministry, Rs. 50,000 crore worth of food produced is wasted every year in the country. India ranks 103 among 119 countries in Global Hunger Index 2018. Wastage of food is not indicative of only hunger or pollution, but also many economic problems in the country, such as inflation. Only government policies are not responsible for the problems we are facing today, but our culture and traditions also form a part. One of our moral responsibilities is not to waste food. Food is also wasted in many phases from farm to fork, for example, during cultivation, handling, transportation, processing, storage and consumption. So, proper utilization by making value-added products and minimization of the food waste can lead to a better economical outcome. Hence, this chapter elaborates the utilization of food waste by making value-added products like energy sources (biofuels), livestock food and other valuable food products and their related advantages and disadvantages.

12.2 Current Status of Food Waste Generation

According to FAO estimates, 1/3 of all food produced globally is lost or goes to waste. Globally, approx. 1.3 billion tons of food is dumped to dustbin every year making one billion people hungry (Gustavsson et al. 2011). Food wastage is generated from different points of supply chain from production to consumption. Wastage of food is equally problematic in both developed and developing countries. In medium- and high-income countries food is to a great extent wasted, meaning that it is thrown away even if it is still suitable for human consumption. Significant food loss and waste do, however, also occur early in the food supply chain. In low-income countries food is mainly lost during the early and middle stages of the food supply chain. Developed countries like USA are also dealing with the food wastage. 8.3-million-ton food is going to garbage and contributing to the carbon emission in the USA (Wrap 2009). According to United States Environmental Protection Agency (USEPA), USA produces approximately four million tonnes of food waste annually. UK also throws away about 25% of household food to the garbage. 20-million-ton garbage is produced by Japan every year (Matsumura et al. 2005). So, the current food waste scenario is perturbing and is required to be counteracted as efficiently as possible (Fig. 12.1).

India is the world's second largest producer of fruits and vegetables but still we are hungry, or malnutrition is haunting us. According to FAO, every third malnourished child is Indian. The reason is that we are throwing fresh fruits and vegetables worth billions due to shortage of proper storage facility. According to the Central Institute of Post-Harvest Engineering and Technology, India, 18% of fruits and vegetables produce in India are wasted due to lack of proper handling and storage facilities. Food wastage is one of biggest problem of India. According to the agriculture ministry of India INR 50,000 crores food is being dumped to garbage every year. Food industry throws the unutilized raw food material. However, these

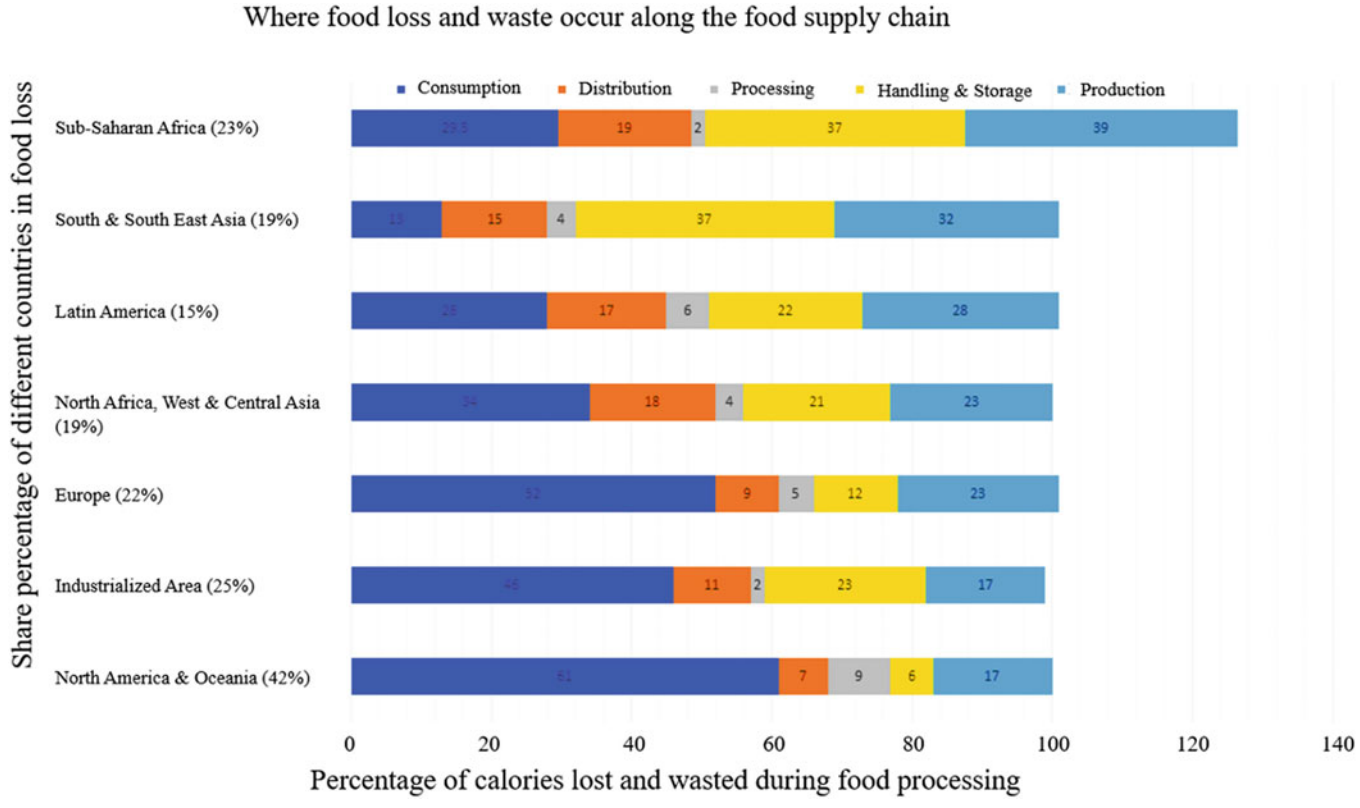

Fig. 12.1 Food loss and waste generation along the food supply chain

are full of nutrition. Wastage of fresh produce leads to loss of income for farmers and higher rates for customers. Food wastage is fast assuming serious dimensions. According to the Food and Agriculture Organization (FAO), a staggering 1.3 billion tonnes of food is being wasted annually. The FAO report further states that one-third of the total global food production is wasted, costing the world economy about $750 billion or Rs. 47 lakh crores (Baig et al. 2018).

Food wastage is an issue that needs global scale attention. According to a report by the National Resources Defence Council (NRDC), 40% of the food goes uneaten in the USA, whereas Asia, India and China cause a loss of 1.3 billion tonnes of food every year. In terms of overall food waste—agricultural produce, poultry and milk—India ranks seventh, with the Russian Federation at the top of the list. India's lower ranking is because most of the countries ranking above it utilize much of their land in raising poultry, while a major chunk of land in India is under agriculture and this explains the highest wastage of cereals, pulses, fruits and vegetables that occurs in India. A recent study conducted by Indian Institute of Management, Kolkata, revealed that only 10% of foods get cold storage facility in India, this factor, accompanied by inappropriate supply chain management, has resulted in India becoming a significant contributor towards food wastage both at pre and post-harvest levels in cereals, pulses, fruits and vegetables (Baig et al. 2018).

The government has made many efforts to reduce food wastage but clearly, the depth of the problem is such that the impact of these efforts is hardly up to the mark. India should also take a cue from global practices that are both unorthodox and innovative in order to tackle food wastage problem. For instance, France has passed unanimous legislation requiring supermarkets to either give unsold food to charity or send it to farmers for use as feed and fertilizer. Similarly, institutions in Canada are recovering unused and unspoiled food from retailers, manufacturers, restaurants and caterers and sending them to charities, in the process of delivering ingredients for over 22,000 meals daily. These powerful initiatives have made a big difference in how these countries have approached a vexing issue. India can effectively use technology to script a new chapter in the prevention of food wastage. The government can speed up research in nanotechnology with the help of which eco-friendly and healthy food preservation applications can be invented that are helpful in preserving food for longer duration and keeping farm produce fresh. In addition to these efforts, the government must make it mandatory for the food retailers across the country to adopt technology standards that allow incentives for the customer to purchase perishable products that are approaching their expiration dates. This will help reduce food wastage, maximize grocery retailer revenue and effectively reduce the global carbon footprint (Bilali and Hassen 2020).

12.3 Minimization and Utilization of Food Waste as Energy Source

Food waste contributes to a large part of municipal solid waste. Due to surplus availability of food waste, it can be a promising source for bioenergy production (such as bio-coal, hydrogen and methane). It is a less energy intensive process and the bio-coal produced by food waste has high energy value. This bio-coal generated by food waste can be used to setup thermal power plant thus can resolve the problem of scarcity of electricity along with utilization of wasted food. Enzymatic hydrolysis and dark fermentation can be used as combined process for the biofuels production. The calorific value of the charcoal produced by food waste has been found good in comparison to the quality of anthracite in many researches. This coal can be an alternative source of conventional coal in thermal power plant. Problem of coal scarcity can be solved to some extent as it is known that natural resources are limited, and it takes years to make coal by natural process. Food wastes from residences and other commercial institutions can also be utilized to produce energy source like ethanol, biodiesel, methane and hydrogen (Hussein et al. 2018).

12.3.1 Ethanol Production by Food Industry Waste

Ethanol production from food waste is gaining popularity day by day. Acid (e.g. sulphuric acid) pre-treated food waste hydrolysate could be used for ethanol production by using immobilized *Saccharomyces cerevisiae* (Gundupalli and Bhattacharyya 2019). Carbohydrates are the major components of food materials. Enzymatic hydrolysis converts the complex carbohydrates into simple sugars and then these sugars are fermented to ethanol (Davis 2008). Some researchers have also been done with simultaneous saccharification and fermentation (SSF) experiments. Production of ethanol from waste is popular among researchers due to ecological and economic reasons because it provides an alternative to petroleum-based fuels and reduces the waste. In past studies, optimum temperature and pH for ethanol production were reported as 38 °C and 5.45, respectively (Le Man et al. 2010).

High solid fermentation is favourable for the ethanol production, however, high ethanol concentration inhibits yeast activity thereby in turn affecting the ethanol yield and efficacy of fermentation (Saricks et al. 1999). Different modified methods have been used in various studies for enhancing the ethanol yield. Integrated vacuum fermentation with product recovery reduces ethanol inhibition in yeast (Table 12.1). So vacuum fermentation enables complete utilization of glucose in the fermentation broth giving higher yield of ethanol productions than conventional fermentation (Huang et al. 2015).

Table 12.1 Studies on utilization of food waste for ethanol production

S. No.	Type of food waste	Enzyme and organism	Treatment	References
1.	Cake waste	Saccharomyces *cerevisiae*	Hydrolysis fermentation	Han et al. (2019)
2.	Local retail store mainly mashed potatoes, sweet corn and bread	Saccharomyces *cerevisiae*	Vacuum fermentation	Huang et al. (2015)
3.	Food waste from dining room	Fungal alpha amylase, glucoamylase and Saccharomyces *cerevisiae*	Enzymatic saccharification fermentation	Alrumman (2016)
4.	Cafeteria food waste	Aspergillus *niger* glucoamylase and Saccharomyces *cerevisiae*	Enzymatic saccharification fermentation	Kim et al. (2008)
5.	Cafeteria food waste	Amyloglucosidase, Carbohydrases and Saccharomyces *cerevisiae*	Enzymatic saccharification fermentation	Moon et al. (2009)
6.	Food waste leachate	Saccharomyces *cerevisiae*	Fermentation	Le Man et al. (2010)

12.3.2 Biodiesel Production by Food Industry Waste

Biofuels necessity is rapidly growing worldwide because natural sources of fuels (petroleum-based fuels) are limited (Heo et al. 2015). Biodiesel is a popular biofuel. They are widely accepted and commercially produced in different countries. Biodiesel production from food waste is another promising area of food waste utilization. Cultivated microalgae are used to produce biodiesel and remove nutrients from various wastewaters. *Tetraselmis suecica* microalgae gives very significant effect on biodiesel production. Lipids as a significant food constituent are required for biofuel production. Thus, industrialization of biodiesel production can provide solution to waste disposal, and energy scarcity problems to a large extent (Karmee and Lin 2014).

Production of biodiesel from edible vegetable oils is more expensive. Using edible oil for biodiesel production is not cost effective and will affect food supply. So, utilization of food waste for extraction of triglycerides and fatty acids will work as a cheaper source for biodiesel production than the fresh vegetables oils. Also, no food security issue will arise. Generally fatty acids are extracted from early stage of food waste liquefaction. Wet and dry extraction methods are used for the fatty acid extraction. Pre-treatment of raw materials is not required in some cases. Wet methods provide many benefits over dry methods by minimizing the loss of short and medium-chained fatty acids as well as reducing the number of steps required, so it yield higher amount of fatty acids as feedstock. Biodiesel produced from the food waste meets the primary standards to be used as the biodiesel (Redzwan et al. 2017).

Food waste leachate was used for oil recovery from food waste which was then utilized for the biodiesel production (Yu et al. 2011). Studies show that production of

Table 12.2 Recent studies on biodiesel production from food waste

S. No.	Type of food waste	Enzyme and organism	Treatment	References
1.	Liquid waste of tofu industry	*Chlorella* spp.	Aerobic and anaerobic degradation	Widayat et al. (2019)
2.	Food waste derived volatile fatty acids	*Rhodococcus* spp. YHY01	Anaerobic digestion	Bhatia et al. (2019)
3.	Sugarcane bagasse	*Chlorella* spp.	Acid hydrolysis	Manzoor et al. (2019)
4.	Primary waste water sludge	Cellulase, *Saccharomyces cerevisiae*	Solvent extraction, fermentation	Ngoie et al. (2019)
5.	Mackerel fish waste		Thermally induced transesterification	Jung et al. (2019)

biodiesel from the oil fraction separated from food waste leachate can be a good alternative source for the biodiesel production (Table 12.2). High acid value is exhibited by the food waste leachate oil fraction so, pre-treatment is necessary. Acid catalyst, for example, sulphric acid and Amberlyst-15, etc. is used for maintaining the acid value of food waste leachate oil fraction (Yu et al. 2011). Process optimization and additional refining are expected to produce high-quality biodiesel from the food waste leachate oil fraction.

Liquid waste is generated from tofu industry in huge amount. Nutrient profile of tofu wastewater is highly favourable for microalgae production. Chlorella sp., microalga can be used as biomass for utilization of tofu waste water and biodiesel production. Studies showed that concentration of tofu waste in microalgae cultivation media had very significant effect on lipid levels of Chlorella sp. (Widayat et al. 2019). Other wastes like rice straw, corn straw, sugarcane bagasse and wheat straw are some major agricultural wastes which can be utilized (Panpatte and Jhala 2019). Sugarcane industry waste could be utilized as the low-cost alternative for the carbon source for cultivating microalgae (Manzoor et al. 2019).

12.3.3 Hydrogen and Methane Gas Production by Food Industry Waste

Steam reforming, thermal cracking of natural gas and electrolysis of water to produce hydrogen gas are cost and energy intensive processes. Again, for the hydrogen production biological methods are safer and cost effective than thermochemical methods (Kim et al. 2008). Additionally, food waste disposal is a serious problem which can be reduced by using food waste for hydrogen production (Table 12.3). This provides the dual benefits of controlling of environmental pollution and providing an alternative energy source. Hydrogen can be produced by two types of fermentation, one is photo fermentation, and another is dark fermentation.

Table 12.3 Recent studies on hydrogen and methane gas production from food industry waste

S. No.	Type of food waste	Inoculum	Treatment	References
1.	Food waste	Anaerobic sludge from waste water treatment plant	Upflow anaerobic biofilter reactor	Hassan et al. (2020)
2.	Food waste and chicken manure	Chicken manure	Batch fermentation	Yusof et al. (2019)
3.	Mixture of tofu, lettuce, rice, pork and steamed bread	Anaerobic sludge from waste water treatment plant	Dark fermentation	Yuan et al. (2019)
4.	Mixture of coconut milk and starch	Hydrogen producing bacteria of coconut milk and sludge	Anaerobic fermentation	Wongthanate and Khumpong (2015)
5.	Rice slurry	Anaerobic digester sludge	Batch fermentation	Fang et al. (2006)
6.	Cheese whey and buffalo manure	Anaerobic digester sludge	Dark fermentation	Yin et al. (2014)
7.	Rice, fish, vegetable and mixed food waste (mixture of fish, vegetables and rice)	Food waste	Anaerobic fermentation	Godday et al. (2014)

For photo fermentation, sunlight is required, and it provides high yield. Photo fermentation for production of hydrogen gas can be achieved by algae, cyanobacteria and photosynthetic bacteria (Kim et al. 2008). The dark fermentation is also known as heterogenic fermentation, generally done by anaerobes and microalgae. They consume the carbohydrate rich substrates and produce hydrogen without the presence of light (Ding 2016). Alkaline, ultrasonication and thermal pre-treatment enhance the solubilization of food waste. So, this can increase the yield of volatile fatty acids, hydrogen and methane production.

Pre-treatment techniques affect the food waste solubilization differently. Alkaline pre-treatment increases the hydrogen yield. Past studies have reported that ultrasonication reduces processing time to 38 h from the 60–80 h needed in normal operation (Menon et al. 2016). Different methods of pre-treatment enhance the different parameters. Alkaline pre-treatment is found more significant for increasing the yield of hydrogen production (Menon et al. 2016). Ultrasonication pre-treatment of food could be used for the reduction in the processing time. Combined process by using the ultrasonication and alkaline pre-treatment has synergistic effect on hydrogen production. For methane production combination of thermal pre-treatment and ultrasonication pose significant effect.

12.3.4 Advantages of Utilization of Food Waste as Energy Source

As India is leading at the second position in population and generating a huge amount of food wastes. Utilizing these wastes to obtain energy can be a better approach towards minimization of the wastes and fossil fuel compensation. Because the fuel is one of the finite resources of nature, its demand is increasing day by day. The production of bioethanol, biodiesel and hydrogen gases adjusts the level of requirement of fuels. The process of energy generation from food wastes is also economically feasible in an easy way (Sarkar et al. 2012).

12.4 Minimization and Utilization of Food Waste as Livestock Feed

Human being depends on livestock for food products like meat, egg and milk. Available modern treatment technologies can be utilized for conversion of food waste into usable product that could be safe, nutritious and value-added feed products. Food waste utilization as animal feed is very promising solution for waste utilization and pollution control. This conserve the resources also as it is known that food resources are very limited. Food waste is instinctively linked to food security. Wastage of food also wastes the resources, which were utilized for the food production like land, labour, water, energy, etc. Thus, the food which is inedible for the human beings can be utilized by the animals as feed. So, this type of utilization can lead to a win situation (Dou et al. 2018).

12.4.1 Poultry Industry

The most used feedstuff for the poultry industry is maize (*Zea mays*). But nowadays, the local demand of maize is exceeding its production yield except in Thailand (Ravindran 2014). So, there is shortage of maize to be used as a feedstuff for the poultry farms. The utilization of by-products of food can fill the gap by its nutritional values equivalent to maize. Rice bran contains 13% protein, 13% fat, 13% crude fibres and is a good source of vitamins and minerals. Similarly, barley also contains 8–16% of protein so these by-products of food industry can be a good substitute to feed the poultry industry (Ravindran 2014). The wastes of some subsectors of food processing industries are shown in Fig. 12.2 in which unmilled grains, low tannin grains from cereal processing industry, detoxified and ground sweet potato tuber from root and tubers processing industry and starch, molasses, oil residues from other industries can substitute the feed to the livestock production of poultry.

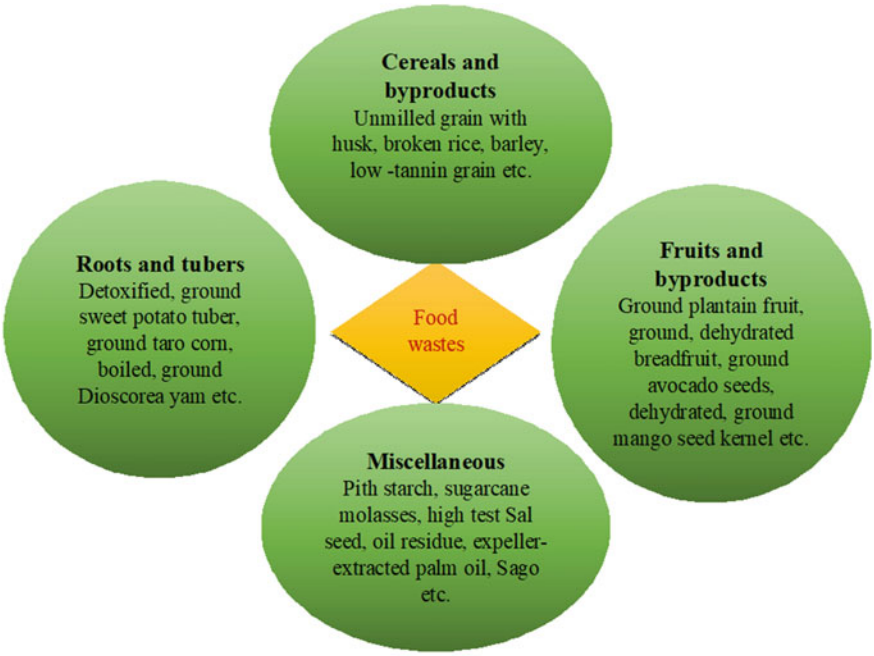

Fig. 12.2 Utilization of food wastes as feedstuffs for poultry

12.4.2 Dairy Cattles

Utilization of food wastes as cattle feed can be another approach to utilize the food wastes rather than disposing it or using it in landfills (Salemdeeb et al. 2017). It is not like that the food waste is directly fed to the cattle there are strict guidelines and regulations to ensure the food safety for the livestock. A proper treatment and analysis should be done before using the food wastes as feed for the cattle to ensure the fulfilment of food safety objectives. Food waste conversion to animal feed provides potential benefits. As feeding municipal food waste to animals is illegal in many countries it is required to utilize safe practices for processing of food waste to animal feed. Enzymatically digested fruits, vegetables, meat and dairy food waste could be used to generate usable food waste products. Pigs were investigated for the enzymatically digested food in respect of their growth, which was found to be good for pigs (Jinno et al. 2018). Studies claim that use of *B. subtilis* fermentation can enhance nutrition of okra (agro-waste from soybean industry), that could be used as low-cost animal feed and could be a part of the human diet (Mok et al. 2019).

The food wastes are highly organic in nature. Many developed countries follow the strategy of reducing, recovering, recycling, landfilling and treating the food waste for the conversion of valuable products. Although landfilling is the least preferable option still it is not fully stopped anywhere in the world. Recycling of the food wastes as animal feed has proved itself to be a good alternative in practice

from centuries which reduces the amount of waste disposal into the environment. It also saves the environment from hazardous effects (Dou et al. 2018).

12.5 Utilization of Fish Processing Industrial Waste

Aquaculture provides good earning to thousands of people as it is the most rapidly growing sector throughout the world. Fish production has increased from 69 million to 93 million tons in last 30 years (FAO 2012). Waste generated from fish processing industry of India is approximately 30–50% which mainly includes skins, bones, fish fillets, whole and gutted fish, fish sticks, shrimp products, etc. (Anon 2005). This waste has very good nutritional properties and can be used as a raw material for the processing of highly nutritious products. These products are an excellent source of protein. The recovery of chemical components from seafood waste materials and fish processing units, which can be used in other segments of the food industry, is a promising area of research and development for the utilization of fish waste by-products. These wastes cause a serious problem of environmental pollution and make the environmental atmosphere unhygienic, prone to various kinds of diseases. The best alternative way is to convert these wastes to value-added bioactive and different products for the use of mankind and other animals.

Food wastes as fish feed could be a better alternative for fish food because it minimizes food waste. Formulated feed pellets from food waste could be used to culture a few freshwater fish species, such as grass carp, grey mullet and tilapia, under polyculture mode. These species occupy different ecological niches, with different feeding modes (i.e. herbivorous, filter feeding, etc.), and therefore all the nutrients derived from the food waste could be efficiently recycled within the ecosystem. Different types of food waste could fill the requirement of different nutrients. So, the appropriate portions of different types of food waste can be used that could fulfil the basic nutritional requirements of lower trophic level fish species. Pig manure serves as pond fertilizer to promote growth of fish (Liang et al. 2016). A research observed fish meal can be replaced by the poultry by-products for culturing hybrid striped bass (Rawles and Gaylord 2006). Food and industrial wastes (from poultry, soy sauce, rice wine, beer and papaya producing/processing industries) could be used for producing fish feed pellets (Kang et al. 2010). Nutrients rich food wastes like meat and fish waste, fruits and vegetables waste could be an alternative for the original raw materials for animal feed (García et al. 2005). Studies indicated that different formulations of food waste based fish feed are beneficial for fish culture due to availability of different nutrition. Enzyme addition, vitamin-mineral premix, probiotic (yeast), and prebiotic fibre could enhance the nutritional quality of fish products. It is found that enzyme supplementation in animal feeds in poultry and swine industries is very effective for enhancing the nutritional quality of their dung which is used as fish feed (García et al. 2005).

Fish waste is a good source of enzymes and bioactive peptides found in the fish gut and can be used for the production of fish sauces, fish feed and fish silage.

Isolation and purification of proteolytic enzymes is an efficient method for the conversion of enzymes into valuable products. These enzymes have a variety of applications in leather industry, food and pharmaceutical industries (Mohanty et al. 2018). Another important utilization of fish waste is fish oil obtained from whole fish or liver. Fish oil is an excellent source of polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that helps in the prevention of blood pressure fluctuation and arrhythmias. Some studies reported that shark liver oil contains a lipid known as squalene, basically a bioactive oil, and has good application in the treatment of diabetes, cancer and tuberculosis. It also has antifungal and antioxidant properties (Stansby 1967). The waste of shell fish is rich in a polysaccharide, chitin, obtained from the crustacean shell waste. Chitin has both food and non-food based application. Food based applications include strong antimicrobial activity, non-toxicity, biodegradable properties, while non-food based applications include dental and surgical uses, photography and cosmetic industry. It acts as an ingredient for the skin cream, shampoo and toothpastes (Rajalekshmi and Mathew 2007).

12.6 Utilization of Fruits and Vegetables Industrial Waste

The production, utilization and consumption of fruits and vegetables have increased day by day due to their high nutritive values and health promoting effects. Fruits and vegetables are consumed in different forms, i.e. raw, minimally processed and fully processed. Fruits and vegetables not only have the high nutritional value but they are also the prime contributors to the food waste among all the horticulture crops. According to study of United Nations Food and Agriculture Organization (FAO), it was estimated that the generation of waste in fruits and vegetables is the highest among all types of foods, approximately up to 60%. The main causes of wastage of fruits and vegetables are over production, post-harvest handling, seasonal variety, climatic variations, physiological changes, etc. (Galanakis 2013). Wastage of fruits and vegetables is not only the loss of edible material but also affects the other resources like agricultural land, water, fertilizer, labour, etc. It also creates an enormous environmental trouble in the form of decomposition of landfills and emission of harmful greenhouse gases (Venkat 2011; Vilari et al. 2017).

Wastage of fresh produce starts from farm level followed through post-harvest handling, storage and during processing. It is estimated that the quantity of this waste is approximately 25–30% varying with the commodity and the type of waste includes leaves, roots, skin, pulp, pomace, seeds, etc. (Panouille et al. 2007). These morphological components are rich source of bioactive compounds which include carotenoids, polyphenols, enzymes and oils having an application in food industry as well as non-food industry like pharmaceuticals and textile industry. Researchers and nutritionists are working hard to find out the solution of reducing the waste and also utilizing this waste into some value-added products which are beneficial for common masses. The conversion of this waste into value-added

Table 12.4 Recent studies on production of other valuable products from food waste

S. No.	Product	Type of food waste	Treatment	References
1.	Bacterial cellulose	Saccharified food waste	*Acetobacter xylinum* KJ1 fermentation	Song et al. (2009)
2.	Chitin	Prawn shell	Cocultivation of *Lactococcus lactis* and *Teredinobacter turnirae*	Aytekin and Elibol (2009)
3.	Fatty acids, sterols, wax esters, diacids, policosanols	Corn, taro, lettuce, bean sprout	Super critical extraction, thermochemical extraction	Yu et al. (2011)
4.	Anthocyanins and phenolics	Blue berry wine pomace	Ultrasound assisted extraction (UAE)	Chemat et al. (2011)
5.	Flavonoids	Satsuma peels	Microwave assisted extraction (MAE)	Dorta et al. (2013)
6.	Pectin	Apple pomace	Enzyme assisted extraction (EAE)	Cheng et al. (2015)
7.	P(3HB-co3HV)	Cheese whey	*Haloferax mediterranei* fermentation	Pais et al. (2016)
8.	Biosorbent for lead removal	Tomato waste and apple juice residue	NaOH to activate surfaces	Heraldy et al. (2018)
9.	Acetic acid and whey protein	Whey cheese	Membrane-integrated process	Pal and Nayak (2016)
10.	Polyhydroxyalkanoates	Carica papaya waste	*Bacillus subtilis* fermentation	Umesh et al. (2017)
11. `	Orange essential oil	Orange peel	UAE, MAE, SFE, enzyme assisted extraction	Gavahian et al. (2018)
12.	Single cell protein	Juice, pulp and peel from oranges and lemon	Fermentation	Mahan et al. (2018)

products is a new beginning towards the sustainable future (Table 12.4) (Ayala-Zavala et al. 2011).

A study has been done to produce citric acid from apple juice industry waste. Apple pomace was found a suitable substrate for the citric acid production (Shojaosadati and Babaeipour 2002). Citric acid has high commercial value. Use of apple pomace as substrate reduces cost of apple juice production as it ensures money for the waste. Citric acid production by this method will be cost effective and it also reduces the environment pollution. Pineapple, mousami, mixed

fruit, sugar cane bagasse and wheat bran can be used as substrate for the citric acid production (Kumar et al. 2003).

Enzymes like alpha-amylases and pectin can be extracted from food waste and can be an effective way of its utilization. Pectin can be extracted from the peel or pomace of the various pectin rich fruits. Pectin is a very valuable product due to its commercial value in food industry. So, before throwing away these parts of food material think twice about the uses of these materials. Citrus fruits peel can be used to produce aroma compounds. Tea waste could be used for the extraction of flavonoids. Using tea leaves for composting is a very old household practice. Lycopene extraction from the tomato waste would be better alternative for lycopene production.

Pineapple waste could be a prominent source of bromelain enzyme. Bromelain, a protease enzyme extracted from pineapple waste could be utilized for meat tenderization (Chaurasiya et al. 2015). Similarly, by-products of seaweeds may be used for extraction of several value-added compounds. Seaweeds are consumed commonly in India and Oriental countries. However, after extraction of agar from them the biomass is usually discarded. The waste biomass can be used to generate high value compounds such as pigments (such as phycoerythrin) that are important in medicine, cosmetics and food industry. Similarly, waste parts of edible plants can be used for synthesis of nanoparticles that are useful in biomedical imaging, sensing, packaging, etc. due to their optophysical properties (Tripathi et al. 2017).

Other than production of ethanol, biodiesel and hydrogen gas, many more valuable compounds can be extracted from the food waste called as bioactive compounds (flavonoids, polysaccharides, caffeine, creatine and carotenoids) (Sadh et al. 2018).

12.7 Utilization of Dairy Industrial Waste

Dairy industry is an emerging sector in India as India is the second highest producer of milk at global level due to increment in the demand of milk and milk products during last few years. During processing of dairy products, industry generates huge waste in the form of by-products that are as nutritious as milk itself. These by-products include buttermilk, whey and their derivatives. Apart from these components water discharged from dairy industry contributes a major part in the environmental and water pollution, thus affecting the ecosystem, agriculture and living organism. However, waste generation and related environmental problems also have increased importance (Chaiudhari and Dhoble 2010). So, the new technologies and alternatives are being developed for the utilization of these by-products and waste to combat the problem of pollution and environmental damage.

Apart from the above problems, food insecurity and nutrition deficiency have arisen as a major problem from past few years. Single cell protein (SCP) is a good source to combat the nutritional deficiency in human diet. Single cell protein is the dried cells of bacteria, yeast, fungi and algae, proteinaceous in nature and can be a

good substitute of dietary supplements. SCP is produced through the fermentation of biomass that is obtained from food wastes with the help of microbial cultures (Nasseri et al. 2011). Many food wastes other than dairy industry have been used for the production of SCP like fruits and vegetables industrial waste, sugarcane industrial waste, cereal industrial waste (rice husk etc.) and non-food industrial waste such as paper mill (Nigam 2000; Bozakuk 2002 and Zubi 2005). But dairy waste has been considered as one of the most promising substrates for the production of SCP as it is a rich source of nutritional components such as carbohydrate, fat, nitrogenous compounds and minerals. Single cell proteins derived from the waste organic products have been proved a very useful technology. It has potential to reduce the organic load at one side and on other side it ultimately increases the production of dietary supplements that are beneficial for the people facing nutritional deficiency (Moeine et al. 2004). Physico-chemical analysis, biochemical analysis and microbial detection inferred a successful yield of biomass for the production of single cell protein.

12.8 Future Prospects

Although scientists, nutritionist and government agencies are trying to utilize as much waste but still there is a need of technologies that are simple and cost effective in nature for the proper utilization of food waste into different useful purposes. Wastage not only occur at the processing level but other factors like lack of awareness, improper chain management, inappropriate technical knowledge and loose government policies are also responsible for the same. It is important to identify a universal process design that can be used to develop and market products developed from food wastes. Apart from all these technologies and ideas, lack of proper knowledge among the common masses is also a very important factor of food waste that can cause a heavy loss to the economy and the resources of that country. There is still a need of economically efficient technologies and awareness among the people to make the natural resources sustainable and safe so that the upcoming generations could be benefitted. An in-depth understanding of recycle/reuse of food waste is very essential so that it can convert into value-added or some useful product which is beneficial for human society. The solution to the problems associated with development and adoption of appropriate technologies and lack of trained manpower will require at realistic time frame and not only central government bodies but also state governments should take various actions for strengthening food waste management in the country.

12.9 Conclusion

World's population is increasing day by day so as also an increment in food demand. Due to the lack of food, food insecurity, nutrition deficiency, hunger, etc. problems are coming into the picture. Proper utilization of food waste is a forward step to

combat these problems because a heavy amount of waste is generated from farm to fork. Instead of dumping, the utilization of unwanted wastes as a low-cost feedstock along with by-products, a better option for production of value-added products and also as health supplement can be considered. Apart from some toxic waste material, most of the waste generated from food industry can be treated with the help of some treatments that can further be used. Food waste is not only used as feed, fodder but also used for the conversion of energy. New emerging technologies and ideas are being developed to increase the utilization of food waste. This utilization not only helps the society but also helps to control the pollution thus saves the environment and ecosystem. Latest techniques, like drying, fermentation and extraction, are giving value addition to the food waste as well as also opens the substitutes for entrepreneurs. This can help to improve the economy of the country and create lot of opportunities for the associated industries. The regulatory agencies and the food processing industries can work hand in hand to develop new processes for waste management and utilization which are commercially viable.

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Ligninolytic Microbes and Their Role in Effluent Management of Pulp and Paper Industry

13

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Abstract

Environmental hazards caused due to pollutants introduced into the environment by utilization of natural resources have been of great concern, with the industrialization at a faster pace. Pulp and paper industries utilizing wood as basic raw material comprise three important constituents: cellulose, lignin and hemicellulose. Lignin and hemicellulose contents are removed from cellulose fibres for production of high quality paper. Lignin is the most recalcitrant and non-hydrolysable component, and is difficult to degradation. Delignification/decolourization of lignin during chemical bleaching leads to generation of highly toxic, mutagenic and carcinogenic pollutants such as chlorophenols, extractable organic halogens and organic halogens, polychlorinated biphenyls and polychlorinated dibenzodioxines affecting environmental communities. Hence, environmental-friendly approaches alternative to traditional bleaching have gained great attention. Microorganisms such as bacteria, actinomycetes and fungi possess unique strategy to overcome the limitation of lignin degradation. Fungi such as white-rot, brown-rot and soft-rot are potent lignin-degrading microorganisms, among them white-rot fungi are widely reported for extensive and rapid degradation due to the presence of complex system for production of extracellular enzymes such as lignin peroxidase, manganese peroxidase and versatile peroxidase. Brown-rot fungi, unlike white-rot fungi generally possess

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nonenzymatic oxidation reaction mechanism that produces hydroxyl radicals via Fenton chemistry. Soft-rot fungi such as *Fusarium*, *Aspergillus, Trichoderma, Penicillium, Alternaria* and *Xylaria* mainly degrade non-woody biomass by following the soft-rot decay process. Bacterial lignin degradation mechanisms are more specific than fungi and possess advantages over fungal degradation, like tolerance to wider range of temperature, pH, oxygen limitations, etc. and easy to genetically manipulate for over-production of lignin-degrading enzyme. Several strains of bacteria such as *Pseudomonas, Burkholderia*, *Bacillus*, *Ochrobactrum*, *Leucobacter*, *Rhodococcus,* etc. have reported for high level ligninolytic enzymes production. This chapter reviews the unique properties of microorganisms for potential application in biobleaching of pulp to reduce the burden of harmful by-products released in environment.

Keywords

Effluent · Pollutants · Waste management · Ligninolytic · Bacteria · Fungus

13.1 Introduction

Pulp and paper industry is the largest consumer of freshwater, generating a large amount of wastewater (effluent) causing a significant impact on the environment worldwide (Mehmood et al. 2019). Paper industry is one of the oldest and core industrial sectors in India, which comprises about 1.6% of total world paper and paperboard production (Kulshrestha 1972; Dey 2014; Singh 2017). Pulp and paper industries produce a significant amount of effluents containing various contaminants depending on the type of processes followed in the industrial plants. The generated effluents are highly toxic and potentially harmful and dangerous, which deserves assiduous disposal approach, and therefore should be treated in wastewater treatment plants for removal of pollutants before being released to the natural environment. In India more than 650 paper mills producing different types of paper products use a wide variety of cellulosic and non-cellulosic raw materials with annual water consumption of 905.8 million m^3 at a rate ranging between 150 and 250 m^3/ton of product and around 695.7 million m^3 wastewater is being discharged annually by this sector (Kumar et al. 2017).

The raw material for Indian pulp and paper industry comes from three primary sources (Figs. 13.1 and 13.2) (Balakrishanan 1999; Beeindia 2015):

1. Forest wood: About 43% of raw material is sourced from bamboo and mixed hardwoods from forest felling, and eucalyptus wood from plantations (both organized plantations and farmers' fields/agroforestry plots).
2. Agricultural residues: About 28% of raw material for pulp and paper industries supplied from bagasse, rice, wheat straws and cotton stalks.
3. Waste paper: About 29% raw material for pulp and paper industries is recovered by recycling of domestic and imported waste paper.

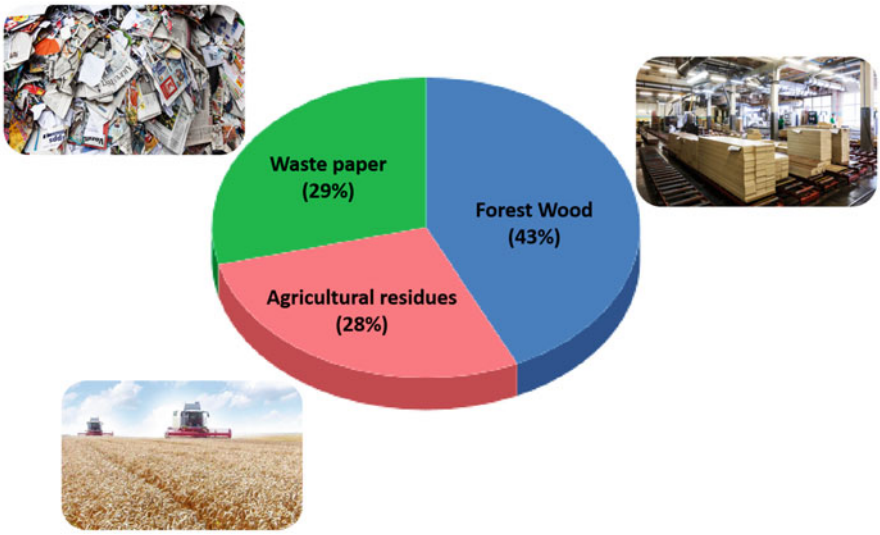

Fig. 13.1 Types of raw material used for pulp and paper industry

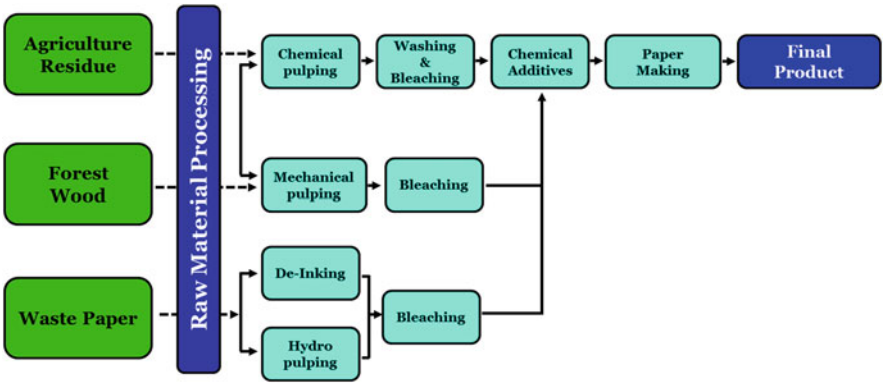

Fig. 13.2 Raw materials and their processing stages in the paper formation. *Source*: Satija (2018)

Among the raw materials, wood is the basic raw material used in paper production, which comprises of three essential constituents: cellulose, lignin and hemicellulose. The paper formed is a thin, nonwoven fabric produced by removing water from a slurry of plant fibres, and then compressing into a thin sheet. Conversion of wood to paper is accomplished by removing the lignin and hemicellulose contents from cellulose fibres. However, presence of too much lignin within the fibres, for instance for newsprint manufacturing by wood pulp is done by simply ripping the

fibres out of the wood, which will not bond well together resulting in the production of a very weak paper. Such paper also gets discoloured on standing, due to chemical changes caused in the lignin by light. Therefore pulp manufacturers prefer to dissolve and remove the lignin out of the wood by chemical solutions (Paliwal et al. 2015). Use of these chemicals in a process for removal of lignin leaves behind vast amounts of by-products such as chlorophenols, polychlorinated biphenyls, dibenzodioxins, etc. which are highly pollutant.

13.1.1 Lignin

Lignin is an aromatic biopolymer most abundantly found in the biosphere, contributing about 30% of total plant biomass (Zhu et al. 2017). It is the most crucial renewable resource of organic carbon on earth (Boerjan et al. 2003). It is a natural composite material providing the strength and rigidity in all the vascular plants (Brown 1985). Lignin is found in the cell wall of plants in association with cellulose and hemicellulose. It is the most recalcitrant component of the plant cell wall (Zhu et al. 2017) because inter-unit bonds in lignin are not hydrolysable and are challenging to degrade either chemically or biologically. Lignin surrounds cellulose in the plant cell wall forming a matrix, which is itself resistant to degradation. The strength and rigidity of stems in higher plants are the results of production and deposit of cellulose, hemicellulose and lignin in the plant cell walls (Fig. 13.3).

The primary precursors for p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units of lignin are p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, respectively (Fengel and Wegener 1989; Brunow 2001) (Fig. 13.4). Lignin is the polymer of these complex phenylpropanoid components cross-linked together through various covalent bonds (e.g. carbon–carbon, ester and ether linkages) (Brunow 2001).

By decreasing water permeation across the cell wall, lignin renders the plant resistant to biodegradation as well as to environmental stresses (Eriksson et al. 1990). Biochemically, lignin is an aromatic, amorphous, heterogeneous, three-dimensional, cross-linked polymer with low viscosity and insoluble in water. The molecular mass of lignin is high (600–1000 kDa), although not uniform, varying greatly within isolated samples (Kirk and Farrell 1987). The molecular mass of lignin is thus difficult to determine, and use of a conventional formula is not possible (Brunow 2001).

Lignin is generally removed from pulp by a chemical and mechanical process, and about 90–95% lignin dissolved in water and generated wastewater is known as black liquor. One of the commonly followed methods for removal of lignin from wood is known as the kraft process. In the kraft process, a solution containing sodium hydroxide and sodium sulphide is used for cooking, and the output of dark solutions of the degraded lignin is known as black liquor.

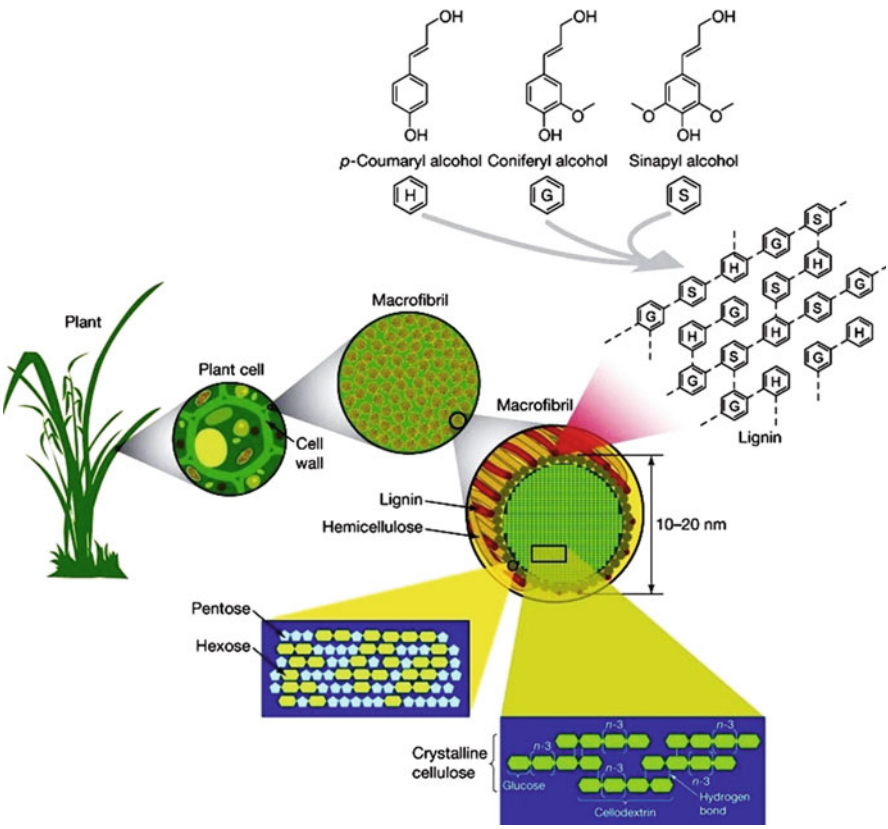

Fig. 13.3 Structure of lignocellulose biomass. *Source*: Rubin 2008

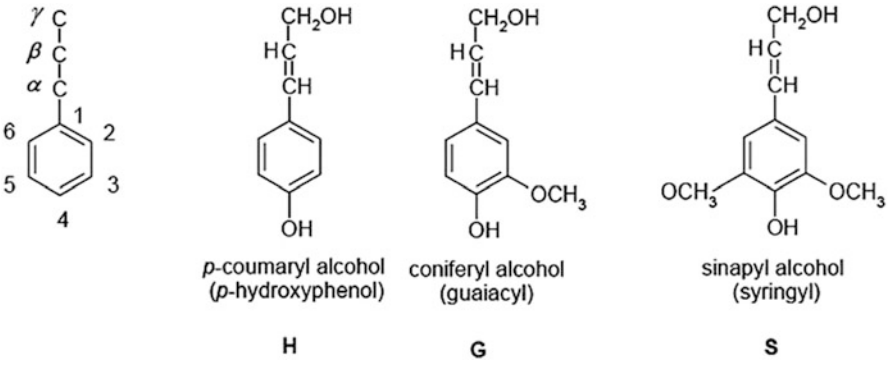

Fig. 13.4 A phenylpropanoid unit and precursors of lignin. Names in parentheses refer to the corresponding phenylpropanoid units in a lignin molecule. *Source*: Buswell et al. 1987

13.1.2 Papermaking Process

The papermaking process takes place in three significant steps: wood pulping, pulp bleaching and papermaking (Fig. 13.5).

13.1.2.1 Wood Pulping

Wood pulping is the initial stage of the papermaking process in which the wood is processed to form the pulp. All the impurities like soil, dust, bark, cellulose, hemicellulose, lignin, etc. are removed during the pulping process. The pulping process is the primary source of the most pollutant of the paper industry. A large amount of water is needed during the pulping process, causing the generation of high amounts of wastewater (Pokhrel and Viraraghavan 2004).

The wood pulping process is followed by several steps described below.

1. *Wood preparation*

In this step, all the impurities like soil, dirt, bark, etc. are removed from woods and are then chipped, separated and cleaned by water.

2. *Wood pulping*

The cleaned wood chips cooked at high temperature, pressure and high alkaline pH solution of sodium hydroxide (NaOH) and sodium sulphide (Na_2S). Approximately 95% of the total lignin is dissolved in pulping liquor (black liquor).

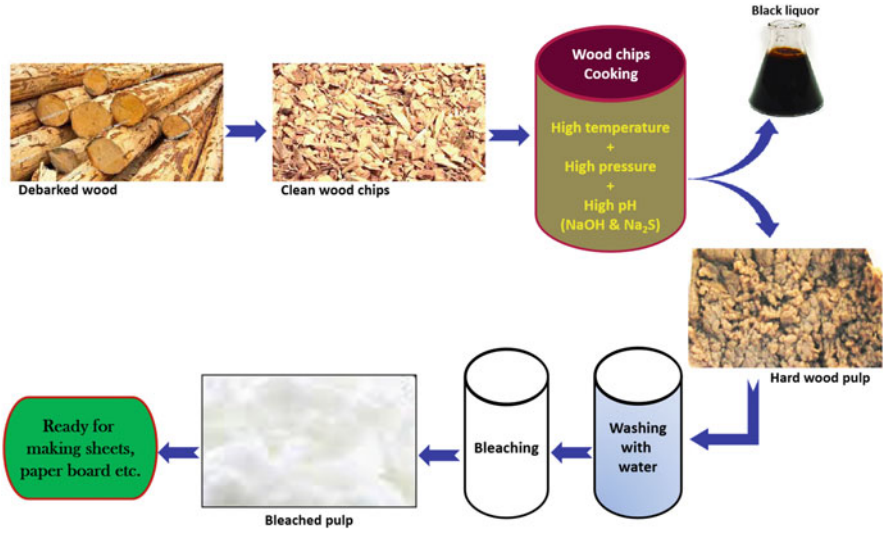

Fig. 13.5 Process of transformation of wood to paper

3. *Pulp washing*

The cooked pulp contains cooking chemicals, lignin and other extractives from the wood which is removed by washing with water.

4. *Pulp screening*

Pulp screening involves sieving of formed pulp to remove pulp knot and uncooked fibres clumped together from the wood pulp.

13.1.2.2 Pulp Bleaching

Pulp bleaching is the process to whiten and brighten the pulp and is done in two steps. Initially, pulp is treated with sodium hydroxide (NaOH) in the presence of oxygen, which removes hydrogen ions from the lignin and then oxygen breaks down the polymer. Subsequently, the pulp is treated with chlorine dioxide (ClO_2), a mixture of NaOH, oxygen (O_2) and peroxide and finally with ClO_2 again to expel remaining lignin.

During the bleaching process, chlorine molecules react with a phenolic constituent of the wood pulp leading to the formation of a large number of toxic chlorinated organic contaminants. High energy and freshwater is required for bleaching process and generate effluents containing a high concentration of compounds like chlorophenols, extractable organic halogens (EOXs) and absorbable organic halogens (AOXs), as well as a small proportion of extremely toxic PCBs (polychlorinated biphenyls) and PCDDs (polychlorinated dibenzodioxines) (Suntio et al. 1988). Chlorinated organic compounds formed due to chlorine compounds used for the bleaching process are released into the environment, which is known to have toxic, mutagenic and carcinogenic effects (Bajpai and Bajpai 1997).

13.1.2.3 Papermaking

This is the final stage of papermaking process in which pulp fibres are mechanically treated to make unique properties as per requirements, and finally pass through continuous moulds/wires to shape smooth and dried sheets.

13.1.3 Environmental Pollution

Lignin is the primary source of pollution in the wastewater formed in pulp and papermaking industries. Lignin is one of the important components of wood, and its degradation by-products, built during the process of cooking and bleaching, are major wastewater contaminants. The pulp is rinsed to free from residues of the liquors and discarded from the mills after preliminary treatments, and is generally runoff into local streams or rivers. To remove all the lignin from the crude pulp, the pulp must be bleached, and the bleaching solutions and washings lead to chlorinated lignin, which are major pollutants when released in the environment. To reduce load of pollution formed during the pulping process, researchers are trying to find

alternatives of wood pulping and bleaching methods to avoid chlorine or pollutants. Poorly treated or untreated effluents are responsible for a considerable amount of pollutants when discharged to watercourse such as a river, lake, ponds, etc. These pollutants can be described by total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD), metals, toxicity and colour (Pokhrel and Viraraghavan 2004). The higher amount of water is utilized in wood pulping and paper production, which causes the generation of large amounts of wastewater (Nemerow and Dasgupta 1991). Industrial effluents are accountable for the thermal impacts, slime growth, scum formation, and furthermore the aesthetic beauty loss in the environment (Pokhrel and Viraraghavan 2004). Paper and pulp mill processes water, contains high chemical diversity of organic pollutants causing high toxicity effects on aquatic communities (death to zooplankton and fish), as well as profoundly affecting the terrestrial ecosystem when discharged into the recipient watercourses (Yen et al. 1997; Pokhrel and Viraraghavan 2004).

Establishment of stringent government rules for controlling pollution, and due to public awareness, paper mills are under pressure to ensure a decrease in the level of chlorinated lignin residue in the effluent through changing the production process and applying improved treatment technologies. Although total chlorine-free (TCF) bleaching using ozone, oxygen and hydrogen peroxide is found to be an alternative to replace conventional chlorine bleaching, implementation of these methods involves high capital investment for process change and also would be economically unfeasible for small-scale pulp paper industries. Increasing awareness about environmental concerns has led the paper industry to look for cleaner production option aimed at the reduced consumption of chlorine and its compounds in the bleaching sequence, which thereby minimizes the discharge of chlorinated compounds in the effluent.

13.2 Biobleaching

The problems caused by chemicals used in bleaching forced industries to consider alternative and new environmentally friendly methods. One such a biological alternative to traditional bleaching was found through the discovery of oxidative enzymes termed as biobleaching. Utilization of lignin-degrading organisms and their enzymes became the highly attractive alternative methods. The ability of microorganisms to break down the lignin molecules has paved the way for providing environmental friendly technologies for the pulp and paper industry.

Biobleaching is an alternative process for chemical bleaching in which microbes or their enzymes are used to remove residual lignin and hemicellulose contents from the cellulosic pulp. Recently, biological methods have been paid more attention to lignin degradation (Mathews et al. 2016; Ebanyenle et al. 2016). Different types of ligninolytic microorganisms such as bacteria, actinomycetes and filamentous fungi are able to biodegrade lignin components to some extent. The high recalcitrant complex structure and the presence of irregular hydrolysable bonds in the lignin

are the cause for restricted metabolization by most of the microorganisms. However, there are microorganisms possessing unique strategy and advantage to overcome the restriction and limitations for degradation of lignin. These ligninolytic microorganisms are known to degrade the lignin, and among them, the fungus is the most efficient lignin-degrading microbes. Apart from the ligninolytic fungal species, several bacterial and actinomycetes also exhibit the ligninolytic activity.

13.3 Fungus and Lignin Degradation

Fungi are well-described microorganisms for lignin degradation, however, among more than one million known species only a few are wood-rotting fungi belonging to the phyla Basidiomycota and Ascomycota and grouped in white-rot, brown-rot and soft-rot fungi (Paliwal et al. 2015) as per their wood degradation capability (Tables 13.1, 13.2, and 13.3). Recently, several basidiomycetous fungal species have been studied intensively, and on the basis of lignin removal and degradation ability, white-rot fungi are the most efficient microbes able to mineralize the lignin extensively. Most of the white-rot fungi grow on hardwoods, while few species such as *Phellinus pini*, *Heterobasidion annosum* and *Phlebia radiata* grow on softwoods (Blanchette 1995).

Lignin degradation requires extracellular enzymes and fungal species have secreted a wide range of ligninolytic enzymes including lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase, which can degrade lignin and another recalcitrant compound (López et al. 2017; Hatakka 2001; Kirk and Farrell 1987). Attack of fungi to lignin is an oxidative and non-specific process leading to decrease in the phenolic, methoxy and aliphatic content of lignin, breaking the aromatic rings, and forms new carbonyl groups. Such changes lead to depolymerization of lignin molecule and production of carbon dioxide (Kirk and Farrell 1987).

13.3.1 White-Rot Fungi

White-rot fungi are widely explored and studied organism for depolymerization of wood components like cellulose, hemicelluloses and lignin (Paliwal et al. 2015). About 60 years ago, Fukuzumi has studied the degradation of lignin through white-rot fungi and confirmed the degradation through *Poria subacida* (Peck) Sacco (Fukuzumi 1960). White-rot fungi and related litter-decomposing fungi can degrade lignin more rapidly and extensively than any other microorganisms (Kirk and Farrell 1987; Hatakka 2001) (Table 13.1). The growth substrates of white-rot fungi are cellulose and hemicelluloses, and lignin is not used as a carbon source. However, they have developed a complex system for the production of the extracellular enzyme, which helps in lignin degradation. Thus, lignin degradation is essentially a secondary metabolic process and occurs at the end of primary growth by secondary metabolism in deficiency of nutrients, such as nitrogen, carbon or sulphur (Kirk and Farrell 1987; Hatakka 2001; Paliwal et al. 2015).

Table 13.1 Studies on activities of white-rot fungi in the treatment of lignocellulosic biomass

Microorganisms	Activities	References
Phanerochaete sordida YK-624	High lignin degradation ability by secretion of lignin peroxidase and manganese peroxidase enzymes	Wang et al. (2020)
Phanerochaete chrysosporium, Lentinula edodes, and *Trametes versicolor*	Synergistic action of electro-Fenton processes and these fungi are superior for lignin degradation (82–89%).	Hou et al. (2020)
Pleurotus ostreatus 3004 CCBAS 278 and *Irpex lacteus 617/93*	Have capability of biodegradation of dental care antimicrobial agents Chlorhexidine and octenidine	Linhartová et al. (2020)
Trametes, *Spongipellis*, *Dichomitous*, *Calocybe*, *Lepista* and *Panus*	Presence of versatile peroxidase	Chaurasia and Bhardwaj (2019), Chen et al. (2010), Moreira et al. (2007), Ruiz-Dueñas et al. (2008)
Pleurotus ostreatus	Degrade lignin through ligninolytic enzymes laccase, LiP, MnP	Metri et al. (2018), Fitria (2008)
Ganoderma applanatum	About 40.9% lignin degradation	Ćilerdžić et al. (2016)
Porodaedalea pini	Lignin-degradation activity through the production of xylanase and endoglucanase	Sunardi et al. (2016)
Lentinus tigrinus LP-7 and *Irpex lacteus KB-1.*	Kappa number reduction and enhancement of brightness	Afrida et al. (2014)
Pholiota adipose–Armillaria gemina	1:2 ratio most effective	Dhiman et al. (2015)
Pycnoporus sanguineus	Biobleaching of kraft pulp of *Eucalyptus globulus* by laccase	Martin-Sampedro et al. (2015)
Trametes versicolor, Trametes hirsute, Trametes velutina, Trametes villosa	Potent lignin-degrading white-rot fungi	Quintana et al. (2015), Bourbonnais et al. (1995), Jönsson et al. (1995), Wu et al. (2011), Wang et al. (2013a), Ahn et al. (2007)
P. ostreatus *P. Pulmonarius*	20-fold increase in hydrolysis	Castoldi et al. (2014)
Irpex lacteus	43.8% of lignin degradation, saccharification efficiency increases sevenfold	Song et al. (2013)
Trametes velutina D10149	85% of lignin removal	Wang et al. (2013a)
Pleurotus eryngii	Versatile peroxidase (VP) was the first time described	Martinez et al. (1996)
Phlebia sp. MG-60	40.7% of lignin degradation after 56 days aerobic incubation	Kamei et al. (2012)

(continued)

Table 13.1 (continued)

Microorganisms	Activities	References
Punctualaria sp. TUFC20056	53.3% lignin removal from bamboo	Suhara et al. (2012)
T. versicolor, *Pycnoporus coccineus*, *T. hirsute*	High laccase production	Wu et al. (2011), Bourbonnais et al. (1995), Jaouani et al. (2005)
Phanerochaete chrysosporium, *Phanerochaete sordida* YK-624, *Trametes versicolor, Coriolus versicolor*, *Schizophyllum commune*, *Tinctoporia borbonica*, *Phlebia radiate, Dichomitus squalens*, *Bjerkandera* sp.	Lignin degradation in pulp and paper mill wastewater	Mäkelä (2009), Katagiri et al. (1997), Eaton et al. (1982), Hirai et al. (1995), Archibald et al. (1997), Kirk et al. (1976), Belsare and Prasad (1988), Fukuzumi (1980), Palma et al. (2000)
Phanerochaete sordida YK-624	Novel lignin peroxidases (YK-LiP2)	Hirai et al. (2005)
Ceriporiopsis subvermispora	11 different isoforms of manganese peroxidase (MnP)	Hofrichter (2002)
Phlebia sp. MG-60	Strong lignin degradation capability especially in a hypersaline environment	Li et al. (2002)
P. chrysosporium and *T. versicolor*	Produce laccase and manganese peroxidase (MnP)	Katagiri et al. (1997)
P. chrysosporium, *T. versicolor*, *P. sordida* YK-624, *Ceriporiopsis subvermispora* and *Bjerkandera* sp. BOS55	Delignify kraft pulp	Moreira et al. (1997), Katagiri et al. (1995), Reid et al. (1982), Hirai et al. (1995), Christov et al. (1996)
Heterobasidion annosum, *Phlebia radiata* and *Phellinus pini*,	Grown on softwoods, involved in lignin degradation	Blanchette (1995)
P. Chrysosporium	Manganese peroxidase (MnP) discovered	Glenn and Gold (1985), Kuwahara et al. (1984), Paszcynski et al. (1985), Paszczyński et al. (1986)
Phanerochaete chrysosporium	First ligninolytic enzyme lignin peroxidase (LiP) isolated	Tien and Kirk (1984)
Pleurotus ostreatus	35% of lignin reduction	Hatakka (1983)
Poria subacida (peck) Sacco	Lignin degradation reported	Fukuzumi (1960)

13.3.1.1 Ligninolytic Enzyme

White-rot fungi are involved in the degradation of cellulose, hemicellulose and lignin by the production of a number of extracellular enzymes including cellulases, xylanases, hemicellulases, laccases and peroxidases, such as lignin peroxidase (LiP),

Table 13.2 Studies on activities of brown-rot fungi in the treatment of lignocellulosic biomass

Microorganisms	Activities	References
Phellinus noxius	Brown root rot pathogen, secretes enzymes for potential degradation of diverse wood substrates	Ibarra Caballero et al. (2020)
Gloeophyllum trabeum, *Piptoporus betulinus, Schizophyllum commune*, *Serpula lacrymans*, *Postia placenta*, *Coniophora puteana* and *Fomes fomentarius*	Typical wood degrading species, commonly found in nature	Peralta et al. (2017)
Serpula lacrymans and *Coniophora puteana*	Most harmful brown-rot fungi	López et al. (2017), Blanchette (1995)
Gloeophyllum trabeum Postia placenta, *Piptoporus betulinus*	Strongly degrade the cellulose, hemicellulose and demethoxylated lignin left behind	Mäkelä et al. (2015), Dey (2014)
Co-transformant strain L#61 of *Gloeophyllum trabeum* KU-41	Most potent strain for high laccase activity	Arimoto et al. (2015)
Fistulina hepatica and *Cylindrobasidium torrendii*	Proved the evolution of brown-rot fungi from white-rot fungi	Floudas et al. (2015)
Gloeophyllum trabeum, *Laetiporus portentosus*, and *Fomitopsis lilacinogilva*	Primarily attack on softwoods	Abdel-Hamid et al. (2013), Sigoillot et al. (2012), Hatakka (2005), Gilbertson (1980)
Gloeophyllum trabeum	Presence of the laccase gene-specific sequences	D'Souza et al. (1996)
Coniophora puteana, Serpula lacrymans, Gloeophyllum trabeum and *Meruliporia incrassata*	Strongly destructive to wood used in building material	Blanchette (1995)
Polyporus ostreiformis	Expression of LiP and MnP, 18.6% lignin removed from rice straw within 3 weeks	Dey et al. (1994)

manganese peroxidase (MnP) and versatile peroxidase (VP) (Hatakka 2001; Datta et al. 2017). Lignin-degrading enzymes are generally classified into two classes: heme peroxidases and phenol oxidases. Lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP) are heme peroxidase enzymes depending on the heme (Fig. 13.6), while phenol oxidases include laccases containing copper (Bugg et al. 2011a.; Falade et al. 2017). Ligninolytic enzymes activity of laccase and peroxidase occurs through depolymerization of the phenolic and non-phenolic lignin polymer, degradation through low molecular weight free radicals such as OH, and by mineralizing the insoluble lignin (Datta et al. 2017).

Manganese peroxidase (MnP) and laccase are the most common lignin-modifying and degrading enzymes produced by almost all species of white-rot

Table 13.3 Studies on activities of soft-rot fungi in the treatment of lignocellulosic biomass

Microorganisms	Activities	References
Daldinia eschscholtzii (SA2 80), *Daldinia eschscholtzii* (SA2 85) *Hypoxylon sp.* (SA2 146), *Hypoxylon investiens* (SA2 149), *Nemania primolutea* (KT2 106)	Lignin degradation range between 5% and 15%. Among all *D. eschscholtzii* exhibited highest degradation capability	Ramadhani et al. (2019)
Neopestalotiopsis sp. B2B	Production of laccase	Kang et al. (2019)
Chrysonilia sitophila	Ligninolytic activity, 20% weight loss of pine wood	Madadi and Abbas (2017), Hatakka (2005)
Neurospora discrete	Two times more degradation of lignin in sugarcane bagasse compared to *P. chrysosporium*	Pamidipati and Ahmed (2017)
Thermoascus aurantiacus	Thermophilic ascomycete, grow in heated parts of wood chip piles	López et al. (2017)
Trichoderma asperellum	Produce ligninolytic enzyme, Xylanase even at alkaline pH	Sridevi et al. (2017)
Aspergillus niger, *A. flavus*	Xylanase enzyme production, reduced kappa number and enhanced brightness	Sridevi et al. (2016), de Alencar Guimaraes et al. (2013)
Trichoderma viride	Lignin removal ability increased by 15% and 11%, respectively, by addition of wet milling and surfactant (Tween 80)	Ghorbani et al. (2015)
Chaetomium globosum, *Ustulina deusta*, *Alternaria alternata*, *Thielavia terrestris* and *Paelomyces* sp.	Common soft-rot fungi, mainly degrade non-woody biomass	Mäkelä et al. (2015), Daniel (1994), Haider et al. (1980), Martínez et al. (2005), Nilsson and Daniel (1989)
Aspergillus niger and *Penicillium chrysogenum*	Biodegradation ability	Hamed (2013)
Alternaria alternata	Cause soft-rot decay	Sigoillot et al. (2012)
Xylaria spp. and *Coccomyces* spp.	Ligninolytic activity with selective delignification	Koide et al. (2005), Liers et al. (2010), Osono and Takeda (2001)
Penicillium chrysogenum, *Fusarium solani*, *F. oxysporum* and *F. proliferatum* in soil, compost and forest litter.	Exhibited lignin degradation ability, but their degradation efficiency is low and mineralized 14 C-labelled lignin up to 27.4%	Rodríguez et al. (1996), Tuomela et al. (2000), Kirk and Farrell (1987)
Thermoascus aurantiacus	Brazilian strain produced high levels of phenol oxidase (PO) and efficiently degraded *Eucalyptus grandis* extractive substances	Machuca et al. (1998)
Botrytis cinerea	Production of extracellular laccases	Thurston (1994)

(continued)

Table 13.3 (continued)

Microorganisms	Activities	References
Aspergillus niger, Trichoderma sp.	Lignin degradation in pulp and paper mill wastewater	Kannan and Oblisami (1990), Prasad and Joyce (1991)
Daldinia, *Hypoxylon*, and *Xylaria*	Common wood degrading fungi, but *D. concentrica* was most potent and caused highest lignin losses (44%)	Nilsson and Daniel (1989)
Fusarium sp.	Degrade lignin components	Higuchi (1980), Iwahara (1980), Buswell et al. (1987)
Graphium sp., *Paecilomyces* sp., *Monodictys* sp., *Thielavia terrestris*, *Papulospora* sp., and *Allescheria* sp.	Lignin depletion characteristics and decayed the standardized blocks of alder, poplar and pine wood	Eslyn et al. (1975)

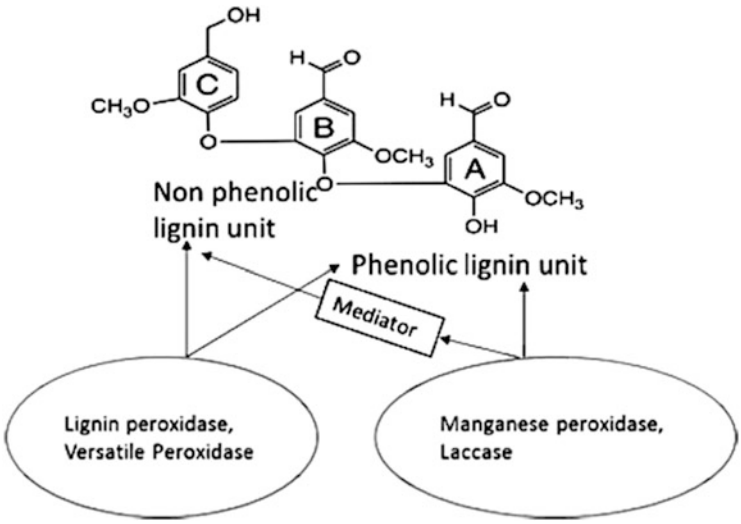

Fig. 13.6 Schematic representation of ligninolytic enzymes and their selective activity on the components of lignin. *Source*: Datta et al. 2017

fungi, while only a few of them produce lignin peroxidase (LiP) (Hatakka 2001). Recently, Su et al. (2018) have reported *Myrothecium verrucaria* as a potent ligninolytic enzyme secreting fungi with high activity levels of laccase (6.61 Ug^{-1}), lignin peroxidase (0.78 Ug^{-1}) and manganese peroxidase (1.31 Ug^{-1}) dry biomass.

Lignin peroxidase (LiP) was the first ligninolytic enzyme isolated from *Phanerochaete chrysosporium* in 1984 (Tien and Kirk 1984). Lignin peroxidase (LiP) exhibited the highest redox potential activity compared to other peroxidases and can directly oxidize the phenolic and non-phenolic structures of lignin without

any mediator (Datta et al. 2017). Haem in peroxidases enzymes (LiP, MnP and VP) make them high redox potential. At the same time, two other research teams (M. Gold's and R. Crawford's groups) have discovered manganese peroxidase (MnP) in the same white-rot fungal species, *P. chrysosporium* (Kuwahara et al. 1984; Paszcynski et al. 1985; Glenn and Gold 1985; Paszczyński et al. 1986). During MnP enzymatic activity, manganese acts as a mediator, and due to its high redox potential, it highly degraded the phenolic compounds of lignin compared to laccase (Datta et al. 2017).

Later on, the third ligninolytic enzyme, versatile peroxidase (VP) was for the first time described in *Pleurotus eryngii* (Martinez et al. 1996). For the first time, VP was purified from the *Bjerkandera*, a wood-rotting fungus and was described to have the ability to transform lignin even without any external mediator (Moreira et al. 2007). It was also recognized to be present in other white-rot fungal species including *Trametes*, *Spongipellis*, *Dichomitus*, *Calocybe*, *Lepista* and *Panus* (Moreira et al. 2007; Ruiz-Dueñas et al. 2008; Chen et al. 2010; Chaurasia and Bhardwaj 2019). Versatile peroxidase (VP) has the combined catalytic activities of both LiP and MnP and shows the high-redox potential activity for non-phenolic compounds like LiP and also able to oxidize Mn^{2+} like the MnP (Abdel-Hamid et al. 2013).

Laccase is a copper-containing enzyme, belonging to the oxidoreductase group of enzyme and oxidizes a wide variety of organic and inorganic substances, including lignin (Datta et al. 2017). In 1883, Yoshida for the first time isolated laccases from the *Rhus vernicifera,* commonly known as Japanese lacquer tree (Yoshida 1883; Thurston 1994; Viswanath et al. 2014) and subsequently in 1896 it was reported as a fungal enzyme by Bertrand and Laborde (Bertrand 1896; Laborde 1896). Laccase is reported in several organisms, but it is highly produced by white-rot fungus and is also reported from some bacterial species (Datta et al. 2017). Among the white-rot fungi, *P. chrysosporium* has deeply studied due to their potent lignin degradation properties. A total of 16 candidate genes have been reported in *P. chrysosporium* in correspondence with lignin degradation which are ten LiP enzymes, five MnP enzymes and one NoP (novel peroxidase) (Levasseur et al. 2008). Ten structurally related genes family *lipA* to *lipJ* encodes the lignin peroxidase (LiP) enzyme. The presence of multiple lip genes in the *P. chrysosporium* is not clearly described; however, oxidation-reduction potential difference among isoenzymes of LiP has been observed (Macarena et al. 2005). Most of the white-rot fungi produce laccase enzyme, interestingly *P. chrysosporium* does not provide it (Larrondo et al. 2003). However, in 2004, Larrondo et al. have described a cluster of four multicopper oxidase genes (*mco1* to *mco4*) in *P. chrysosporium* (Larrondo et al. 2004). Crystal structures study of LiP and MnP determines that both these enzymes and other peroxidases are structurally related to each other indicating the divergent evolution (Edwards et al. 1993; Sundaramoorthy et al. 1994; Banci et al. 1999). Manganese peroxidase (MnP) is often produced in multiple forms and is only a single fungal species *Ceriporiopsis subvermispora* and up to 11 different isoforms have been described (Hofrichter 2002).

Earlier studies have reported that several white-rot fungi such as *Phanerochaete chrysosporium* (Eaton et al. 1982; Katagiri et al. 1997), *P. sordida* YK-624 (Hirai

et al. 1995), *Trametes versicolor* and *Coriolus versicolor* (Kirk et al. 1976; Archibald et al. 1997), *Schizophyllum commune* (Belsare and Prasad 1988), *Tinctoporia borbonica* (Fukuzumi 1980), *Phlebia radiata* and *Dichomitus squalens* (Mäkelä 2009) and *Bjerkandera* sp. (Palma et al. 2000) degrade the lignin in pulp and paper mill wastewater but, only some of them such as *P. chrysosporium* (Katagiri et al. 1995), *Trametes versicolor* (Reid et al. 1982), *P. sordida* YK-624 (Hirai et al. 1995), *Ceriporiopsis subvermispora* (Christov et al. 1996) and *Bjerkandera* sp. BOS55 (Moreira et al. 1997) have been claimed to possess the ability to delignify kraft pulp.

Hirai et al. (2005) isolated and characterized a novel lignin peroxidase (YK-LiP2) from white-rot fungus *P. sordida* YK-624. The absorption spectrum of identified enzyme YK-LiP2 from *P. sordida* YK-624 was same as LiP from *P. chrysosporium.* However, the degradation capacity of YK-LiP2 from *P. sordida* for dimeric lignin model compounds was higher than the LiP from *P. chrysosporium. P. chrysosporium* has produced several ligninolytic enzymes, but it is not capable of producing the versatile peroxidase (Chaurasia and Bhardwaj 2019). It has been reported that *P. chrysosporium* and *Trametes* species could secrete the oxidative enzyme and make them highly selective towards lignin (Zhang et al. 2012; Knežević et al. 2013; Zeng et al. 2013). Katagiri et al. (1997) have investigated the production of ligninolytic enzyme for biobleaching of unbleached softwood kraft pulps by *P. chrysosporium* and *Trametes versicolor* and described that both the fungal species were able to produce the laccase and manganese peroxidase (MnP). However, production of lignin peroxidase was not observed (Katagiri et al. 1997). In comparison with *T. versicolor, P. chrysosporium* showed higher delignification activity for unbleached softwood kraft pulps. During delignification of softwood kraft pulps, MnP production by *P. chrysosporium* was much less than that of unbleached hardwood kraft pulp.

Apart from *P. chrysosporium* several other white-rot fungal species play a significant role in delignification (Table 13.1). Several species of *Trametes* such as *T. versicolor, T. hirsute, T. velutina, T. villosa* have also been reported as potent lignin-degrading white-rot fungi (Quintana et al. 2015; Wang et al. 2013a; Wu et al. 2011; Ahn et al. 2007; Bourbonnais et al. 1995; Jönsson et al. 1995). In 1989, Archibald et al. have described that *T. versicolor* have the capability of delignifying and substantially brightening the unbleached kraft pulps (Archibald et al. 1997). Archibald et al. (1997) investigated the delignification enzyme families secreted by *T. versicolor* which include lignin peroxidases (LiP), manganese peroxidases (MnP), laccases and cellobiose dehydrogenases (CDH). They have also purified two laccase isozymes (laccase I and II) and they have reported that in the presence of the mediator both laccase isozymes were able to delignify the kraft pulp (Archibald et al. 1997). The secreted MnP activity by *T. versicolor* and the presence of substantial available Mn (II) ions are necessary for lignin degradation and pulp brightening. They have also reported that purified MnP, supplied with Mn (II), H_2O_2 and Mn (II)-complexing agents can delignify pulp (Archibald et al. 1997).

Bourbonnais and Paice (1992) demonstrated that bleaching of Kraft pulp by laccase enzyme from fungus *T. versicolor* in the presence of 2,2′-azinobis-

(3-ethylbenzthiazoline-6-sulphonate) (ABTS) led to methanol release and delignification of the pulp. The methanol release was produced by demethylation of the pulp during the delignifying process. Several other researchers have also reported that *T. versicolor*, *Pycnoporus coccineus* and *T. hirsute* are high laccase enzyme-producing fungi (Bourbonnais et al. 1995; Jaouani et al. 2005; Wu et al. 2011). Quintana et al. (2015) have reported that an enzymatic biobleaching sequence was developed in a combination of laccase from *Trametes villosa* with violuric acid (VA) followed by pressured H_2O_2 treatment, and found better bleaching and desired dissolving pulp requirements such as improved brightness, reduced hemicellulose, insignificant cellulose degradation, brightness stability against moist heat ageing, etc. (Quintana et al. 2015; Chaurasia and Bhardwaj 2019).

Xylanase is a hemicellulase enzyme which hydrolyses xylan to xylose, widely used in feed processing and pulp and paper industry. Researchers have proved that pretreatment of agro-industrial residues pulp with xylanase produced by *Aspergillus niger* (de Alencar Guimaraes et al. 2013; Sridevi et al. 2016), *A. flavus* (de Alencar Guimaraes et al. 2013), *Lentinus tigrinus* LP-7 and *Irpex lacteus* KB-1.1 (Afrida et al. 2014) causes reduction of kappa number and enhancement of brightness (Chaurasia and Bhardwaj 2019). Song et al. (2013) have reported that 43.8% of lignin degradation was obtained after 42 days of non-sterile fungal pretreatment with *Irpex lacteus* of corn stover, saccharification efficiency was increased sevenfold after enzymatic hydrolysis (Song et al. 2013). Sunardi et al. (2016) have reported that *Porodaedalea pini* showed lignin-degradation activity through the production of xylanase and endoglucanase (Sunardi et al. 2016). Kappa number is a parameter for the bleaching ability, relative hardness, or degree of delignification of pulp.

In 2018, Metri et al. used *Pleurotus ostreatus* to degrade the lignin in palm midrib and reported that fungus used lignin for their growth, due to which the lignin level decreases (Metri et al. 2018). Their finding revealed the previous result confirming that *P. ostreatus* belong to such microbial group which can thoroughly degrade the lignin in CO_2 and water by producing a family of enzymes including the phenoloxidase laccase, lignin peroxidase (LiP) and manganese peroxidase (MnP) (Fitria 2008; Metri et al. 2018). An earlier study has proved that *P. ostreatus* can reduce lignin content about 34% in 5 weeks pretreated wheat straw with *P. ostreatus*. Still, only 12% lignin reduction was observed in untreated samples (Hatakka 1983). Scanning electron microscopy and Fourier transform infrared spectroscopy (FTIR) analysis evidenced that *P. ostreatus* and *P. pulmonarius* pretreatment *Eucalyptus grandis* sawdust enhanced the more extensively and selective lignin degradation (Castoldi et al. 2014). It has also been proved that *P. ostreatus* and *P. pulmonarius* are responsible for the improvement of hydrolysis and sugar reduction approximately 20-fold (Castoldi et al. 2014).

Li et al. (2002) isolated white-rot fungi *Phlebia* sp. MG-60 from mangrove stands in Okinawa, Japan and employed for the first time to bleach hardwood kraft pulp (UKP). They found that *Phlebia* sp. MG-60 was strongly capable to degrade lignin than *P. chrysosporium,* especially in a hypersaline environment (Li et al. 2002). Later on, Kamei et al. (2012) used *Phlebia* sp. MG-60 for aerobic delignification, anaerobic saccharification and fermentation of oak wood and found that *Phlebia*

sp. MG-60 selectively degrades lignin under aerobic solid-state fermentation conditions, and 40.7% of initial lignin was degraded after 56 days aerobic incubation. Under semi-aerobic liquid culture conditions, ethanol was directly produced from delignified wood (Kamei et al. 2012).

In 2015, beneficial effects of synergism between two or three fungal/bacterial cocultured lignocellulose-degrading microorganisms were explored and found that saccharification activity was significantly increased by cocultured fungal combination of *Neosartorya fischeri–Myceliophthora* thermophile and other fungal combination *Trichoderma longibrachiatum–Phanerochaete chrysosporium* by up to three- and ~sevenfold than their monocultures (Taha et al. 2015). Dhiman et al. (2015) also investigated the high saccharification yield by the cocktail of two fungal species. They reported that 1:2 ratio for *Pholiota adipose–Armillaria gemina* was the best combination for better yield of cocktail characteristics.

Studies on the Mn-oxidizing peroxidases and laccases from *Ganoderma applanatum* have reported that enzymes expression was more excellent in submerged cultivation than solid-state and showed degradation of lignin (40.9%), hemicellulose (32.7%) and cellulose (27.4%) during the oak sawdust fermentation. Oak sawdust stimulated maximum activities of Mn-dependent and Mn-independent peroxidases while wheat straw favoured more significant laccase activity than Oak sawdust (Ćilerdžić et al. 2016). Suhara et al. (2012) isolated 51 fungal white-rot basidiomycete from decayed bamboo culms (*Phyllostachys pubescens*), among them *Punctularia* sp. TUFC20056 showed high lignin degradation capability by removing 53.3% of lignin from bamboo.

13.3.2 Brown-Rot Fungi

Brown-rot is another group of most common and destructive wood decay fungi comprising of approximately 10% of all wood-rotting basidiomycetes. Typical wood degrading brown-rot fungi species are *Gloeophyllum trabeum*, *Piptoporus betulinus, Schizophyllum commune*, *Serpula lacrymans*, *Postia placenta*, *Coniophora puteana* (known as the 'cellar fungus') and *Fomes fomentarius* which are commonly found in nature (Peralta et al. 2017). Brown-rot such as *Gloeophyllum trabeum*, *Laetiporus portentosus* and *Fomitopsis lilacinogilva* are generally found in coniferous ecosystems and primarily attack softwoods (Gilbertson 1980; Hatakka 2005; Sigoillot et al. 2012; Abdel-Hamid et al. 2013). Comparative and functional genomics indicated that evolution of brown-rot fungi was accompanied by losses in key enzymes, especially cellulases and lignin-modifying enzymes implicated in biomass breakdown in white rot (López et al. 2017). Recently comparative studies on *Fistulina hepatica* and *Cylindrobasidium torrendii* have proved that the brown-rot evolved from white-rot fungi by losing multiple functional genes involved in cellulose and lignin degradation (Floudas et al. 2015) (Table 13.2).

Unlike white-rot fungi, brown-rot fungi are less efficient in lignin degradation (Datta et al. 2017), but the degradation of wood polysaccharides such as cellulose and hemicellulose is much quicker which rapidly loses its strengthening properties

(Madadi and Abbas 2017). Consequently, in advanced stage of wood decay through brown-rot fungi, wood shrinks, becomes crumbly and converts to brown colour due to the lignin oxidation and cracks into roughly cubical pieces (Gilbertson 1980; Monrroy et al. 2011). Brown-rot fungi cannot degrade the lignin completely, and to some extent, the residual lignins can be dealkylated, demethoxylated and demethylated, however, their aromatic rings still remain without degradation (Sigoillot et al. 2012). Unlike white-rot fungi that produce different ligninolytic enzymes for lignin degradation, the brown-rot fungi do not produce lignin-degrading enzymes; however, they have other mechanisms for lignin modification and depletion from wood. Expressions of lignin-degrading enzymes such as LiP and MnP have also been reported in the brown-rot fungus *Polyporus ostreiformis*, revealing 18.6% lignin removal from rice straw within 3 weeks (Dey et al. 1994). D'Souza et al. (1996) have also detected the presence of the laccase gene-specific sequences in brown-rot fungus *Gloeophyllum trabeum*.

Due to the lack of exoglucanases, the digestion process of the plant wood by the brown-rot fungi is completely different, i.e. nonenzymatic process (Goodell 2003). In contrast to white-rot fungi, wood degradation process of brown-rot fungi such as *Gloeophyllum trabeum, Postia placenta* and *Piptoporus betulinus* actively degrade the cellulose and hemicellulose and demethoxylated lignin left behind (Dey 2014; Mäkelä et al. 2015). *G. trabeum* significantly releases alkali-soluble lignin mainly in the first week during its growth on pine sawdust (Agosin et al. 1989). *Gloeophyllum trabeum* is the most studied brown-rot fungi and plays an important role in the wood used in construction together with *Coniophora puteana* (Boletales) and *Serpula lacrymans* (Blanchette 1995). The brown-rot fungus *Gloeophyllum trabeum* KU-41 strongly degrades the Japanese cedar wood. Arimoto et al. have developed a gene transformation system for *G. trabeum* KU-41 for strong biofuel production from Japanese cedar wood and found that co-transformant strain L#61 was a potent strain for high laccase activity among all obtained 44 co-transformants (Arimoto et al. 2015).

The brown-rot fungi produce nonenzymatic, low molecular agents which are responsible for early stages of wood decay (Goodell 2003). These initiators are of low molecular weight compounds, diffusible, extracellular oxidants (free radicals), like phenolates, glycopeptides or iron-chelatingg compounds, e.g. siderophores, oxalate and simple aromatic compounds, etc. These initiators are capable of penetrating the wood cell wall and depolymerizing cellulose, making it accessible to further degradation (Wang and Gao 2003). The degradation of wood started by the readily diffusion of the initiators from hyphae and operating at a distance from the hyphae after penetration in wood (Shimada et al. 1997; Goodell et al. 1997; Evans et al. 1994; Wood 1994; Espejo and Agosin 1991; Fekete et al. 1989).

Brown-rot fungi start lignin degradation through nonenzymatic oxidation reaction process that produces hydroxyl radicals via Fenton chemistry ($Fe + H O \rightarrow Fe + OH^{\cdot} + OH^{-}$) (Kirk et al. 1991; Kerem et al. 1998, 1999). Brown-red fungi partially oxidize lignin via demethylation of the aromatic ring of phenolic and non-phenolic compounds (Blanchette 1984; Datta et al. 2017), resulting in aromatic

hydroxylation and splitting of the ring, and increase in the phenolic hydroxyl content of reaction mixture (Kirk and Farrell 1987; Hatakka and Hammel 2011).

Some brown-rot fungi are able to accumulate the oxalic acid, causing a significant reduction of pH and generating the hydroxyl radicals using the oxalic acid as a proton donor for enzymatic and nonenzymatic hydrolysis of polysaccharides and as a chelator for a Fe (II)-H_2O_2 system (Goodell et al. 1997). This process does not occur with *G. trabeum*, because it could be producing the enzymes that degrade oxalate (Espejo and Agosin 1991). *G. Trabeum* exhibited different attributes for rapid degradation of aliphatic polyether through extracellular one-electron oxidation (Jellison et al. 1991), resulting in simple aromatic compounds such as 4,5-dimethoxy-catechol and 2,5-dimethoxyhydroquinone (Enoki et al. 1997) and 2,5-dimethoxy-1,4-benzoquinone (Paszczynski et al. 1999). These compounds may serve as oxygen-reducing agents, ferric chelators and compounds of redox-cycling (Kerem et al. 1999).

Several brown-rot fungi such as *Coniophora puteana, Serpula lacrymans, Gloeophyllum trabeum* and *Meruliporia incrassata* are strongly destructive to the wood used in the building and other structures, and due to lignin modification, wood is converted to dark, shrink, typically broken into small cubical parts which can easily fragment into brown powder (Blanchette 1995). *S. lacrymans* and *C. puteana* are the most harmful brown-rot fungi mainly found in the wood of temperate regions which generally prefer softwood to hardwood as substrates (Blanchette 1995; López et al. 2017).

13.3.3 Soft-Rot Fungi

Soft-rot fungi such as *Chaetomium globosum*, *Ustulina deusta*, *Alternaria alternata*, *Thielavia terrestris* and *Paecilomyces* spp. belonging to Ascomycetes and Deuteromycetes (Haider et al. 1980; Nilsson and Daniel 1989; Daniel 1994; Martínez et al. 2005) mainly degrade non-woody biomass (Mäkelä et al. 2015). Wood degradation process in soft-rot fungi is not as well described as in the white-rot and brown-rot (Blanchette et al. 2002), and they follow the soft-rot decay processes which are their lifestyle characteristics. Soft-rot decay occurs by Ascomycetes and Deuteromycetes; however, facultative soft-rot decay has also been reported scarcely in some basidiomycetes (Sigoillot et al. 2012) (Table 13.3).

Several decades ago, different soft-rot fungi such as *Graphium* sp., *Paecilomyces* sp., *Monodictys* sp., *Thielavia terrestris*, *Papulospora* sp. and *Allescheria* sp. isolated from pulp chip storage piles were used to decay the standardized blocks of alder, poplar and pine wood. All these fungi were responsible for the depletion of lignin however; carbohydrates were depleted faster than lignin in the alder and poplars. In the case of pine both *Paecilomyces* sp. and *T. terrestris* removed lignin faster than carbohydrates, because both fungal species have more characteristic of white-rot fungi (Eslyn et al. 1975). Other soil fungi such as *Fusarium* sp. have also been reported to degrade the lignin components, however; their contribution to the biosphere's polymer conversion was not described in detail (Higuchi 1980; Iwahara

1980; Buswell et al. 1987). Wood degradation by the soft-rot fungi usually occurs in wet environmental condition following a characteristic decay patterns (Mäkelä et al. 2015). Several studies have been carried out to investigate the changes in the structural and chemical composition of degraded wood by soft-rot fungi (Hamed 2013; Blanchette 1995; Rodríguez et al. 1996; Tuomela et al. 2000; Hofrichter and Fritsche 1996).

Soft-rot fungi preferably degrade wood polysaccharides like cellulose and hemicellulose (Sigoillot et al. 2012); moreover, the ligninolytic activity has also been reported in the ascomycete fungi such as *Xylaria* spp. and *Coccomyces* spp. with selective delignification; however, their enzymatic system has not been explored broadly (Osono and Takeda 2001; Koide et al. 2005; Liers et al. 2010). Lignin degradation occurs very slowly (Nilsson and Daniel 1989; Daniel and Nilsson 1998), because their lignin-degrading enzyme LiPs or laccases may not show the potential oxidative activity on recalcitrant guaiacyl lignin, but on syringyl lignin (Rodríguez et al. 1996). In the wet environments, this limited action of the soft-rot fungi makes the wood consistently soft, while in the dry environment wood becomes brown and crumbly (Eriksson et al. 1990).

Soft-rot fungi most frequently prefer the hardwoods for the degradation, and only slightly degradation occurs in the softwoods. Degradation generally occurs in the moist and aquatic conditions area, due to which the soft-rot fungi is mainly found on waterlogged woods, utility poles and archaeological wood (Martínez et al. 2005). In comparison with white-rot and brown-rot fungi, soft-rot fungi can survive in adverse environmental conditions and tolerate a wide range of temperature, humidity, pH conditions and oxygen limitation (Aarti et al. 2015; López et al. 2017). It directly acts on a large number of wood substrates in soils and other environments (López et al. 2017). Soft-rot fungi are particularly active in such an adverse condition where the activity of white-rot and brown-rot fungi generally decreases, indicating they are more commonly found in the hardwood than in softwood (López et al. 2017).

Several Ascomycetes from the Xylariales order such as the genera of *Daldinia*, *Hypoxylon* and *Xylaria* previously were in the white-rot fungi group but due to the typical type II soft-rot activity currently, it is classified as soft-rot fungi and is primarily found on the hardwood (Hatakka 2005; López et al. 2017). Among all these, *Daldinia concentrica* was reported to be the most potent wood-degrading fungus and able to cause more than 53% weight loss in birch wood and highest lignin loss (44%) was observed when the weight loss was 77% in 4-month inoculation. However, low degradation was also reported by this fungus in the pinewood with only 2.5% weight loss (Nilsson and Daniel 1989). Sigoillot et al. have reported that common anamorphic fungi such as *Alternaria alternata* also cause soft-rot decay (Sigoillot et al. 2012). Other studies have also described that few species of *Eutypella* produce soft-rot decay in the early stage of wood decay, while white-rot decay in the late stages (Worrall et al. 1997; Pildain et al. 2005).

Deuteromycetes and certain ascomycetes microfungi such as *Penicillium chrysogenum*, *Fusarium solani*, *F. oxysporum* and *F. proliferatum* mainly degrade the polysaccharide in soil, compost and forest litter. They also exhibited the lignin degradation ability, but their degradation efficiency is low compared to white-rot

fungi (Kirk and Farrell 1987; Rodríguez et al. 1996; Tuomela et al. 2000) and mineralized 14 C-labelled lignin up to 27.4%, prepared from milled wheat straw. The red mould of bread (*Chrysonilia sitophila*) also has the lignin degradation capability, and 20% weight loss of pine wood was reported in 3 months, with 18% and 25% loss of carbohydrate and lignin, respectively (Madadi and Abbas 2017; Hatakka 2005). Recently, Pamidipati and Ahmed have reported that *Neurospora discreta* can degrade the lignin up to twice as much in sugarcane bagasse compared to well-known white-rot fungus *Phanerochaete chrysosporium* and produces about 1.5 times the amount of lignin degradation of products in the submerged culture (Pamidipati and Ahmed 2017; Madadi and Abbas 2017). The production of extracellular laccases in the plant pathogen *Botrytis cinerea* was observed by Thurston in 1994. This fungal species also shows the soft-rot like decay in several horticultural crop plants like *Cucumis* and *Daucus,* including 'noble rot' and 'grey rot' of *Vitis vinifera* (Thurston 1994).

Hamed in 2013 investigated the biodegradation ability of wood by two artificially infested soft-rot fungi, *Aspergillus niger* and *Penicillium chrysogenum* and through scanning electron microscope (SEM) evaluation they confirmed that in comparison with the hardwood, softwood is more resistant to fungal attack in early stages. However, in the later stage of infection degradation occurs rigorously (Hamed 2013). Recently, the biodegrading capability of the lignin through *Aspergillus flavus* and *Emericella nidulans* was studied and reported approximately 14.4–21% reduction of alkali lignin in different mediums (Barapatre and Jha 2017). The extracellular ligninolytic enzymes (peroxidases and oxidases) produced by soft-rot fungi may be effective than white-rot fungi, although they have some unique characteristics. For instance, the thermophilic ascomycete *Thermoascus aurantiacus* generally grow in heated parts of wood chip piles and also abundant in low-cost agro-industrial and forest residues/wastes (López et al. 2017). Another Brazilian strain of *T. aurantiacus* produced high levels of phenoloxidase (PO) and efficiently degraded the *Eucalyptus grandis* extractive substances and biobleached the kraft pulp of Eucalyptus wood. Furthermore, this fungal species grows rapidly on a solid medium that contains various compounds related to high lignin such as guaiacol, vanillin and tannic acid (Machuca et al. 1998). In 2015, Ghorbani et al. have reported that lignin degradation performance by soft-rot fungi can be improved by slight modification. Lignin removal by *Trichoderma viride* increases about 15% and 11%, respectively, by the addition of wet milling and surfactant (Tween 80) (Ghorbani et al. 2015). Another species *Trichoderma asperellum* have reported producing the ligninolytic enzyme, xylanase even at alkaline pH, and could be an effective biobleaching agent for pulp (Sridevi et al. 2017).

13.4 Bacteria and Lignin Degradation

Bacteria generally show cellulolytic and pectinolytic activities and their performance of lignin degradation is low in comparison with fungi (Blanchette 1995; Daniel and Nilsson 1998). Lignin degradation mechanism of bacteria is more specific than

fungi, and they are able to cleave only one type of bond at a time in the lignin polymer (Vicuña et al. 1993). Lignocellulose degradation by bacteria commonly occurs in the mixed culture or in the culture of bacterial and fungal together (Vicuña et al. 1993; Daniel and Nilsson 1998). However, wood degradation by bacteria has some advantages over fungal degradation, such as the bacteria can tolerate a wider range of temperature, pH and oxygen limitations than fungi (Daniel and Nilsson 1998). Another advantage is that in bacteria, genetic manipulation for overexpression of gene for lignin-degrading enzyme production is easy compared to fungi (Suman et al. 2016). Several studies have been carried out on the biodegradation of lignin through bacteria. Recently, several lignin-degrading bacteria have been characterized, which comes under a broad taxonomic group from Qinling, China (Yang et al. 2017) (Table 13.4).

It has been reported that strains of *Streptomyces, Pseudomonas*, *Rhodococcus* and *Bacillus* have the capability to decompose the lignin (Lee et al. 2019). Several bacteria such as *Pseudomonas* sp., *Ochrobactrum* sp. and *Burkholderia* sp. strains have shown high value of ligninolytic enzyme activity, particularly, extremely high LiP activity in *Burkholderia* sp. H1 (Yang et al. 2017). It has been already reported that extracellular ligninolytic enzymes, including laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) are required for lignin degradation (Singh et al. 2013). Previously, these enzymes were reported in most of the fungal species mainly in white-rot fungi; however, increasing number of ligninolytic enzyme secreted by bacteria has also been reported in recent studies (Singh et al. 2013; Yang et al. 2017; Chaurasia and Bhardwaj 2019). Recently, Xu et al. (2018) have isolated *Klebsiella pneumoniae* NX-1, *Pseudomonas putida* NX-1 and *Ochrobactrum tritici* NX-1 from leaf mould samples which exhibited potential ligninolytic activities, among them *P. putida* NX-1 showed high laccase, lignin peroxidase and Mn-peroxidase activities. Rahman et al. (2013) have identified *Bacillus* Sp. SHC1, *Ochrobactrum* sp. SCH2 and *Leucobacter* sp. SHC3 from palm oil plantation soils, which were found to be producing all three ligninolytic enzymes, viz. laccase, lignin peroxidase and manganese peroxidase. *Bacillus* sp. SHC1 was reported as high amount of manganese peroxidase and lignin peroxidase producing; however maximum laccase production was observed in the *Ochrobactrum* sp. Scheme 2 (Rahman et al. 2013).

Lignin degradation ability of the *Pseudomonas* sp. has been studied broadly and has been proved that *Pseudomonas* sp. might be a potent lignin-degrading bacteria (Sasikumar et al. 2014; Yang et al. 2017). Salvachúa et al. (2015) found that *Pesudomonas* strains and *Acinetobacter* ADP1 have the ability for depolymerization of high molecular lignin species and catabolize a part of low molecular weight aromatics. In an earlier study, Yang et al. (2012) have demonstrated the lignin-degrading ability of *Pseudomonas putida* mt-2 through depolymerization of high molecular weight lignin (Yang et al. 2012). Three dyp-type peroxidases were reported in the *Pseudomonas fluorescens* Pf-5 strain which could cause release of the low molecular weight fragment of lignin (Rahmanpour and Bugg 2015). Another ligninolytic soil bacterium *Rhodococcus jostii* RHA1 has dye-decolorizing peroxidase (DypB) capable of catalysing the peroxide-dependent oxidation of divalent

Table 13.4 Studies on activities of bacteria in the treatment of lignocellulosic biomass

Microorganism	Activities	References
Microbacterium phyllosphaerae and *Agrobacterium sp.*	Lignin-oxidizing enzymes and aromatic degradation gene clusters are involved in lignin degradation	Granja-Travez et al. (2020)
Bacillus cereus WGB1	Ligninolytic bacterium, potential degradation of methylene blue	Mary et al. (2020)
Paenibacillus lautus strains S18, S20, S36	Grow with lignin as a sole carbon source	Tahir et al. (2019)
Streptomyces griseorubens LH-3	Pulp brightness increases by 14.5% after treatment with purified thermostable endo-xylanase and kappa number reduction by 24.5%	Wu et al. (2018)
Klebsiella pneumoniae NX-1, *Pseudomonas putida* NX-1, *Ochrobactrum tritici* NX-1	Potential ligninolytic activities	Xu et al. (2018)
Rhodococcus opacus PD630	Sole carbon source for growth	Kosa and Ragauskas (2013), He et al. (2017)
Clostridium thermocellum	Decreased b-O-4 linkage and increased S/G index	Akinosho (2017)
Bacillus ligniniphilus	Release of monomeric aromatic compounds	Zhu et al. (2017)
Pseudomonas sp., Ochrobactrum sp., Burkholderia ginsengisoli	High value of ligninolytic enzyme activity	Yang et al. (2017)
Burkholderia sp. H1	Extremely high LiP activity	Yang et al. (2017)
Pseudomonas monteilii, Raoultella planticola, *Lelliottia amnigena*, *Lelliottia nimipressuralis*	Have laccase activity	Yang et al. (2017)
Pseudomonas plecoglossicida, *P. citronellolis* strain DSM 50332 and NBRC 103043, *P. monteilii, Ochrobactrum anthropic, Leclercia adecarboxylata*	Highest MnP activity in *Pseudomonas plecoglossicida*, however other strains also have high MnP activity	Yang et al. (2017)
Burkholderia ginsengisoli, Ochrobactrum haematophilum, P. plecoglossicida, P. citronellolis, P. monteilii	High LiP activity	Yang et al. (2017)
Rhizobia sp. YS-1r	15% lignin degradation of acid insoluble in switchgrass	Jackson et al. (2017)
Cupriavidus basilensis	Break down kraft lignin	Shi et al. (2017)
Pseudomonas putida KT2440	Extremely high lac activity	Mazurkewich et al. (2016), Yang et al. (2017)

(continued)

Table 13.4 (continued)

Microorganism	Activities	References
Pseudomonas sp.	High MnP activity	Yang et al. (2017), Rahmanpour and Bugg (2015), Salvachúa et al. (2015)
Paenibacillus glucanolyticus	Decreased average molecular weight of lignin	Mathews et al. (2016)
Trabulsiella sp.	Biodegradation of lignin, isolated from termite gut	Suman et al. (2016)
P. glucanolyticus SLM1	Facultative anaerobic, lignin degradation under aerobic and anaerobic conditions	Mathews et al. (2016)
Acetoanaerobium sp. WJDL-Y2	Maximum KL degradation capability is 24·9%	Duan et al. (2016)
Burkholderia sp. H1	High ligninolytic activity and degrade alkali lignin and Klason lignin	Kumar et al. (2015)
Pesudomonas strains and *Acinetobacter* ADP1	Depolymerize high molecular lignin	Salvachúa et al. (2015)
P. fluorescens Pf-5	Dyp-type peroxidases, release low molecular weight fragment of lignin	Rahmanpour and Bugg (2015)
Bacillus tequilensis SN4	Thermo-alkali-stable laccase potential to biobleach softwood pulp, active at high temperature (90 °C) and also stable at a higher pH (9.0–10.0)	Sondhi et al. (2015)
Bacterial cocultures *Aeromonas hydrophila–Pseudomonas poae* and *Klebsiella oxytoca–Bacillus amyloliquefaciens*	Degradation increase 6.6-fold and ~sevenfold, respectively	Taha et al. (2015)
Acinetobacter ADP1 *Rhodococcus jostii RHA1* *Pseudomonas putida* *Amycolatopsis sp.*	Nearly 30% of initial lignin can be depolymerized and catabolized	Salvachúa et al. (2015)
Bacillus sp. CS-1 and CS-2	Capability of alkali lignin degradation	Chang et al. (2014)
Klebsiella sp. strain BRL6-2	Have small arsenal of genes encoding lignocellulolytic enzyme	Woo et al. (2014)
Pseudomonas sp.	Potent lignin-degrading bacteria	Sasikumar et al. (2014)
Paenibacillus sp. strain LD-1	Reduced the pollution parameters such as colour by 68%, lignin 54%, phenol 86%, BOD 83% and COD 78%	Raj et al. (2014)
Bacillus Sp. SHC1, *Ochrobactrum* sp. SCH2, *Leucobacter* sp. SHC3	Produce ligninolytic enzymes such as laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP)	Rahman et al. (2013)

(continued)

Table 13.4 (continued)

Microorganism	Activities	References
Rhodococcus jostii RHA1	Dye-decolourizing peroxidase (DypB)	Singh et al. (2013)
Bacterial consortium LDC	Lignin break down up to 60.9% under static culture conditions within 15 days	Wang et al. (2013b)
Bacterial strain C6 (*Bacillus pumilus*) and strain B7 (*Bacillus atrophaeus*)	High laccase activity	Huang et al. (2013)
Ochrobactrum sp. and *Rhizobium* sp.	Depolymerize lignin and produce low molecular weight oxalic acid and protocatechic acid metabolites	Taylor et al. (2012)
Pseudomonas putida mt-2	Depolymerization of high molecular weight lignin	Yang et al. (2012)
Streptomyces, Nocardia, and Rhodococcus	Lignin peroxidase for lignin degradation	Leisola et al. (2012), Bugg et al. (2011b)
Amycolatopsis sp. 75iv2 ATCC 39116 (formerly *Streptomyces setonii* and *S. griseus* 75vi2)	Extracellular heme-dependent enzyme activity against lignin model	Brown et al. (2011)
Pandoraea norimbergensis Bacillus sp.	Degrade ligninolytic indicator dyes	Bandounas et al. (2011)
Thermobifida fusca	Thermophilic bacteria, dyp-type peroxidases	Adav et al. (2010)
Streptomyces viridosporus T7A	Best studied for production of lignin peroxidase (LiP) and lignin degradation	Niladevi and Prema (2008)
Streptomyces psammoticus, and *S. chromofliscus*	Produce extracellular LiP	Niladevi and Prema (2008)
S. Psammoticus	Have MnP activity	Niladevi and Prema (2008)
Sphingomonas paucimobilis SYK-6	Catabolic pathways for lignin components breakdown studied	Masai et al. (2007)
Aneurinibacillus aneurinilyticus	Reduced colour by 58% and lignin content by 43%	Raj et al. (2007)
Burkholderia sp. strain VE22	Isolated from lower termite (*Coptotermes formosanus*) gut, degrade the aromatics	Harazono et al. (2003)
Endospore coat protein CotA of *Bacillus subtilis*, *Azospirillum lipoferum, Marinomonas mediterranea*	Showed the laccase activity	Martins et al. (2002)
Actinomycetes	Digest and modify the lignin structure	Buswell et al. (1987)

manganese (Singh et al. 2013). Recently, a second class of enzymes in the soil bacteria *Sphingobacterium*— a manganese superoxide dismutase (MnSOD1 and MnSOD2) partially purified extracellular fractions showed high lignin degradation activity (Rashid et al. 2015).

Yang et al. (2017) have identified different strains of *Pseudomonas,* and most of them had higher laccase activity. *Pseudomonas putida* KT2440 strain showed the highest Lac activity and catabolized the lignin. Their cleavage paths have also been studied (Mazurkewich et al. 2016). A bacterial consortium LDC was selected from reeds pond sludge which could degrade 60.9% lignin in reeds at 30 °C under static culture conditions within 15 days. This bacterial consortium was classified into various bacterial species such as *Pseudomonas* sp. (25.2%), *Desulfomicrobium* (10.9%), *Clostridiales* (9.1%), *Microbacterium* (7.8%), *Geovibrio thiophilus* (5.1%), *Azoarcus* sp. (5.1%), *Thauera* (5.1%), *Paenibacillus* sp. (5.1%), *Acinetobacter* sp. (3.1%), *Cohnellasp*. (2.2%) and uncultured bacterium (21.3%) (Wang et al. 2013b). The bacteria strain C6 (*Bacillus pumilus*) and strain B7 (*Bacillus atrophaeus*) isolated from soils of a rich biodiversity rainforest in Peru were reported to have high laccase activity (Huang et al. 2013). A novel extracellular thermo-alkali-stable laccase (SN4 laccase) enzyme was reported by Sondhi et al. (2015) from *Bacillus tequilensis* SN4 which has the potential to biobleach softwood pulp and is active at high temperature (90 °C) and also stable at a higher pH 9.0–10.0 (Sondhi et al. 2015). Martins et al. have reported the laccase activity in the endospore coat protein CotA of *Bacillus subtilis* and they have also found that apart from *B. subtilis* laccase activity was also in the *Azospirillum lipoferum* a soil bacterium and the marine bacteria *Marinomonas mediterranea* (Martins et al. 2002).

Raj et al. (2007) have identified a potent lignin removal bacteria *Aneurinibacillus aneurinilyticus* from sludge of pulp and paper mill and reported that these bacteria do not use the kraft lignin as a sole carbon source but, after 6 days, reduced colour by 58% and the lignin content by 43% from kraft lignin-mineral salt media supplemented with glucose at pH 7.6 and 30 °C. Later they have identified other potent laccase-producing bacteria *Paenibacillus* sp. strain LD-1 from the contaminated soil sample through lignin enrichment method. This bacterium showed potential bioremediation of highly hazardous pulp and paper mill effluent and it was found that this bacteria significantly reduced the pollution parameters such as colour by 68%, lignin 54%, phenol 86%, BOD 83% and COD 78% at 34 ± 1 °C and 120 rpm for 144 h (Raj et al. 2014). Mathews et al. have also isolated a facultative anaerobic bacterial strain *Paenibacillus glucanolyticus* SLM1 from pulp mill waste and can degrade the lignin under aerobic and anaerobic conditions (Mathews et al. 2016). Recently, Tahir et al. (2019) have confirmed the lignin degradation property of the *Paenibacillus lautus* strains S18, S20 and S36 isolated from decaying oil palm empty fruit bunches (OPEFB).

Some previous studies have confirmed that high MnP activity was in most of the *Pseudomonas* sp. (Rahmanpour and Bugg 2015; Salvachúa et al. 2015), later on it was also confirmed by Yang et al. (2017). Highest MnP activity was in *Pseudomonas plecoglossicida* strain NBRC 103162; however, other *Pseudomonas* sp. such as *Pseudomonas citronellolis* strain DSM 50332, *Pseudomonas citronellolis* strain

NBRC 103043 and *Pseudomonas monteilii* also have high MnP activity. Noteworthy, higher MnP activity was also found in *Ochrobactrum anthropic* strain ATCC 49188 and *Leclercia adecarboxylata* strain NBRC 102595 (Yang et al. 2017). Laccase activity has been reported in several bacterial strains such as *Pseudomonas monteilii, Raoultella planticola* strain NBRC 14939, *Lelliottia amnigena* strain JCM1237, *Lelliottia nimipressuralis* strain LMG 10245. In general, high LiP activity was reported in *Burkholderia ginsengisoli* strain NBRC 100965, *Ochrobactrum haematophilum* strain CCUG 38531 and several *Pseudomonas* sp. such as *Pseudomonas plecoglossicida* strain NBRC 103162, *Pseudomonas citronellolis* strain DSM 50332, *Pseudomonas monteilii* (Yang et al. 2017).

The bacteria *Burkholderia* sp. H1 isolated from rotten wood samples showed high ligninolytic activity and degrades alkali lignin and Klason lignin in wheat straw (Kumar et al. 2015). Another bacterial strain *Burkholderia* sp. strain VE22 isolated from lower termite (*Coptotermes formosanus*) gut could also degrade the aromatics (Harazono et al. 2003). In 2017, Jackson et al. have reported that *Rhizobia* sp. YS-1r also have lignin degradation activity and could degrade acid-insoluble lignin in switchgrass up to 15%. However, *Rhizobia* sp. YS-1r exhibited low potential for degradation of raw lignin compared to *Burkholderia* sp. H1 (Jackson et al. 2017). Moreover, lignin degradation activity of *Burkholderia* sp. H1 was lower than *Ganoderma applanatum* BEOFB 411 fungal strain that had a maximum rate of degradation about 35% during cultivation for 14 days in wheat straw (Ćilerdžić et al. 2016). The *Cupriavidus basilensis* B-8 has also been reported to break down the kraft lignin from 15.1 KDa to 1.65 KDa for 7 days, and degraded lignin fragments were used as a carbon source for bacterial metabolism (Shi et al. 2017).

Duan et al. isolated the lignin-degrading bacterial strain, *Acetoanaerobium* sp. WJDL-Y2 from the sludge of a pulp and paper mill (Duan et al. 2016). GC-MS analysis of kraft lignin-degraded products revealed that the bacterial strain oxidized the lignin structural units p-hydroxyphenyl, guaiacyl and syringyl, and low-molecular-weight aromatic compounds such as benzene-propanoic acid, syringic acid and ferulic acid (Duan et al. 2016). Apart from *Burkholderia* sp. VE22 strain, *Trabulsiella* sp. was the other termite gut bacteria isolated from termite (*Odontotermes obesus*) gut and has been reported to degrade the alkyl lignin which was confirmed by the presence of some aromatic compounds in GC-MS analysis of the degraded product (Suman et al. 2016).

Two bacterial strains of *Bacillus* sp. CS-1 and CS-2 were isolated from forest soils in Japan exhibiting the capability of alkali lignin degradation (Chang et al. 2014). The bacterial species *Ochrobactrum* and *Rhizobium* were isolated from woodland soil and have reported to depolymerize lignin molecules and produce low molecular weight oxalic acid and protocatechuic acid metabolites from wheat straw lignocellulose (Taylor et al. 2012). A small arsenal of genes encoding lignocellulolytic enzymes have been reported from the *Klebsiella* sp. strain BRL6-2, isolated from tropical forest soils in the USA (Woo et al. 2014). *Enterobacter aerogenes* ATCC 29007 was used to assess the effects on cell growth, 2,3-butanediol production and enzyme activity of compounds derived from lignocellulosic biomass (Lee et al. 2015). Salvachúa et al. (2015) have demonstrated that a

bacterial subset can depolymerize and catabolize lignin initially up to 30%, particularly by *Amycolatopsis* sp., *Acinetobacter* ADP1, two *Pseudomonas putida* strains, and *Rhodococcus jostii*.

Several decades earlier, Actinomycetes have reported to digest and modify the lignin structure extensively; however, their degradation pattern differed with that of white-rot fungi (Buswell et al. 1987). Actinomycetes are the strong ligninolytic enzyme producers and among them *Streptomyces viridosporus* T7A was best studied for the production of lignin peroxidase (LiP) and lignin degradation. Additionally, other strains of *Streptomyces* such as *S. psammoticus* and *S. chromofliscus* have also reported producing the extracellular lignin peroxidase (LiP) (Niladevi and Prema 2008). Apart from the LiP, *Streptomyces* strains are also a good source of laccase, but the information about the production of MnP is less. However, MnP activity has been reported in some actinobacteriurn such as *S. psammoticus* (Niladevi and Prema 2008). Other strains such as *S. coelicolor* A3(2) and *S. badius* ATCC 39117 also showed lignin decomposition activity (Majumdar et al. 2014; McCarthy 1987). Bacteria from some genera, such as *Streptomyces, Nocardia, and Rhodococcus,* have been shown to degrade lignin with bacterial lignin peroxidase by radiochemical assay $_{14}$C-labelled lignins (Leisola et al. 2012; Bugg et al. 2011b). Masai et al. (2007) extensively studied the catabolic pathways for the breakdown of lignin components in *Sphingomonas paucimobilis* SYK-6. Later on dyp-type peroxidases have been reported in a secretome of cellulose-degrading thermophilic bacterium *Thermobifida fusca* belonging to Actinobacteria (Adav et al. 2010). Extracellular heme-dependent enzyme activity has been reported against lignin model in the soil bacterium *Amycolatopsis* sp. 75iv2 ATCC 39116 formerly *Streptomyces setonii* and *S. griseus* 75vi2 (Brown et al. 2011). Recently, a thermostable endo-xylanase enzyme from the *Streptomyces griseorubens* LH-3 for biobleaching of eucalyptus kraft pulp was studied and found that pulp brightness increased up to 14.5% after treatment with purified xylanase and kappa number by 24.5% (Wu et al. 2018).

Bandounas et al. (2011) identified three bacterial species *Pandoraea norimbergensis* LD001, *Pseudomonas* sp. LD002 and *Bacillus* sp. LD003 and allowed to grow on high- and low-molecular-weight lignin fractions and degradation of ligninolytic indicator dyes. They found that *Pandoraea norimbergensis* LD001 and *Pseudomonas* sp. LD002 were efficiently growing but their decolourizing capability of dye was low, during the growth of *Bacillus* sp. LD003 was slow but decolourized the dye efficiently (Bandounas et al. 2011).

Microbial synergistic effects on saccharification were also studied, and it was observed that dual and triple microbial combinations significantly affect the saccharification. Bacterial cocultures *Aeromonas hydrophila–Pseudomonas poae* and *Klebsiella oxytoca–Bacillus amyloliquefaciens* led to increased saccharification up to 6.6- and ~ sevenfold, respectively (Taha et al. 2015).

13.5 Research Gaps and Future Outlook

Wood is the basic raw material for paper production, comprising three essential constituents: cellulose, lignin and hemicellulose. The lignin presence in the paper leads to discolouration of paper on standing by the chemical changes in lignin in the presence of light. Therefore, pulp manufacturers prefer to dissolve and remove the lignin out of the wood by chemical solutions (Paliwal et al. 2015). Lignin and hemicellulose contents are removed from cellulose fibres during the wood to paper conversion. Since several decades, pulp and paper industries are converting lignocellulose into valuable fibres, lignin burning for energy. The second-generation biofuels production technology began commercialized in 2015, capable of producing the chemicals and biofuels from the cellulose and hemicellulose (Xu et al. 2019; Nguyen et al. 2017). The complex structure of lignin makes it very hard to transform into valuable products and is considered as waste in biorefineries and needs to be removed, due to its inhibitory effects on fermentative bacteria (Lee et al. 2019). Depolymerization and fragmentation of the lignin are predominant strategies for the production of any valuable product such as paper, biofuels, etc. (Xu et al. 2019). In the pulp and paper industries, most of the lignin content is removed during cooking stage at high alkaline and high temperature, and the remaining residue is decolourized during bleaching process leading to the generation of highly toxic, mutagenic and carcinogenic by-products which are released in the environment.

Biological treatment of the lignin is one of the most effective and environment-friendly approaches for lignin valorization. During the last several decades, wood-rooting fungi have reported as potential lignin-degrading microorganism belonging mainly to phyla Basidiomycota, Ascomycota. Progress on fungal degradation of lignin has been made, and few of them are in the commercial processes. However, the main issue with fungal delignification is the duration required to achieve higher delignification percentages, which is more than 13 days and can vary up to 40 or 50 days, depending on the strain involved. The effectiveness and reduction of the delignification process duration have been improved to some extent by treatment with alkali before initiation of fungal delignification, and are also reported to increase yield in the glucose and bioethanol production. Bacterial degradation has some advantages over fungal degradation, such as the bacteria can tolerate a wider range of temperature, pH, and oxygen limitations than fungi (Daniel and Nilsson 1998). Another advantage is that in bacteria genetic manipulation for overexpression of gene for production of lignin-degrading enzymes is easy compared to that in fungi (Suman et al. 2016). Lignin degradation through crude and purified/semi-purified ligninolytic enzymes such as laccase, MnP and LiP also is of great concern. Synergistic effects of the combination of two or more enzymes exhibited enhanced delignification. Alkali pretreatment facilitates and improves the delignification process, similar to the microbial delignification. The advantage of enzymatic delignification over fungal delignification is the duration of time required to achieve the same delignification which is less varying between 1 and 4 days. Nevertheless, the enzymatic degradation of lignin is a high-cost process due to difficulties in producing ligninolytic enzymes on a large scale.

Although the role of microorganisms in delignification has several advantages and is beneficial to the surrounding environment in reducing the pollutants, implementation of these environmentally friendly methods is hindered due to several factors. The different methodologies of delignification process have specific advantages; however, these delignification processes cannot compete with the conventional methods in terms of cost and duration required for delignification. Extensive research to explore and identify effective and potential strains is one of the key aspects which could play a significant role to replace the existing conventional methods. Whilst, improvement in the methodologies of utilizing these microorganisms for delignification, to bring down the cost and time-span involved in the process, is crucial for upbringing and implementation of such environmental friendly technologies. In addition, it may be emphasized that these ligninolytic enzymes produced by fungal species have the potential to produce second-generation biofuels. Research with a multidimensional approach to explore the lignin-degrading microorganisms, ligninolytic enzymes and synergetic relationship, for their role in delignification in the industrial process and biofuel production could pave the way for implementation effectively and provide insight in the economic efficiency.

13.6 Conclusions

This paper reviews on the microorganisms possessing properties to degrade lignin, a component removed through various processing methods by pulp and paper industries, leading to the generation of wastewater containing large number of chlorinated toxic organic contaminants, which are highly toxic, mutagenic and carcinogenic. This large amount of wastewater generated in the form of effluents contains high chemical diversity of organic pollutants causing high toxicity effects on aquatic communities, as well as profoundly affecting the terrestrial ecosystem when discharged in recipient watercourses. One among the alternatives is the use of microbes or their enzymes like laccase, lignin peroxidase and manganese peroxidase, which are potential to remove or break down the lignin residuals and hemicellulose contents from the cellulosic pulp. Microorganisms possessing these enzymes could be a potential source for environmentally friendly technologies for the pulp and paper industries in future. Wood rotting white-rot fungi have been widely studied and reported, and among them, *Phanerochaete* sp. and *Trametes* sp. have been shown to possess strong ligninolytic properties by several researchers. Apart from white-rot fungi, several species of brown-rot and soft-rot fungi are also involved in the biobleaching process. In addition, most of the bacteria and Actinomycetes have reported as strong ligninolytic enzymes producer, among them species of the *Pseudomonas, Klebsiella, Bacillus* and *Streptomyces* could play an important role in lignin breakdown. Microbial based bleaching is an eco-friendly, safe and free from the fear of toxicity, mutagenic and carcinogenic effects of effluents on environmental communities. Use of such microbial-based

applications is the need of today and could lead to a decrease in pollutants in wastewater generated and released into the natural environment by industry.

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Production of Polyhydroxyalkanoates Using Waste as Raw Materials

14

Lalit Kumar, Lalit R. Kumar, Nishu Giri, and Brijendra Kumar Kashyap

Abstract

Bioplastics (PHA) arise as a potential candidate to substitute the petroleum-based non-biodegradable conventional synthetic plastics since both are thermoplastics, moldable and can be tailor-made for several applications. This chapter discusses biological methods for the production of PHAs (polyhydroxy alkanoates) using waste sources. Biosynthetic pathways for PHA production, different fermentation strategies, different types of waste substrates have been discussed in the chapter, along with different PHA extraction methods reported in the literature. Comparison between pure culture and mixed culture, use of genetic engineering for PHA production and techno-economic evaluation for PHA production have also been discussed.

Keywords

Bioplastics · Waste sources · PHA extraction · Mixed culture · Pure culture

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14.1 Introduction

Plastics are an essential part of the packaging for all industries. However, they have a disadvantage that the accumulation of recalcitrant plastics in the environment has posed a serve environmental problem (Yadav et al. 2019). Solutions to plastic waste management include incineration, source reduction, recycling, and bio or photo-degradation. However, most of them have problems associated with them. In such a scenario, biodegradable plastics offer the best solution to the current environmental problems. Polyhydroxyalkanoate (PHA), also known as biopolymers, are polymers of alkanoic acids synthesized by various micro-organisms as reserves of carbon and energy, generally in the presence of excess carbon source with limited nutritional conditions (Zheng et al. 2020). Polyhydroxy butyrate (PHB) is the most commonly produced, and hence most studied PHA (Yeo et al. 2018; Sirohi et al. 2020).

14.2 Applications of Bioplastic

Bioplastics (PHA) arises as a potential candidate to substitute the petroleum-based non-biodegradable conventional synthetic plastics since both are thermoplastics, moldable and can be tailor-made for several applications. The current high cost of PHA restricts its application until now in medical and pharmaceuticals. However, due to the advancement of production technology using cheap substrate and improved recovery processes, the applications of PHA are spreading (Chee et al. 2010; Raza et al. 2018). Some of the significant major areas of potential applications of PHA are summarized in Fig. 14.1. Since biodegradable plastics have applications in biomedical, packaging, and food industry, the PHA market will benefit. The PHA global market is expected to rise from the US $57 million in 2019 to the US $98 million in 2024, characterized by a compound annual growth rate (CAGR) of 11.2% (Surendran et al. 2020).

14.3 Upstream Processing for PHA Production

14.3.1 Biosynthesis of PHA

PHA can be produced either by chemical means or by biological approaches. PHA production by biological means leads to a higher molecular weight compared with that achieved with chemical methods. However, PHA production by biological means does not have much control over the monomer structures in the PHA polymers. Monomer structure in PHA polymers is influenced by PHA polymerase enzyme. PHA biosynthesis is conducted by micro-organisms that can grow on sustainable carbon sources such as starch, glucose, sucrose, fatty acids, and even in wastewater at room temperature and atmospheric pressure. Due to which, PHA biosynthesis by microbes is considered more environmentally friendly and sustainable approach (Koller et al. 2017; Marques Monteiro Amaro et al. 2019).

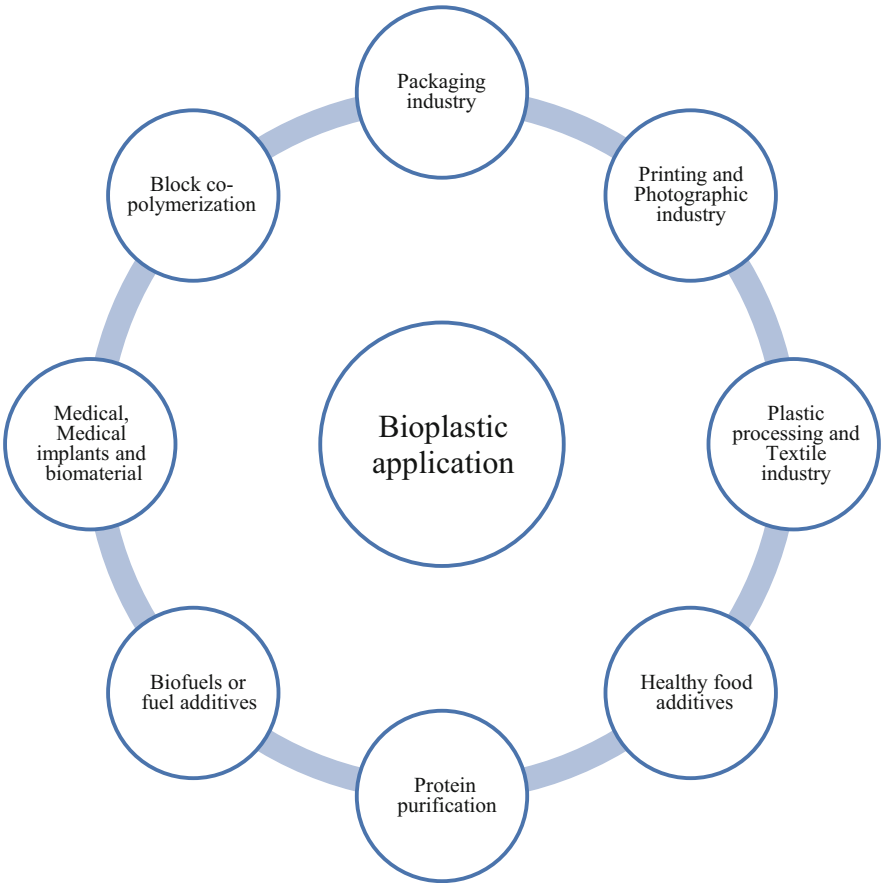

Fig. 14.1 Potential applications of PHA in numerous fields

The in-depth study was done for metabolic pathways of PHA biosynthesis in *Cupriavidus necator* (formerly known as *Ralstonia eutropha*). This pathway is shared in a broad range of bacteria. Usually, three simple steps are involved in PHA production. A β-ketothiolase catalyzes the two molecules of acetyl-CoA with the formation of a carbon-carbon bond. NADPH-dependent acetoacetyl-CoA reductase catalyzes the reduction of acetoacetyl-CoA formed in the first reaction to 3-hydroxybutyryl-CoA (Lee 1996). PHB is produced by polymerization of (R)-3-hydroxybutyryl-CoA molecules by the PHB synthase leading to PHB granules (Srivastava and Tripathi 2013). In the three-step PHB synthesis pathway, two acetyl-CoA molecules are combined to form acetoacetyl-CoA in a condensation reaction catalyzed by β-ketothiolase. An NADPH-dependent acetoacetyl-CoA reductase then carries out its conversion to 3-hydroxybutyryl-CoA. The third and the final step is the polymerization reaction catalyzed by PHB synthase.

A high concentration of intracellular Acetyl-CoA is desired for PHB accumulation as TCA cycle tries to lower the concentration of acetyl-CoA. Also, intracellular CoA tries to favor reaction in the backward direction for the formation of Acetyl-CoA using β-ketothiolase and TCA cycle. Hence the high concentration of acetyl-coA and low concentration of CoA are desired for PHB accumulation. In Sect. 14.3.2, factors affecting PHA accumulation would be discussed.

14.3.2 Raw Materials for PHA Production

The carbon source has been known to account for the significant fraction of upstream PHA production cost (Amache et al. 2013). From an economic perspective, the cost of carbon substrate accounts for 30–40% of the production cost (Andler et al. 2019). Therefore, the choice of carbon source has an underlining economic impact on PHA production and its extraction. It is imperative to choose renewable, inexpensive, and readily available carbon substrates that can support the microbiological growth of the PHA-producing microbe while keeping the cost bar at lower ends. Microbes are known to have tremendous potential to produce PHA using a wide range of inexpensive complex waste effluents like used plant oils, fatty acids, hydrocarbons, and simple carbohydrates. In the renewable feedstocks class, the three most used sources are agricultural grains and associated waste, forestry biomass, and industrial effluent waste material. Every year a large volume of waste is generated by the agricultural sector and food processing industries. These waste streams provide potential avenues to capitalize on the organic content present in this waste stream for PHA production. The use of these waste streams reduces the cost of PHA production and reduces the load on the waste disposal industry. The most common types of industrial waste streams are *cheese whey* and *molasses* obtained from sugar canes or beet (Koller et al. 2012). Many studies have tested PHA production using whey and molasses. Though these wastes contain a high amount of organic content, some degree of pretreatment is required before they can be used with microbes for PHA production (Alcaraz Cercós 2015).

14.3.2.1 Different Waste Streams for PHA Production

The selection of waste stream as a source of nutrient medium for PHA production is mainly dependent upon the geographic location of the PHA production plant (Jiang et al. 2011). Different waste streams that are being tested for their potential as a source of carbon for PHA production are, (1) Lipids and oil like a waste, (2) Crude Glycerol from Biofuel Industry waste, (3) Whey from Dairy Industry, (4) Lignocellulosic biomass, (5) Bagasse from Sugar Industry waste.

Lipids and Oil Like a Waste

Lipids of different origins can be used as a substrate for PHA production. Lipids obtained from waste cooking oil generated at restaurants and similar food processing-related operations. Animal fate is another source of lipids which can be obtained from slaughterhouses and other meat processing-related operations (Surendran et al. 2020).

Crude Glycerol from Biofuel Industry

Crude glycerol is a by-product of the biodiesel industry, which is produced in large quantities. Due to its carbon content, it presents exciting opportunities for PHA production using crude glycerol as a carbon source (Gözke et al. 2012).

Whey from Dairy Industry

Whey is the surplus waste material generated during cheese production. A surplus amount of whey is generated in many regions of the world. The surge in surplus among whey creates the proper disposal of this waste stream, which is known to have significantly high organic content reflected by its very high ranges of biological oxygen demand values. Lactose is the major component of whey that can serve as a carbon source for microbial growth and PHA production (Koller et al. 2012).

Lignocellulosic Biomass

The agriculture sector, wood processing, and paper production industry are commonly known to be the highest producer of lignocellulosic waste. The lignocellulosic waste streams are abundant in hexose and pentoses. A meticulously selected microbial strain is required to capitalize on this waste for PHA production. Unmetabolized sugars can quickly accumulate in the growth medium during fermentation, thereby causing feed-back inhibition that can negatively impact microbial growth and PHA production (Kumar et al. 2008).

Bagasse from Sugars Industry

Bagasse is a major waste stream generated in the sugar industry. Due to their high organic content and simple six-carbon carbohydrates, it can be served as a potential source of carbon substrate in PHA production, making it economically competitive (Koller et al. 2008).

14.3.3 Factors Affecting PHA Production

14.3.3.1 Carbon Content

For the accumulation of PHA, carbon content should be in excess. In carbon limiting conditions, micro-organisms start utilizing PHA as a carbon source. PHA gets degraded by PHA depolymerase and the metabolic flux shifts for the production of acetyl-CoA, which will participate in TCA. Hence carbon content should be in excess for PHA accumulation (Lam 2010). However, above a specific value, carbon content can have an inhibitory effect on PHA accumulation and biomass growth (Yan et al. 2008).

14.3.3.2 Nitrogen Content

It has been reported that under excess nitrogen conditions, enzymes for TCA cycle are active, and participation of acetyl-CoA in TCA cycle results in low accumulation of PHA. Moreover, the relative concentration of CoA increases as compared to Acetyl-CoA under excess nitrogen content, resulting in a shift in the TCA cycle's metabolic flux. Hence nitrogen content should be in limiting conditions, and an

optimum C:N ratio should be present for PHA accumulation inside the cells (Lam 2010). However, C:N ratio depends on micro-organism and culture employed for PHA accumulation.

When activated sludge is used as a waste source, an increase in COD concentrations has resulted in high PHA yields. However, inhibition of PHA was found when high nutrient content (due to an increase in nitrogen content) was present (Lam 2010).

14.3.3.3 DO (Dissolved Oxygen)

It has been reported that high oxygen concentration favours the TCA cycle for energy generation, which causes PHA yield to decrease. Low oxygen lowers the activity of enzymes such as citrate synthase and iso-citrate dehydrogenase. Low oxygen results in a relative increase in Acetyl-CoA concentration compared to CoA, shifting the metabolic flux in the direction of PHB production (Lam 2010). Hence, oxygen limiting conditions usually favour PHB production. The micro-aerophilic reactor has been reported to yield high PHA yields (62%) (Satoh et al. 1998).

14.3.3.4 pH

pH used during fermentation for PHA accumulation depends on organism to organism, and characteristics of waste material employed as raw material. However, for mixed cultures, optimum pH used was between 7 and 9 (Yan et al. 2008; Chua et al. 2003).

14.3.4 Strategies for PHA Production

The effectiveness of PHA production processes from wastes is strongly dependent on culture selection. Almost every complex waste substrate in consideration for PHA production requires some pretreatment of the complex carbohydrates present in the waste stream into a simple metabolizable carbon source. Many studies have been devoted to the pretreatment of complex organic waste substrates in volatile fatty acids (acetic acid, propionic acids, and butyric acids) using anaerobic fermentation techniques. It further adds multiple steps in the PHA production process. Overall, there are four steps in PHA production using complex waste streams. (1) Pretreatment of waste streams; (2) Enrichment of the microbe(s) to adapt in the complex waste substrate medium; (3) Upstream production of PHA; and (4) Extraction and purification of PHA.

Simple sugars (glucose, galactose, glycerol, etc.), alkanols, and alkanoates can be directly utilized as substrates for cell growth and PHA accumulation. Pretreatment of waste sources (agricultural, industrial or municipal) is done to convert complex material into simpler substrates and removal of unwanted materials that can inhibit the growth of microbes during fermenter.

14.3.4.1 Pretreatment for Whey

Whey contains fat (0.3% w/w), protein (0.9% w/w), ash (0.5% w/w), nitrogenous compounds (1% w/w), minerals and vitamins (0.8%) besides lactose (5%). Firstly

lactose protein is concentrated to 80–85% (w/w) through ultra-filtration where lactose is obtained in permeate. Then metals or minerals present with lactose are removed through reverse osmosis membrane (Koller et al. 2012). After that, lactose is passed through immobilized lactase to result in glucose and galactose, which can serve as a substrate for microbial cell growth and PHA accumulation.

14.3.4.2 Pretreatment for Sludge

Municipal sludge is passed through the anaerobic reactor for pretreatment where complex organic substrates like lipids, carbohydrates, protein are converted into simpler molecules like ethanol, glucose, lactic acid, cellobiose, etc. This conversion takes place through extra-cellular enzymes released by microbes present in an anaerobic reactor like *Clostridium thermocellum*. Alcohols produced during hydrolysis steps are converted to butyric acid, propionic acid, valeric acid, caproic acid, etc. These serve as a substrate for cell growth and PHA accumulation. Figures 14.2 and 14.3 display the pretreatment of lignocellulosic wastes and crude glycerol, respectively.

14.3.4.3 Pretreatment of Cellulosic Waste

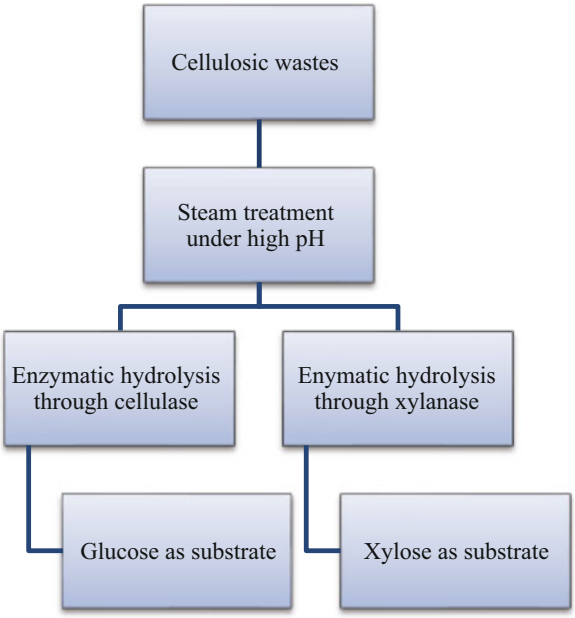

Fig. 14.2 Pretreatment for lingocellulosic wastes

14.3.4.4 Pretreatment of Crude Glycerol

Fig. 14.3 Pretreatment of crude glycerol

14.3.5 PHA Production Using Waste Sources

From an economic point of view, the cost of the substrate (mainly carbon source) contributes significantly to PHA's overall production cost. In the past decade, a wide variety of low-cost carbon substrates (e.g., renewable carbon sources), such as wastewater, municipal wastes, agricultural and industrial residues or by-products (e.g., starch, tapioca hydrolysate, whey, molasses, malt, and soy wastes), crude carbon substrates (food wastes or by-products) have been tested for PHA production by pure cultures due to their low price and potential availability (Lee and Gilmore 2005). Table 14.1 summarizes the use of waste material as a carbon source and nitrogen source for PHA production. It is evident from Table 14.1 that PHA content varied between 35% and 90%, which indicates that the application of waste sources as raw materials for PHA production is apt as Industrial fermentation PHA yield is between 25 and 30% only.

14.3.6 Use of Mixed Culture for PHA Production

The production of biodegradable PHA's from microbial cells by mixed culture cultivation is the primary consideration these days. Table 14.2 summarizes the use of mixed culture for PHA production. The reported PHA yields from mixed cultures were between 40 and 77%, indicating that their application for PHA production is apt.

14.3.7 Operating Mode for Fermentation

The PHA production strategy varies along with other factors chosen for PHA production, including the source of carbon substrate (hexoses or complex carbohydrates), microbial strain(s) chosen, and mode of fermentation (batch or fed-batch) (Amache et al. 2013). The fermentation can be performed in one phase or multiple phases (as in sequence batch reaction, SBR). Mixed microbial biomass utilizes an open system septic fermentation technique whose composition varies as per the carbon substrate in the medium and the physicochemical conditions of the fermentation (Valentino et al. 2015).

Table 14.1 Reported studies for PHA accumulation using waste as raw materials

Micro-organism	Waste material used as carbon source	Type of polymer	PHA content (%)	Reference
Ralstonia eutropha NCIMB 11599	Saccharified waste potato starch	PHB	46	Kamilah et al. (2013)
Bacillus cereus strain	Pea shell slurry	PHB	41	Kumar et al. (2009))
Ralstonia eutropha ATCC 17699	Crude glycerol	PHB	55	Gözke et al. (2012)
Ralstonia eutropha	Fermented palm oil mill effluent	PHA	90	Chee et al. (2010)
Ralstonia eutropha	Waste animal fats	PHA	80	Riedel et al. (2015)
Pseudomonas citronellolis	Apple pulp waste	PHA	30	Rebocho et al. (2019)
Ralsthonia eutropha	Pineapple peel waste	PHA	–	Vega-Castro et al. (2016)
Rhodospirillum rubrum	Syngas from Household feedstock	PHB	16	Revelles et al. (2017)
Pseudomonas pseudoflava	Synthetic waste	PHBV	55–60	Reddy et al. (2017)
Haloferax mediterranei	Whey	PHBV	66	Koller (2015)

*PHBV** co-polymer of Poly-hydroxy-butyrate and Poly-hydroxy-valerate.

Table 14.2 Reported studies for PHA accumulation using mixed cultures

Waste material as carbon source	Type of polymer	PHA content (%)	Reference
Fermented paper mill wastewater	PHA	77	Jiang et al. (2012)
	PHA	67.6	Queirós et al. (2014)
Fermented sugar cane molasses	PHA	75	Albuquerque et al. (2010)
Fermented food waste	PHA	40	Reddy and Mohan (2012)
Municipal solid waste	PHBV	–	Colombo et al. (2017)
Fermented cheese whey	PHA	60–70	Colombo et al. (2016)
Mixed microbial culture	PHA	65–70	Huang et al. (2017)
Anaerobically treated brewery wastewater	PHA	72.6	Tamang et al. (2019)
Nutrient rich wet oxidation liquors	PHBV	41	Wijeyekoon et al. (2018)

*PHBV** co-polymer of Poly-hydroxy-butyrate and Poly-hydroxy-valerate.

14.3.7.1 Sequencing Batch Reactor

The sequencing batch reactor is employed when sludge is used as waste carbon source. The sequencing batch reactor allows for nitrification and denitrification for nitrogen present in the sludge. COD reduction and phosphorous removal from the sludge also takes place during SBR. Usually, SBR is employed for biomass enrichment step for PHA production (Zeng et al. 2018).

The operation of sequencing batch reactor can be divided into various phases (Fig. 14.4):

Filling Phase During the fill phase, the sink collects influent wastewater. The influent produces food as a carbon source to the microbes in the activated sludge, creating an environment for biochemical reactions. A mechanical mixer is active for mixing but aeration off. Therefore, an anoxic condition is present, which encourages denitrification as denitrifying bacteria are anoxic/anaerobic. During the fill phase, anaerobic conditions can also be achieved, resulting in the biomass undergoing a release of phosphorous. The biomass reabsorbs this release once SBR operation enters into reaction phase.

Reaction Phase During this phase, no wastewater enters the sink, and the mechanical mixing and aeration units are on. Nitrification occurs at this step as nitrifying bacteria are aerobic in nature, and the microbes utilize organic load present in the sludge for growth and enhancement of PHA accumulating capacity. Phosphorus released during the filling phase is utilized here.

Settling Phase During this phase, activated sludge is allowed to settle under flocculation. No flow enters or leaves the sink, and no aeration and mixing take place. The activated sludge tends to settle as a flocculent mass, forming a clear separation with the clear supernatant. The sludge mass is called the sludge blanket.

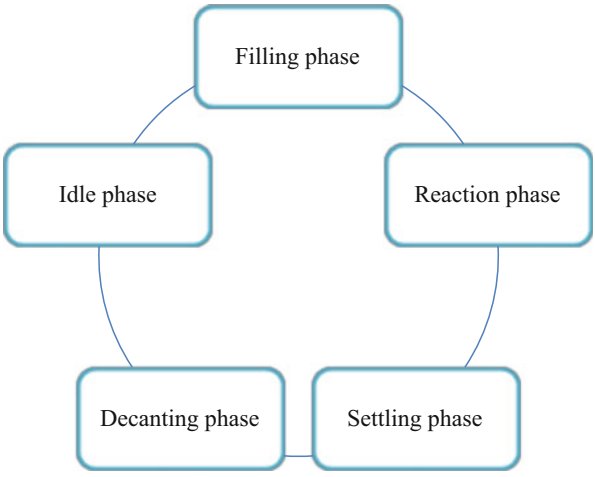

Fig. 14.4 Various stages of sequencing batch reactor

During this phase, denitrification takes place as denitrifying bacteria are anaerobic in nature.

Decanting During this phase, a decanter is used to withdraw the clear supernatant effluent/treated water. Once the settling phase is completed, a signal is sent to the decanter to initiate the opening of an effluent discharge valve.

14.3.7.2 Feast Famine Cycle-Completely Aerobic or Anaerobic/Aerobic Combination

PHA production has been observed in activated sludge obtained from wastewater treatment plants (WWTPs) operating for the removal of phosphates. Such mixed culture systems are operated using cycles of alternating aerobic and anaerobic operations, also referred to as feast famine (FF) process. The PHA production in the FF process is a result of nutrient limitation stress imposed on microbes during famine phase reducing their intracellular metabolic resources (RNA and enzymes) which allows them to instinctively and exclusively store nutrients when they are exposed to nutrients in feast phase (Kourmentza et al. 2017). In fact, PHA production is only favored in nutrient limiting conditions for the growth of the microbe and abundance of carbon substrate resulting in imbalanced growth conditions in the medium. Aerobic activated sludge biomass has been reported to store a higher amount of PHA favoring prioritized accumulation than biomass growth after prolonged starvation (Reis et al. 2011).

Furthermore, during the famine phase, the microbes with an intrinsic ability to store intracellular PHA have the edge over the other microbes, which lack this capability such that the sludge microbial biomass is selectively enriched in terms of relative abundance of PHA producing microbes. During the famine when external carbon source is depleted, the microbes tend to lean on their internal reserves of carbon (PHA, lipids, and other inclusion bodies) for the source of carbon and energy to carry out necessary maintenance activities and survive. The approach of transient nutrient availability for enhanced PHA accumulation is often termed as "Aerobic Dynamic Feed" approach or FF regime. Researchers have reported mixed microbial biomass selection with a high potential of PHA accumulation using the ADF technique. In aerobic–anaerobic cycles, a similar concept coupled with the presence of oxygen as the nutrient element for enrichment. In the absence of oxygen (final electron acceptor), the microbes are provided with an excess carbon source, which further enforces the tendency of channelling of carbon for PHA accumulation instead of biomass growth. In the following aerobic cycle, the microbial biomass capitalizes on the presence of oxygen and residual carbon to achieve microbial growth and maintenance via regular oxidative phosphorylation routes. Therefore, aerobic–anaerobic techniques and the FF regime put a tremendous amount of metabolic pressure on the microbe to do PHA accumulation as a survival mechanism.

14.3.7.3 Fed-Batch for PHA Accumulation

A batch or a fed-batch strategy is employed for the PHA accumulation step. The fed-batch strategy consists of two phases. During the first phase, excess nutrients such as C, N, P are provided for the growth of microbes. During the second phase, excess carbon content with limiting N content is provided as feed for enhanced PHA accumulation. Although the batch mode is economical and has low operating costs, a fed-batch strategy is better as in batch mode; carbon content can be exhausted at one point of time during fermentation. The accumulated PHA reserve is used as a carbon source for growth, and PHA degradation starts resulting in low PHA yield. SBR with continuous feed mode has been reported for PHA accumulation (Lam 2010). Nevertheless, hydraulic retention time should be optimized for enhanced PHA production.

14.4 Downstream Operation for PHA Recovery and Purification

The fermentation broth is subjected to centrifugation or filtration for separation of liquid and solid biomass containing PHA. Solid biomass is further subjected to drying using lyophilization or thermal methods. Extraction of PHA is commonly performed using solvent extraction method like chloroform. The use of expensive solvents makes the extraction process expensive and detrimental to the environment (Chee et al. 2010). Many different alternatives are being developed to recover PHA like cell lysis using caustic chemicals (sodium hydroxide, and sodium hypochlorite). During these procedures, cell biomass is treated with sodium hypochlorite solution after concentrating the biomass using centrifugation. The intense chemical action of hypochlorite degrades the PHA molecule decreasing their integrity and molecular weight. On the other hand, the use of slighter agents like surfactant results in less pure PHA product. It is recommended to use an integrated approach of using surfactants and sodium hypochlorite, to obtain a highly pure PHA product. Though such approaches do result in enhanced recovery and purity, a certain degree of molecular degradation is also observed by dispersion of hypochlorite and chloroform in the extraction solution (Chee et al. 2010).

Another alternative method for PHA extraction is the use of enzymatic hydrolysis. Enzymatic approaches, by their nature, are gentle on PHA products and selectively act on the biomass. Enzymes like cellulases, lysozymes, and proteases can be used simultaneously to disintegrate the biomass and extract the PHA molecules. Enzymes often require mild operational conditions of pH and temperature, making it very eco-friendly. The enzymatic extraction is often assisted by a thermal shock to the cell biomass to facilitate the PHA extraction process. Overall, the enzymatic approach can result in high product yields and purity. However, the use of enzymatic methods is too costly at the Industrials scale. Cell disruption using mechanical methods like homogenizer and sonication can be employed for PHA extraction. However, at large scale, homogenization can be employed only for high biomass concentration while sonication is not effective at large scales. Table 14.3 summarizes

Table 14.3 Advantages and disadvantages of methods employed for PHA extraction

Method	Advantage	Disadvantage
Solvent extraction	High purity (>99%), high yield (>90%), no polymer degradation, removal of endotoxin	High capital and operation cost, not eco-friendly, toxicity for human health.
Surfactants	Retains original molecular weight, no biomass dry process	Low purity, high cost, require large volume of wastewater
NaClO	High purity, no biomass dry process	Reduce molecular weight
Acids	High yield and purity, low cost	May degrade molecular weight
Alkaline	High yield and purity, low cost	May degrade molecular weight
Surfactant-chelate	High yield and purity	Produce large volume of wastewater
Enzyme	High yield and purity	High cost of enzyme
Homogenizer	No chemical, no pretreatment, applicable for large scale with high biomass concentration	Poor disruption at low biomass concentration, formation of fine cellular debris that interference in recovery, micronization of PHA
Ultra-sonication	Low cost, pollution free, combination with other extraction methods leads to high purity	Difficult to apply on large scale
Supercritical fluid	Simple, rapid, inexpensive, non-toxic for human health, eco-friendly	Requires strict process parameter, need further chemical for high purity, recovery mechanism under research
Dissolved air flotation	No chemical, less contamination	Consecutive batch flotation steps required

Table 14.4 Comparison between pure culture and mixed culture for PHA production

Step	Pure culture	Mixed culture
Upstream	High PHA yields	Variable PHA yields
	High volumetric productivity	Relatively low volumetric productivity
	High operational costs	Lower operational costs
Recovery	High recovery yield	Low recovery yield
	Low extraction costs	High extraction costs
Scale	Industrial/commercial	Pilot

the advantages and disadvantages of various methods employed for PHA extraction (Alcaraz Cercós 2015; Kumar et al. 2018; Samorì et al. 2015).

14.5 Economic Evaluation for PHA Production

The industrial cost for the production of PHA is 16$/kg, while for synthetic plastics, it is $1–2/kg (Gurieff and Lant 2007). If pure culture is used, raw materials contribute 40–50% of the total cost, while pretreatments of waste occupy most of the cost for mixed cultures (Amache et al. 2013). Table 14.4 compares mixed culture and pure culture for PHA production (Alcaraz Cercós 2015). It is evident from

Table 14.4 that there are advantages and disadvantages for PHA production from either culture. Mixed culture has low operational costs in the fermentation step due to no requirement of sterilization and no procurement of raw materials. However, their pretreatment costs for the breakdown of complex matter into simpler units like hydrolysis or enzymatic digestion requires a high cost (Mannina et al. 2020). Furthermore, the yields obtained from mixed culture vary due to the presence of different types of micro-organisms (some are PHA accumulating, and some are not) and inert materials in mixed culture. However, the cost of raw materials is saved in mixed cultures making the process economical (Mannina et al. 2020). However, the downstream process for mixed culture results in high extraction costs. There are a lot of inert materials, wood, ash, etc., from waste sources making the purification difficult and expensive (Pavan et al. 2019; Mannina et al. 2019). Besides, recovery for pure culture is easier and results in high yield. The average PHB yield in Industries is around 25–30% of dry weight, further increasing extraction and purification costs. The downstream operation is also dependent on PHA accumulation during the fermentation step. Some of the micro-organisms have high PHA accumulation capacity and resulted in high PHA yields lowering the extraction costs. For example, *Enterobacter aerogenes* have resulted in 90% PHA yield from domestic wastewater. Therefore, there is a need to optimize PHA's production cost by optimal selection of micro-organisms with high PHA accumulating capacity and optimal selection of raw materials and waste streams depending on the construction site of production plant (Jiang et al. 2011).

In one of the studies, economic assessment of the entire PHB production process was carried where production was done using *Cupriavidus necator* and citric molasses as carbon source and employing propylene carbonate as solvent coupled with different extraction pretreatment methods. The alternatives that employ high pressure and heat to pretreat the biomass showed lower production costs (US$ 4.28/kg) and better economic indicators among evaluated scenarios (Pavan et al. 2019).

14.6 Recent Advancements for PHA Production

In native bacteria, glucose is converted to acetyl-CoA by two enzymes named PhaA (β-ketothiolase) and PhaB (acetoacetyl-CoA reductase) into 3-HydroxyButyryl CoA, followed by polymerization by PhaC enzyme (also known as PHA synthase) into PHB. In the starvation situation, the PHA is depolymerized by PhaZ enzyme (also known as PHA depolymerase) to 3HB and consumed by cells as carbon and energy. PhaC enzyme can be cloned and expressed in other bacteria such as *E. coli*. *E. Coli* is micro-organisms that can assimilate a wide range of carbon sources, which usually cheaper than glucose, like sucrose, carbohydrate, etc. *E. Coli* is also fast-growing bacteria and is not a PHA producing micro-organisms. Thus, it does not have a PhaZ enzyme. In their pathway, the acetyl-CoA is produced during metabolism. By borrowing the enzyme from native PHA-producing bacteria, the biosynthesis of PHA can be performed in *E. coli*. Omitting the PhaZ makes recombinant *E. coli* accumulate a massive amount of PHA in their body, reducing extraction costs

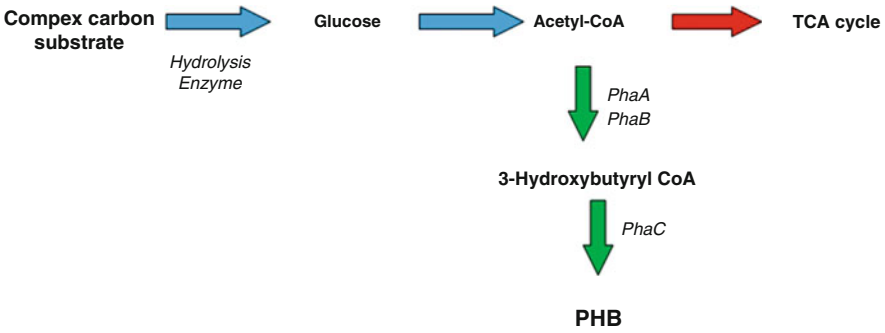

Fig. 14.5 Mechanism for enhanced PHB production in recombinant *E. coli*

for Industrial production of PHA. One of the studies reported a PHA yield of 80% using recombinant *E. coli*, indicating recombinant organisms can be used for enhanced PHA accumulation reserve (Chen and Jiang 2017). Figure 14.5 displays the use of genetic engineering for enhanced PHB production in *E. coli*.

14.7 Conclusion

Following conclusions can be drawn from the chapter:

1. PHAs are readily biodegradable, more environment-friendly than conventional plastics and possess a wide range of applications.
2. PHA accumulated during fermentation affects downstream costs. High PHA yields during fermentation require less downstream operations and fewer chemicals for extraction than low PHA yields in fermentation.
3. Waste streams can be employed for PHA production as they act as inexpensive carbon sources to uneconomical carbon sources such as glucose. Moreover, the technology used for PHA production from waste sources provides alternatives to disposal and treatment of waste streams. The process also treats pathogenic bacteria present in municipal and industrial wastes.
4. Mixed cultures from activated sludge can be employed for PHA production as high PHA contents (co-polymer) have been obtained using mixed cultures. Moreover, strains can be identified and isolated from sludge for industrial PHA production. The knowledge of PHA accumulation by these organisms in their native environment will be helpful for industrial applications. However, downstream operations for mixed cultures result in low extraction yields and have high purification costs due to inert materials requiring a large amount of solvents. Additionally, pretreatment cost for mixed culture is high.
5. Fed-batch mode is better than batch mode for enhanced PHA accumulation. During the fed-batch mode, carbon feeding with the limitation of other nutrients can be maintained for enhanced PHA accumulation. Continuous feed mode of the

SBR system can be employed for PHA production. However, the hydraulic retention time for continuous mode should be investigated.

6. Recombinant strains have resulted in enhanced PHA production. They can be employed for the industrial production of PHA. Organisms like *E. coli* have a high growth rate, can utilize a wide range of substrates and lack PHA depolymerase enzyme, which is responsible for PHA degradation.
7. A thorough study of waste composition (including sludge) is necessary for PHA accumulation as sludge has various nutrients present like nitrogen, phosphorus, ammonia, and trace elements. Hence optimization of nutrients is essential. The increase in COD concentrations has resulted in high PHA yields, but PHA inhibition was found when high nutrient content (due to an increase in nitrogen content) was present.

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Newer Aspects of Waste-to-Valorization Technologies in Food Industry

15

Soumya Rathore and Anand Kumar Pandey

Abstract

Wastage of food is a major issue around the globe. In countries like India, where a huge population is facing the problem of hunger each day, food wastage possesses a significant threat to the environment and food security. Besides the pollution and hazardous aspects, wastes obtained from food industry also have nutritive components that can be converted into some valuable and useful products of higher value. By-products from food processing industries are an important source of sugar, minerals, organic acids, dietary fibre, bioactive compounds such as polyphenols, protein isolates, single cell protein (SCP), biofuels, etc. Although it is not easy to stop the complete production of waste from food industry, techniques and technologies can be used for reusing and recycling the waste as food, feed and fodder. This chapter deals with some newer techniques and approaches through which management of industrial waste can be improved in terms of cost, retention of nutritive and valuable components and minimizing the pollution. The collaboration of regulatory agencies and food processing industries can also develop new technologies for waste utilization, which are beneficial for the common masses and are commercially viable.

Keywords

Food waste · Food wastage · waste management · Food waste to energy generation

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15.1 Introduction

Food is a first and basic requirement for life on earth. From ancient time to the present day, humans used different methods to obtain and secure their food. Initially, man used two methods to fulfill their food needs, i.e. hunting and agriculture but as time passed, many new methods came into the picture for the production and preservation of food. Today, food production has taken the shape of full-fledged industrial form that supplies most of the energy food consumed by the world population. The food-processing sector is one of the most important industry sectors throughout the globe. India is also coming under the category of top food-producing countries in the world. It is the largest producer of milk, exporter of spices, livestock population, the second-largest producer of fruits and vegetables, the third-largest producer of food grains and fish followed by other nations. Although the production of food on the planet has substantially increased from past decades and it is increasing day by day, still a harsh reality exists in the form of food insecurity in the major parts of the globe. According to FAO, 795 million people are suffering from undernourishment throughout the world in which 15 million are coming from the developed regions of the world. Apart from undernourishment, malnutrition, hunger, food crisis and food insecurity are the factors that possess a great threat to the major population of the world (Xie et al. 2019).

There are many strands that contribute to the food crisis and food insecurity in which food wastage has a prime role among all of them. Human beings produce large quantities of food waste on daily basis from small quantity of kitchen waste to huge amount of wastage during social events and functions and in hotels and restaurants. In addition, wastes are generated in producing the goods and services we utilize. Variations in food production, distribution and consumption have also led to exorbitant food waste around the world (Truong et al. 2019). Food loss and food wastage generation produce an impact at an environmental, social and economic level. Sometimes food waste contains many nutritious and valuable components that are beneficial for the health and well-being of humans. Discharge of food material occurs along the entire food supply chain (FSC), and it involves all sectors of waste management from collection to disposal. Therefore there is an urgent need to take appropriate measures to reduce food waste burden by adopting standard techniques of waste utilization and/or waste management practices (Shi et al. 2020). Several conventional physiochemical and biological techniques are already available and many have been developed recently. Conversion of the waste to value-added products or waste valorization can be a promising approach for waste utilization. Extraction of bioactive compounds, sugars, fats, oils and enzymes from food waste can be a highly beneficial mode of food waste utilization. It will not only reduce the economic loss but will also provide high value products that can be of great benefit for food as well as pharmaceutical industry. Hence this chapter puts forward the current scenario of food wastage and different measures are being taken to combat this situation along with the techniques and technologies being utilized for the conversion of food waste into bio-energy or value-added products.

15.2 Food Wastage

Food wastage includes the amount or quantity of food that is discarded or left uneaten. Wastage of food can occur not only at the end of the chain but also during the manufacturing, retailing, improper handling and improper storage practices. Improper consumption is also a major factor in food wastage. Heavy amount of food is wasted in restaurants, hotels, parties, marriage and cultural functions (Goswami 2018).

How much amount of food is produced on the planet every year and how much food is wasted every year? It is quite tough to give an accurate answer to these questions. Some studies have shown that the production of food throughout the world increased substantially in the twentieth century due to advanced agriculture techniques, fertilizers, irrigation and novel food processing and preservation techniques. According to GAP (Global Agricultural Productivity) index of 2018, from the last five years, the growth rate of agricultural productivity is so slow that it will not be able to meet the future need of approx. 10 billion people for food, feed and biofuels by 2050 (FAO 2018). Though there is an increment in the world's food production year by year, still food insecurity, hunger crises and malnourishment are a harsh reality of today's scenario. One of the primary reasons behind these problems is food wastage. A large amount of food is wasted or discarded around the world, according to FAO report 280–300 kg/capita/year is wasted in developed regions around the globe and 120–170 kg/capita/year comes from developing countries (Refsgaard and Magnussen 2009).

15.3 Food Wastage Crises in India

The production of food in India is constantly increasing year by year. Having a million hectares of arable land, India ranks as one of the topmost food producing countries among the world. India is among the top producers of several crops such as wheat, rice, pulses, sugarcane and cotton. It is the highest producer of milk and second most senior producer of fruits and vegetables. In 2013, India contributed 25% to the world's pulses production, the highest for any other country, 22% to the rice production and 13% to the wheat production. It also accounted for about 25% of the total quantity of cotton produced, besides being the second-highest exporter of cotton for the past several years (Deshpande 2017). During 2017–2018 crop year, food grain production was estimated on record to be 284.83 million tons. The food and grocery market in India is the sixth largest in the world (Fig. 15.1). The food processing industry contributes 32% to this food market and is one of the largest industries in the country, contributing 13% of total exports and 6% of industrial investment (Ministry of Agriculture, Government of India, MOSPI, BCG, Crisil 2018).

Along with the increase in food production and processing rates, rate of food wastage is also increasing exponentially and posing adverse effects on the population. Several population sectors are unable to procure food in required amount due to

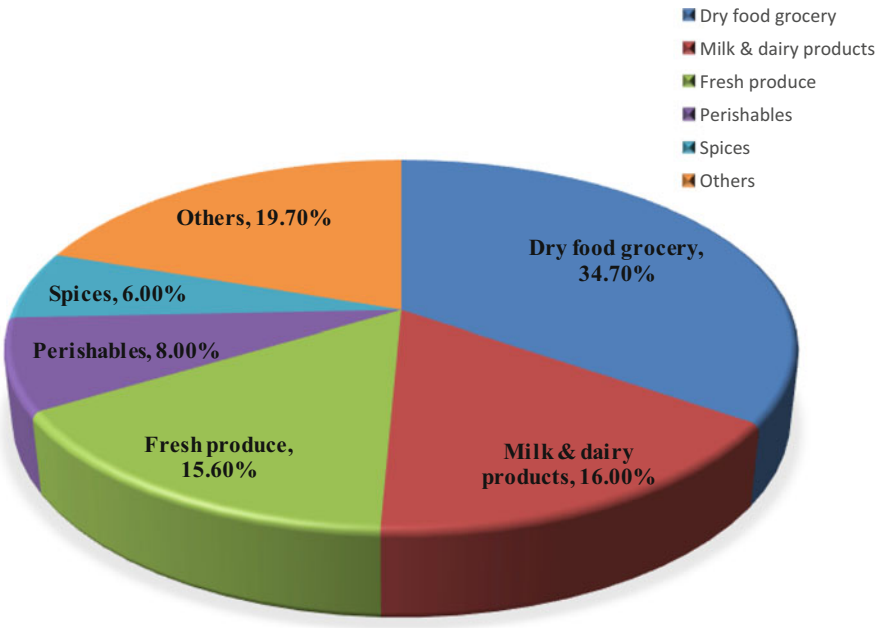

Fig. 15.1 Major food categories in food processing industries. Source: Global Agricultural Productivity 2019

the increasing wastage and remain malnutritioned resulting in a consistent poor ranking of the country in Global Hunger Index year to year. A report released by FAO in 2017 "The State of Food Security and Nutrition in the World" states that around 14.5% population of India are undernourished, especially women and children. According to estimation by United Nations, India is wasting or losing nearly 40% of its total food production which results in massive financial loss of around one lakh crore rupees per annum (Hency Thacker 2018).

15.4 Causes of Food Wastage

Apart from the various steps in the supply chain, some other factors have a strong influence on the food wastage worldwide. One of them is urbanization which increases production and development of proper supply chain to fulfil the requirements of food among the masses living in urban areas. This requires improved transportation systems, appropriate storage conditions and better sales management to avoid the wastage and losses of food (Escaler and Teng 2011). Often lack of such facilities results in massive food loss and wastage. Another factor is the change in eating habits, which mainly depends on the economic status and culture of the population of that particular country. Increase in income and cultural beliefs is directly proportional to the change in diet pattern, especially in developing

Table 15.1 Causes of food wastage

Pre-harvest losses	Weather variation, insects and disease, variety of crops, quality of soil and scarcity of water
Cultivation and harvest	Labour intensive agriculture, especially in developing countries, lack of advanced agricultural technologies, limited resources
Post-harvest handling	Improper handling, improper storage facility (e.g. lack of cold storage), inappropriate transportation, mechanical damage (cutting, bruising, etc.)
Physical infrastructure	Lack of storage, uneven distribution, spillage
Processing facilities	Lack of proper technology, inadequate machinery, insufficient system design, food safety issues
Food trimming	Automated and manual trimming
Distribution	Poor transportation facility, inaccurate demand and supply forecasting, poor record-keeping, rejection of shipments
High retail grading standards	Rejection of products due to low quality standards especially in developed countries
Supply chain	Packaging and labelling errors, contamination, cold chain failures
Consumer behaviour	Financial status, choice, ethical values, culture, educational level
Household practices	Inadequate planning of meal, leftover in every meal, portion size of the meal

Source: Moustakas et al. 2019

countries. However, this changing pattern of income and diet is rather low in developed countries. The third factor includes the increment in commercial globalization and the quick dispersal of mass distribution both in developed and developing countries. In many countries of Asia, Africa and South America, supermarkets are rapidly replacing the retailers.

It has been estimated that nearly 30–40% of food is wasted in both developed and developing nations, though sources are differing with the nations. Wastage of food occurs during the various pre-processing, processing and post-processing steps. The multiple causes of food wastage in both developed and developing countries are shown in Table 15.1.

15.5 Impacts of Food Wastage

15.5.1 Environmental Impact

The excess amount of food produced which never gets consumed due to any reason generates greenhouse emissions that convey harmful effects to the environment. To analyse the environmental impact of wastage of any food, one can consider three parameters: carbon footprint, ecological footprint and water footprint (Zhong et al. 2019).

a. The carbon footprint represents greenhouse gas emissions (GHGs) generated during production. In the specific case of the agri-food sector, GHGs comprised

primarily of CO_2 generated using fossil fuels, methane (CH_4) derived from livestock enteric fermentation and emissions of nitrous oxide (N_2O) caused by the use of nitrogen-based fertilizers. An accurate calculation of the carbon footprint of wasted food must necessarily consider all the stages of the food supply chain, along with the life cycle assessment (LCA) method.

b. The ecological footprint is a measure used to analyse the effect of consumption of a population on the environment. It quantifies the total area of land and water ecosystems needed to sustainably provide all the resources used and to sustainably absorb all the emissions produced. The ecological footprint is a composite indicator, measuring the various ways environmental resources are handled through a single unit of measure, which includes specific conversion and equivalence factors: the global hectare.
c. The water footprint or virtual water content is a specific indicator of the use of freshwater and is devised to convey both the actual quantities of water resources used and the way the water is used. In the case of food production, the water used in the industrial production stage is considered, as well as the evapotranspiration of irrigated agriculture.

15.5.2 Economic Impact

The economic impact of food waste is estimated by calculating the loss obtained during the agriculture, processing and distribution. To calculate the commercial waste, generally two main ways are taken into consideration, i.e. cost of production and price of existing goods in the market. In the former case, resources necessary to produce food are directly proportional to the value of the goods. Therefore, the economic impact could be estimated as the "value that is lost with waste", while in latter case product's value does not depend on the cost of production but on its consumption, represented by market's price. Therefore, the economic impact of waste could be estimated using "the price of the individual goods" as a calculation criterion (Zhong et al. 2019).

15.5.3 Social Impact

Food wastes and loss do not affect only the present population but the future population also. The food processing industries use a great deal of money and resources including land, labour, energy and other agricultural inputs, so the capital loss will affect the present population and the loss of resources will lead to unavailability of food to the future populations (Roka 2019). Vegetables, fruits, cereals and meat processing industries are the major sectors generating a large volume of food wastes resulting in the economic loss (Buzby et al. 2014).

15.6 Food Waste to Energy Generation

Waste to energy (WtE) generation is the concept of waste valorization and energy recovering process from the waste in the form of electricity and heat. The examples of renewable energy generation are biogas, bioethanol and syngas. These concepts are beneficial in the process of drying and packaging in the industry as they provide an economical source of energy (Bosmans et al. 2013). There are two major interlinked factors on which the evolution of creative technologies for WtE depends: (1) type of waste to be controlled or recovered and (2) accessible legislation. The regulatory authorities responsible for controlling environmental pollution are subsiding the industries to apply the eco-friendly techniques/methods for managing the waste. In addition, the type of technique used for waste management is also influenced by the physiochemical properties of the waste (Van Passel et al. 2013). The conventional energy resources are gradually reaching to the edge of the terminus. In this condition, the renewable energy can be a great alternative to compensate the fuel consumption and using food wastes as raw material makes it the best approach of food waste utilization and energy generation.

The energy from food wastes is shown in Fig. 15.2.

a. Thermal and thermochemical technology
b. Biological technology

15.6.1 Thermal and Thermochemical Methods

Thermal conversion technologies are based on the generation of heat energy through the oxidative combustion of waste biomass. Direct combustion and incineration are the two methods used for the conversion. Incineration is considered as the most appropriate thermal conversion over direct combustion as the latter involves the open burning of biomass that results in the release of harmful compounds like furans.

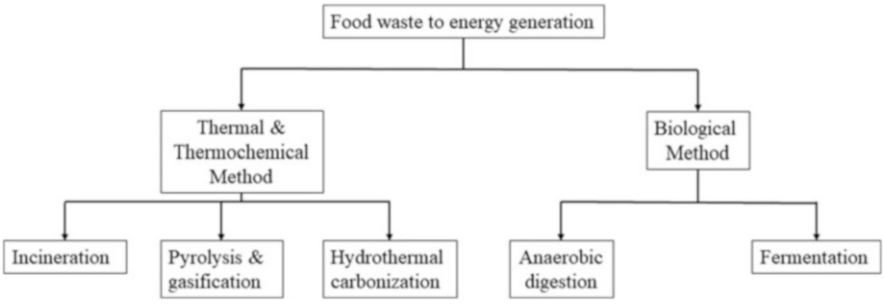

Fig. 15.2 Different methods for energy generation from food wastes

They were thus causing environmental pollution and other health impacts on humans (Scarlat et al. 2015).

Unlike incineration and open combustion, thermochemical conversion technologies employ a series of chemical reactions occurring at different temperatures. They may require partial oxidation as in gasification or proceed in the absence of oxygen as in pyrolysis. These conversion technologies are temperature dependent and progress through overlapping spatial and temporal stages of drying and degassing, pyrolysis and gasification and finally full oxidative combustion that turns the organic waste into ash. All these technologies require strict control of process conditions in specially designed reactors that can separate temperature accordingly. Without temperature separation and proper air rationing, thermochemical reactions do not occur ultimately, turning the process into incineration or combustion (Scarlat et al. 2015).

15.6.1.1 Incineration

The technique through which the food waste is combusted to generate the heat and energy is called incineration. It involves the oxidative combustion of waste material in an incinerator that is simultaneously destructing the pathogenic waste material and generating thermal energy. This heat is used to operate the steam turbines and to heat the heat exchangers (Autret et al. 2007). It also reduces the volume of waste, its toxicity and recovering of the mineral or chemical content of waste. Through incineration, the amount of food waste is reduced up to 80–85%, which makes it suitable for disposal. During incineration, the biomass is converted either directly into CO_2 and water vapour or indirectly into CO, H_2 and Char. Though incineration is a simple and commonly used technique, still some challenges like high capital, maintenance and operating cost of food waste plant are important ones. In some instances, high moisture content of food waste also creates some problems as it makes continuous and optimal plant operation challenging to achieve owing to the requirement of additional fuel to support the process (UNEP 2013). However, the development of advanced processes has improved the overall efficiency and reduced the challenges of the process. Utilization of new metal alloys, high-performance exhaust gas cleaning systems and dioxin destruction are some of the advancements that support the overall improvement of the incineration process (Porteous 2001).

15.6.1.2 Pyrolysis and Gasification

Pyrolysis is a process of thermal degradation of biomass in an anaerobic environment within the temperature range of approximately 400–900 °C (optimum temperature can be 700 °C) producing gas, liquid and solid products. Some variables like rate of heating, wastage type, time of contact, temperature, pressure scale influence the yield and composition of products generated through pyrolysis process (Velghe et al. 2011). Pyrolysis can be categorized into three forms depending on different operating conditions: slow, fast and flash pyrolysis. It is an appealing and attractive way for the production of fuel from solid biomass that has various applications in the production of chemicals, heat and power. Methanol can also be recovered from organic liquid fraction through pyrolysis that can further be distilled for different

industrial applications. Despite the advantages of pyrolysis, it is an expensive technology that requires high investment costs before it can be carried out commercially for energy harnessing (Wu et al. 2017).

In gasification technique, the food waste is partially oxidized at high temperature (800–900 °C) to generate the mixture of combustible gases which can be used as fuel gases (McKendry 2002). These gases have low calorific values and can be used for direct burning or as a fuel for gas turbines and engines. These gases also help in the production of chemicals like methanol as they can be used as a feedstock for methanol production. If the gasification process is compared with pyrolysis and combustion, it has been found that higher recovery of energy and heat capacity has occurred in the gasification process (Sansaniwal et al. 2017). One of the most significant merits of the gasification process is the production of syngas rich in hydrogen, which is the essential requirement for the production of some valuable chemicals and fuels (Young 2010). This process is also considered more appropriate than others due to its advantages like economical acceptability, conjoining of operating conditions particularly in the ratio of temperature and equivalence and the features of specific reactor like plasma reactor, fixed bed reactor, etc. used in the production of syngas (Arena 2012). Though the gasification process has many advantages, still the elements of the solid waste affect its accomplishment and play an important role in the performance of the process. The principal aspects of the waste for gasification are composition of solid waste, inorganic matter (ash content), lower heating value, size of the particles and its density, moisture and volatile matter content. Apart from these elements, other contaminants like heavy metals, alkalies, etc., also affect the performance of gasification process (Zevenhoven Onderwater et al. 2001). Thus, gasification of biowaste is deliberated to be an ideal route for the conversion of diverse biomass feedstocks varying from wastes of agriculture, industrial, kitchen, food and farm.

15.6.1.3 Hydrothermal Carbonization

Hydrothermal carbonization (HTC) is used on the wastes containing a high moisture content (80–90%), which makes the food wastes an energy rich resource by applying autogenous pressure and low temperature (180–350°) (Berge et al. 2011). HTC first came in light in 1913 to produce the coal from cellulose. Still, it has attracted the attention of scientists and technologists rapidly from the last few decades as the advanced version of thermal technology (Libra et al. 2011). HTC process has several merits over other methods of waste to energy conversion like the reduction of a large amount of waste, short footmarks of treatment and no odours after the completion of the process (Li et al. 2013). Other additional benefits associated with the HTC are the shorter period for biological process (only a few hours) and high temperature that helps to retard the growth and activity of pathogens, inactivates organic contaminants and recovery of nutrients from nitrogen-containing species (Libra et al. 2011). The reason behind the consideration of HTC process in managing the food waste is the avoidance of intense energy used in drying as well as carbon segregation to reduce environmental pollution. When food waste is anaerobically digested or fermented, some of the original carbon in the substrate is converted into

CO and lost to the atmosphere. With HTC, however, most of the original carbon present in food waste remains integrated into the final hydrochar product (Titirici et al. 2007).

15.6.2 Biological Methods

15.6.2.1 Anaerobic Digestion

Anaerobic digestion (AD) is the process of biochemical conversion of organic matter with the help of specialized bacteria/yeast resulting in the formation of biogas consisting mainly of carbon dioxide and methane gas and trace amounts of other gases such as nitrogen (N), oxygen (O) and hydrogen sulfide (H_2S). The mixture of methane and carbon dioxide is referred to as biogas. Anaerobic digestion of food wastes in controlled conditions can convert it into biogas and soil fertilizers (Chanakya et al. 2007). AD has been considered as an attractive technology for the decomposition of organic matter from the past few decades in many developed countries like Europe. Researches were emphasized on this technique for food waste treatment due to its environmental benefits like high energy production, recovering of nutrients from waste and a large volume of reducing waste (Widayat et al. 2019). Food wastes consist mostly of organic components making AD a good choice for alternative energy generation. However, in some instances, food waste contains a higher concentration of salt resulting in obstruction of the process due to the presence of sodium, potassium and magnesium salts. To overcome this obstruction, co-digestion of this kind of waste is done to decrease the concentration of salts and smoothening of the process (Chang et al. 2007). AD leads to the overall conversion of food waste into CH and CO and therefore contributes to the reduction of organic matter and recovery of energy from organic carbon in a cost-effective manner.

15.6.2.2 Fermentation

This one is another approach for energy generation from food wastes. It includes the conversion of biomass into bioethanol with the action of fermentable sugars present in food waste and certain microbes like yeast or bacteria. The principal component for the production of bioethanol is the polysaccharide which is present in cellulose and hemicellulose component of food waste. The production of bioethanol can be done by using different substrates like banana peels, wastes of potato peels, wastes of citrus fruits, etc. (Tewari et al. 1986). Due to the complexity of polysaccharides, sometimes pre-treatments are required for the smooth fermentation process. Various acids, alkalis and enzymes can be used for the pre-treatments of polysaccharides to increase the digestibility of cellulose. Enzyme hydrolysis is one of the frequently used pre-treatments in the production of ethanol from food waste. The average energy content of 8.3–11.6 kJ/g TS could be estimated for ethanol produced from food waste based on 26.9 MJ/kg energy content of ethanol (Pham et al. 2015).

15.7 Recent Developments and Strategies for the Utilization and Management of Food Wastes

Solid waste management is one of the critical services every city government must provide with widely variable service levels, costs and environmental impacts. Solid waste generation is also increasing faster than any other environmental pollutant, including CO_2. As the world population becomes more urbanized and affluent, the increase of waste generation is putting enormous pressure on local governments, primarily in the rapidly growing cities of Africa, Latin America, Southeast Asia, China and India.

According to Widayat et al. (2019), a large amount of waste is produced by food processing industry every year throughout the globe which includes residues of different foods like fruits and vegetables industry, meat, poultry and fish industry, dairy industry distilleries and breweries, etc. The waste obtained from the industry contains many components that have good nutritional value, so these wastes can be used to make value-added products which in turn can be used for human consumption, animal feed and as a substrate for various microbial/enzymatic processes.

Figure 15.3 shows the 4-R concept, i.e. reduce, reuse, recycle and recover to manage\utilize the food waste as well as reducing the environmental impact of the waste. Wastage of food can be reduced using novel processing and preservation techniques. Although sometimes the extent of food wastage is influenced by some other factors like poor household practices, consumer behaviour/attitude, culture, etc. so, reuse and recycle of food waste or by-products is another way to control or

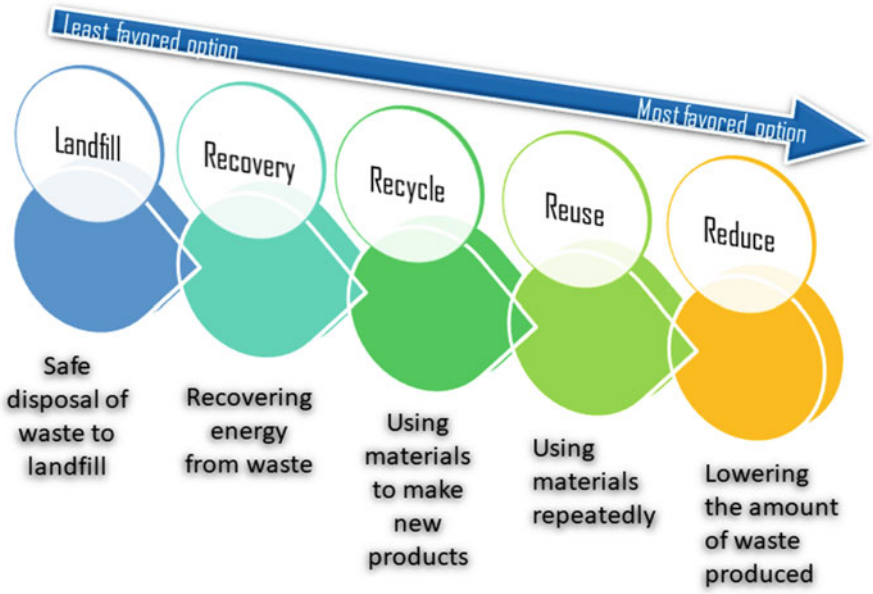

Fig. 15.3 Basic strategies for food waste utilization

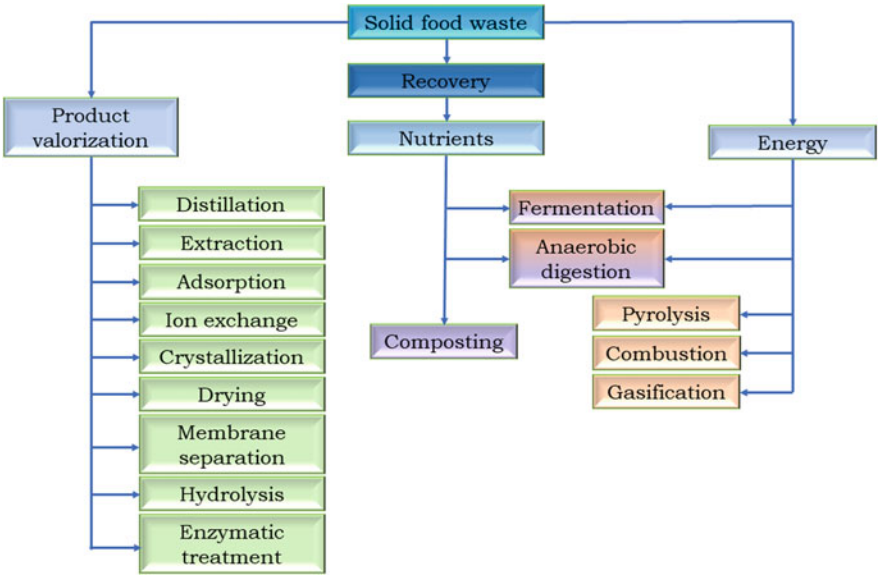

Fig. 15.4 Different techniques for the valorization of food wastes

utilize the food waste (Jayathilakan et al. 2012). Some studies of recent years show that food waste is also a good source for generating energy. So, the recovery of energy from food waste is a good alternative.

It is a big economic burden on agro-food industries to manage the solid waste generated from them before final disposal (Hussein et al. 2018). Proper waste management helps to improve resource efficiency and savings. In Fig. 15.4, there are various technologies shown to recover the solid food waste generated from the food industry. This will focus on valorization technologies and products that can be recovered from food processing by-product and wastes, followed by an outline of the conversion of food waste to renewable energy.

15.7.1 Food Waste Valorization Techniques

The development of sustainability is based on a circular economy model and has an essential role in the global agenda. Durability provides an opportunity for food agro-industry to generate indirect income and reduce food waste by using different technologies of food valorization on the form of bioactive compounds and nutrients. The valorization of food wastes and by-products has become a significant subject of research to improve the sustainability of the food chain. According to agro-food wastes minimization and recycling network (AWARENET), valorization is the increment of the economic as well as the technical value of the waste generated by agro-food sector. It includes different separation techniques such as mechanical, diffusional and chemical separation as well as biological methods and extraction of bioactive compounds (Table 15.2).

Table 15.2 Different techniques of recovery of solid wastes from food processing industries

Separation methods	Techniques	Applications
Mechanical separation	Mechanical extraction	Solid–liquid phase: extraction of juice from fruits
	Mechanical pressing	Separation of fats and oils from oilseeds
Diffusional separation	Screening	a. Remove solid material from food waste b. Separated solids converted into valuable products by drying c. Widely used in winery, distillery and canning plants
	Distillation	a. Separation of solvent mixtures b. Removal of volatile mixtures c. Essential oil production d. Production of alcohol e. Solid by-products starchy in nature
	Floatation	a. Separation of suspended matters from aqueous solution b. Recovery of proteins from protein-rich diet such as soybean c. Production of protein concentrates
	Crystallization	a. Formation of solid particles within a homogenous phase b. Isolation of organic and inorganic constituents from food waste c. Lactose recovery
	Adsorption	a. Recovery of polyphenolics used as functional food ingredients and as natural antioxidants b. Recovery of pectin and phenolic compounds from apple pomace c. Recovery of aromatic components
	Evaporation	a. De-watering of salt streams b. Concentration of highly contaminated wastewaters c. Concentration of saline effluents (e.g. wastewater from fish and meat industry)
	Ion exchange	Production of whey powder through demineralization Lactose production
	Drying	a. Freeze drying: fruits and vegetables, meat, instant coffee products b. Spray drying: milk powders, whey powders c. Flash drying: fibres from tomato pulp
	Membrane separation	a. Clarification of juice b. Purification of water c. Whey demineralization
Chemical separation	Hydrolysis	a. Conversion of starch into sugars b. Conversion of animal fats or vegetable oils to glycerol and fatty acids

(continued)

Table 15.2 (continued)

Separation methods	Techniques	Applications
		c. Conversion of proteins, fats, oils or carbohydrates through enzymes
	Precipitation	a. Separation of soluble phosphorus b. Recovery of protein c. Recovery of sugar from molasses
Biochemical methods	Pasteurization	a. HTST (high temperature short time) b. LTLT (low temperature long time)
	Fermentation	Particular microorganisms involved, e.g. yeast and fungi Ethanol production from glucose
	Enzymatic treatment	Degradation of food waste consisting of proteins, lipids and carbohydrates Extraction of oils or other valuable compounds from seeds, skins and peels
Separation of bioactive compounds from plant material	Conventional methods	Soxhlet extraction Conventional solid/liquid extraction
	Non-conventional methods	a. Ultrasound for the extraction of bioactive compounds from herbs and grape peel b. Enzyme assisted extraction of edible oils c. Extrusions for oilseeds d. Ohmic heating for oil extraction supercritical fluid extraction for the extraction of volatile components from spices

15.7.2 Products Recovered from Food Industry Wastes and By-Products

Annually, a large amount of waste is generated from food processing industries which could be a promising source of functional compounds, nutraceuticals and high nutrition that are beneficial for people. This food waste possesses a strong negative effect on the environment due to its low biological stability. Microbial decomposition of food waste can cause an unfavourable consequence on the environment and humans. Effective and productive use of by-products influences both the economy and environmental pollution of the nation. Proper waste management plays a vital role in the growth of food industries. Therefore, the recovery of by-products into beneficial health product can provide economic benefit to labour, stakeholder and country. The food industries are categorized as follows:

15.7.2.1 Fruits and Vegetables Industry

A large amount of waste is generated from fruits and vegetables during processing (juice production, wine production, etc.) and their preservation. According to Awarenet (2004), in the production of sugar from beetroot, approximately 30% solid waste is obtained, which sometimes reaches around 85%. Waste obtained from

fruit and vegetable industry includes seeds, leaves, stem, roots, tubers, etc. and sometimes whole fruit or vegetable. They are rich in cell wall material. The origin of by-products from fruits and vegetable industry and its characterization is as follows:

1. Fruit and vegetable juices: Many fruits and vegetables are used to produce juices and generate a massive amount of waste ranging from 5% to 75% depending upon a variety of fruit, but the average solid waste is 30–40% (Vilarino et al. 2017). The maximum portion of waste is obtained during the pressing process of juice which is known as "pomace" that consists of peels, stones, seeds. Pomace contains valuable compounds like pectin, dietary fibre, pigments, cellulose, lactic acid and natural sweeteners depending on the type and variety of fruits and vegetables (Shojaosadati and Babaeipour 2002). The uses and applications of the pomace obtained from the fruits and vegetables are:
   a. Extraction and purification of pectin further used in various food products (jam, jelly, marmalade, etc.) as a gelling agent.
   b. Extraction of protein-degrading enzymes (papain in papaya or bromelain in pineapple) which are used as meat tenderizers.
   c. Extraction of flavanones from oranges or citrus fruits pomace used in the pharmaceutical industry.
   d. Extraction of a bioactive terpenoid pigment from tomato pomace.
   e. Extraction of polyphenols from apple pomace.
   f. Extraction of oil from the stones of some fruits (mango, apricot, peach) having culinary or cosmetics applications.
2. Wine production: Wine is a trendy beverage worldwide. Though many fruits are used for the preparation of wine, grapefruit comes on the top of them. Waste generation during wine processing is approximately 20–30% of the processed material in which 2–8% is generated during harvesting and crushing process and 10–20% in pressing and separation process (Sadh et al. 2018). The principle by-products of grape wine industry are stem and pomace that contains a good number of antioxidants, hydrocolloids, citric acid, tartaric acid and dietary fibres. Resveratrol, an antioxidant extracted from the grape stems has a good use as nutraceuticals, cosmetics and biopharmaceuticals. Some other antioxidants like procyanidins also obtained from the grape pomace have meaningful use in the production of grape seed oil.
3. Processing and preservation of fruits and vegetables: Processing and preservation include various processes during which a large amount of waste is produced like trimming, peeling, decoring, coring, etc. The amount of waste depends on the type of fruit and vegetable ranging from 1% of raw material for cranberries to 20–30% for broccoli or carrot. Waste consists of peel, stem, leaves, stalks having good nutritional value. Valuable phenolics and bioactive components can be extracted by peels of different vegetables. Stones and kernels of fruits contain natural oils having good significance in food, cosmetic and pharmaceutical industries. Many natural pigments can be recovered from the peel of fruits and can be used as natural colour in different food items.

4. Sugar and starch production: Sugar is mainly produced from beet (in Europe) or cane (in warmer climates). Beets are first flamed by water and separated from leaves, weeds, beet tails and soil. They are then sliced and pressed, leading to sugar beet pulp. This pulp can be dried and used as animal feed or as a raw material (rich in cellulose) for paper production. The pulp contains valuable components such as protein, prebiotic, cellulose, pectin or hemicellulose. Ferulic acid can also be extracted from the pulp and converted to vanillin through biological methods.

Starch is extracted from corn (60%), wheat (20%) and potato (20%). Starch production generates around 8 million tons of waste every year. They are mainly produced during rasping of the vegetables and extraction of starch, resulting in a mixture of pulp (2%), potato juice (76%) rich in sugar and protein and starch (20%). Proteins can be separated from potato juice by coagulation and used for animal feed. Potato juice is then concentrated and used as a fertilizer. Pulp is used for animal feed, but also for ethanol production. Production of dietary fibre has also been proposed (Sadh et al. 2018).

15.7.2.2 Grain Processing Industry Wastes

Grains and grain products are the essential requirements as food for human beings (Russ and Meyer-Pittroff 2004). The processing industries of grains produce a large amount of wastes in the form of rice bran, wheat bran, rice hull, etc. These by-products are processed to animal feeds as they contain high energy, high protein and high fibre (Ajila et al. 2012).

15.7.2.3 Brewery and Winery Industry Wastes

Wine production is an integral part of agricultural and beverage industry producing a vast amount of waste in the form of wastewater during fermentation and further processing. The discharge of untreated effluent changes the physicochemical properties of the soil. But this wastewater can be utilized by applying the concept of biorefinery for the generation of energy using winery wastes as substrate. This idea is still under process. The winery wastes can also be bioconverted to obtain more value-added products (Zacharof 2017).

15.7.2.4 Marine Food Processing Industry Wastes

In coastline areas, a tremendous amount of waste is produced from fisheries, seafood processing industries and aquaculture. These wastes are highly spoilable as they cause environmental hazards but contain protein, chitin (second-largest found natural polymer on earth) and carotenoids. The fish processing industrial wastes can utilize various enzymes (Klomklao et al. 2005), protein hydrolysates (Šližyte et al. 2005), collagen extract (Fernández-Díaz et al. 2001) and gelatine extract (Choi and Regenstein 2000). Different bioactive compounds obtained from wastes of marine processing industries and their current uses are shown in Table 15.3.

Table 15.3 Bioactive compounds from marine processing industry wastes and their current applications

Waste type	Bioactive compound	Applications	References
Crustacean processing waste	Astaxanthin	Nutrient compound and colour additive	Jiao et al. (2015)
Cartilage of shark and crustacean processing waste	Chondroitin sulphate and glucosamine	Targeted to improve joint health and alleviate symptoms of arthritis, dietary supplements	Jiao et al. (2015)
Crustacean processing waste	Chitin, chitosan and their derivatives	Food and functional food ingredient	Dutta et al. (2004)
Fish processing waste	Collagen	Used in pharmaceutical preparations and cosmetics. Also utilized in edible casings in the meat industry	Jiao et al. (2015)
Shellfish Processing	Protein	Used in pharmaceutical, functional food ingredients	Truong et al. 2019)

15.7.2.5 Meat Processing Industry Wastes

Slaughterhouse is the central area of the meat industry from where a large number of animal by-products such as blood, bones, hoofs, horns, skin, viscera and other things are generated which are unfit for disposal as such and require costly treatment (Ryder et al. 2015).

There are the following applications of wastes generated by meat processing industries (Id and Library 2016):

1. Food applications (antioxidants, opioid activities (Chang et al. 2007), bioactive peptides (Ofori and Hsieh 2014))
2. Technological applications (immunoglobulins, gelatine and emulsifier (Chemistry 2000), colour enhancer)
3. Feed and pet food applications (protein hydrolysates (Gilbert et al. 2008))
4. Energy generation application (biodiesel from animal fat (Bhatti et al. 2008))
5. Medical and pharmaceutical applications (dressing of skin burns) (Jayathilakan et al. 2012)
6. Chemical applications (body lotions, creams, rubber, softener, lubricants, plasticizers)

15.7.2.6 Dairy Processing Industry Wastes

Milk and cheese production industries are one of the major parts of the food processing industry which produces a huge amount of liquid discharge in the form of whey, carrying a high organic load. Due to this load, the BOD and COD of the whey become higher as much as 30,000–50,000 ppm and 60,000–80,000 ppm, respectively, which makes it inappropriate for the disposal as such into the environment (Zavareze et al. 2010). Approximately more than 160 million tons of whey is

produced per annum, with an increasing growth rate of 1–2% per year. The whey contains 55% of milk nutrients and lactose (4.5–5% w/v), soluble proteins (0.6–0.8% w/v), lipids (0.4–0.5% w/v), vitamins (B complex and others), mineral salts (8–10% of dry extract), lactic acid (0.05% w/v) and citric acid, non-protein nitrogen compounds like β-lactoglobulin, α-lactoglobulin, immunoglobulins, serum albumin and lactoferrin. Its proper utilization can be of great benefit (Keri Marshall 2004). Thus, the valorization of whey can give various useful and economically remunerative compounds (Das et al. 2016).

15.7.2.7 Different Roles of Whey Proteins

The derivatives of whey are present in the form of concentrate, isolate and hydrolysate playing the roles of therapeutics (Marshall 2004) and food additives in different foods.

Anti-Inflammatory and Antioxidants

Whey protein acts as the precursor of glutathione antioxidant repressing the effects of free radicals creating oxidative stress. Whey is treated with high pressure to obtain more bioactive peptides, to elevate intracellular glutathione level and to decrease in vitro generation of IL-8. This interleukin is responsible for mediating pathogenicity of respiratory tract diseases (Piccolomini et al. 2012). Native and pressure-treated whey hydrolysate inhibits the generation of reactive oxygen species (ROS) and raises the ferric ion reducing antioxidant power (FRAP) depending on the dose. It has also been seen that the intake of hyperbaric treated whey increases the expression of HSP70 in rats (De Moura et al. 2013).

Immunomodulation

The concentrate of whey protein elevates the innate immunity of mucosa in suckling rats (Pérez-Cano et al. 2007). It also affects the parameters of blood and proliferation and migration of immune cells in mice (Badr et al. 2012a, b). The whey protein concentrate is also used to combat the rigorousness of inflammation caused by psoriasis which is a chronic autoimmune skin disease (Fig. 15.5) (Prussick et al. 2013).

Anticancer

Many researches have been carried out to show the efficiency of whey hydrolysate against cancer (Attaallah et al. 2012). The experiment was done on melanoma B16F10 cells to analyse the upshots of whey protein (Castro et al. 2009). In these cells, the expression of caspase-3 which is responsible for mediating the cell death through apoptosis was increased considerably when cultured in the medium containing whey protein isolate (Takata et al. 2001).

Antidiabetic

Proline, which is a crucial constituent of whey protein, helps in glycaemic therapy and controlling the vascular inflammation in diabetes (Jain 2012). Whey protein stops the diabetic wounds by inhibiting the access of the cytokines responsible for

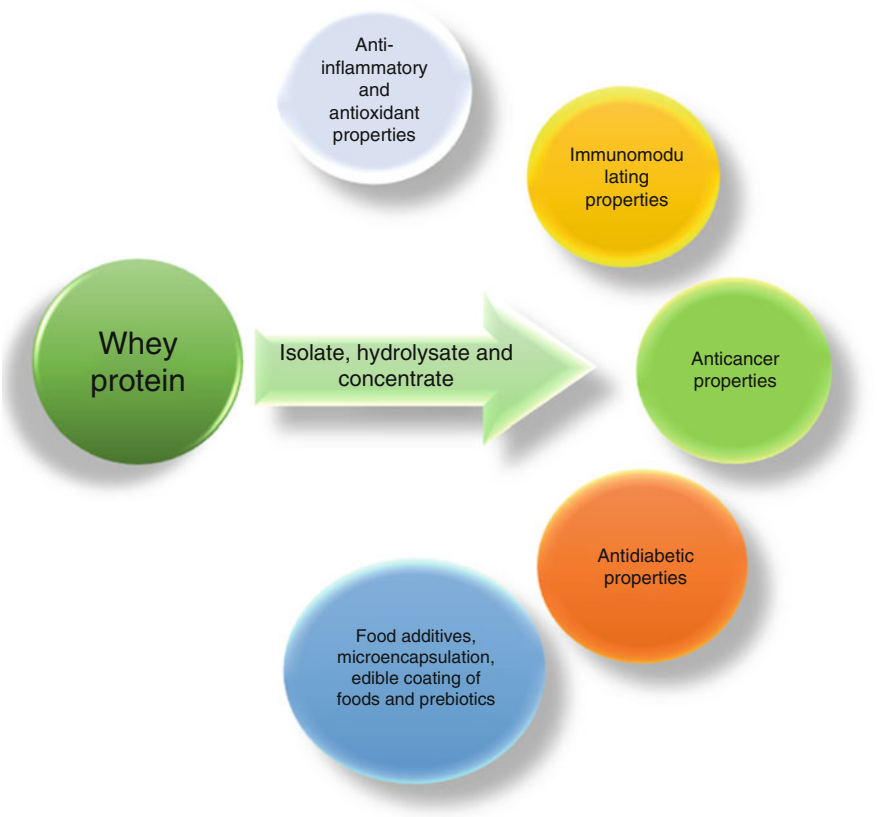

Fig. 15.5 Different roles of whey proteins

inflammation. It was also demonstrated that the hydrolysate and isolate of whey affect the secretion of insulin, as both provoke the higher insulin secretion (Mortensen et al. 2012). Post-meal glycaemia can be lowered by consuming whey protein through both mechanisms, insulin-dependent and insulin-independent (Akhavan et al. 2014).

Apart from these applications, whey protein hydrolysate and isolate are also used as food additives, microencapsulation (Agustín et al. 2016), edible coating of foods (Cagru et al. 2004), prebiotics (Coppa et al. 2006), etc.

15.8 Challenges and Research in Food Waste Management

The present scenario of food waste management is not appropriate due to the lack of advanced methods of waste collection and disposal. Others factors that are responsible for poor waste management are the unavailability of qualified professionals, lack of training in waste management and lack of accountability in current waste

management systems. Limited environmental awareness combined with low motivation has inhibited innovation and the adoption of new technologies that could transform waste management. Public attitudes to waste are also a major barrier to improving the management of food waste. Apart from the above challenges, one more important hurdle is the government finance regulatory framework that is associated with the financial assistance or budgets for covering the development of techniques for collecting, storing, treating and disposing of the waste (Khajuria et al. 2010).

Food insecurity, hunger and food waste may persist, at least in part, because of uneven capitalist accumulation and financial speculation in the global food system as well as economic and social inequality associated with poverty and income inequality. To this end, future research should examine the structure and development of food waste reduction schemes within the cultural and historical context of the urban political economy at particular locals.

Specific recommendations will have to be developed for more effective approaches to international development financing for solid waste management, to open up faster, better funded and more flexible credit lines, which recognize the need to deliver rapid improvements to waste management systems on the ground and on compiling the evidence base for successful financing of waste management and resource recovery infrastructure, identifying good practices and developing good practice recommendations, aimed at both developed and developing countries. Two further areas of focus are research on how to achieve behaviour change and more effective approaches for the producers of products and other stakeholders in the supply chain to take more responsibility for waste management associated with their products and wastes in developing countries.

15.9 Conclusion and Future Prospects

The rate at which the global population is increasing, the need and demand of food will also increase, which will increase the production of food wastes from households and food processing industries. Huge amount of food loss occurs with food waste that does not seem to favour the food sustainability in the future. The use of the above techniques and strategies can reduce the food loss and production of food wastes up to a greater extent which will help the population to use the resources more economically and beneficially. The techniques which are currently in use provide various options for the utilization of food wastes to produce many bioactive compounds and feed. But the best benefit of utilizing food wastes is the generation of energy. In both, developing and developed countries, the demand of fossil fuels will increase tremendously and utilizing the wastes for energy production seems to be a highly promising approach in coming future.

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Xylanase in Waste Management and Its Industrial Applications

16

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Abstract

Xylan is the second most abundant polysaccharide found on earth. It is a heteropolysaccharide. The different monomeric units in xylan are linked via glycosidic and ester bonds. The enzyme xylanase cleaves the β-1, 4-glycoside linkages present in xylan to release different monomers. Xylanase is a highly diverse and abundant enzyme present in different microorganisms—bacteria, fungi, yeast, and algae. It is an industrially important enzyme which finds applications in different industrial sectors like paper and pulp industry, animal food and feed, textile industry, pharmaceutical sectors, rayon production, cellophane production, and chemicals like cellulose esters (acetates, nitrates,

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propionates, and butyrates) and cellulose ethers (carboxymethyl cellulose and methyl and ethyl cellulose). Biofuels have been the choice as an alternative to the non-renewable source of energy. Food crops—corn, wheat, sugar are mostly used as a raw material for the production of biofuels. Bioethanol and biodiesel are the first-generation biofuels generated using sugars in fermentation. Nowadays, non-food crops (food crop waste, organic waste, and no value food) are used as raw materials for biofuel production and generally called as second-generation biofuels. The agriculture and agro-allied sector industries (breweries, pulp and paper, textile, and timber) generate a huge volume of hemicellulosic byproducts which accumulates in the environment and causes environmental pollution and other hazards. These byproducts are considered as waste if not converted to valuable products. Moreover, these byproducts are biodegradable in nature and therefore can be recycled. The valuable products which can be produced from these byproducts are biofuels as a source of bioenergy, animal feeds, and other enzymes like xylanases. The enzyme xylanase has been isolated from a wide variety of microbial systems and agro-waste products, and its growth conditions have been optimized under diverse environmental conditions for maximal stable enzyme production. Moreover, studies at the molecular level, including gene cloning, purification and expression of an enzyme have also been conducted. This chapter gives an overview of Xylanase, its production methods followed by industrial applications, current status and future prospective.

Keywords

Xylan · Xylanase · Bioenergy · Waste management · Endo-β-xylanases · β-xylosidases · Exo-β-xylanases · Xylan hydrolysis

16.1 Introduction

Lignocellulosic plant biomass contains cellulose and hemicelluloses in maximum amount; cellulose being the highest component followed by hemicelluloses (Wang et al. 2019) and lignin (Fig. 16.1) (Malhotra and Chapadgaonkar 2018). Cellulose is an insoluble fibre of β-1, 4-glucan. Hemicellulose is a non-cellulosic polysaccharide of glucans, mannans, and xylans. Xylan is one of the principal constituents of hemicellulose. Chemically, xylan is a polysaccharide, a principal constituent of the plant cell wall, having monomeric units of xylose linked by β-1, 4-glycosidic bonds and side chains having different monosaccharide residues (Kulkarni et al. 1999; Beg et al. 2001; Knapik et al. 2019). Xylan is sandwiched between the lignin and cellulose layers in the plant cell wall. The covalent and non-covalent interactions between lignin and xylan are responsible for maintaining the integrity of cellulose and prevent the degradation of cellulose by enzyme cellulase and help in the fibre cohesion (Beg et al. 2000).

Xylans are categorized into four main categories based on the type of side chain present in them, as described in Table 16.1 (Burlacu et al. 2016).

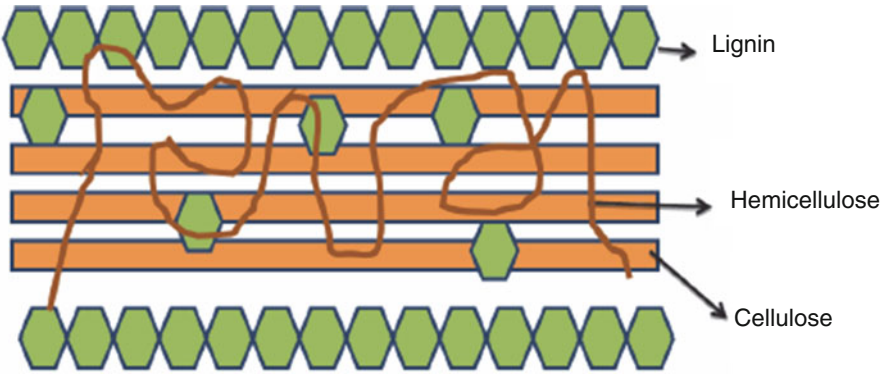

Fig. 16.1 Lignin, cellulose, and hemicellulose fractions in lignocellulosic complex (Malhotra and Chapadgaonkar 2018)

Table 16.1 Classification of xylans

S. No	Xylan type	Side chain
1.	Arabinoxylan	α-L-arabinofuranosyl substituent
2.	Glucuronoxylan	α-D-glucuronic acid
3.	Glucuronoarabinoxylan	α-D-glucuronic and α-L-arabinose
4.	Galactoglucuronoarabinoxylan	α-D-galactopyranosyl residues

16.2 Xylanase

Enzymes are the biological macromolecules produced by many species of bacteria, actinomycetes, fungi, and yeasts. Xylanase is a complex of different enzymes—endo-1, 4-β-D-xylanases (EC 3.2.1.8), β-D-xylosidases (E.C.3.2.1.37), α-glucuronidase (EC 3.2.1.139) acetyl xylan esterase (EC 3.1.1.72), α-L-arabinofuranosidases (E.C.3.2.1.55), *p*-coumaric esterase (3.1.1.B10), and ferulic acid esterase (EC 3.1.1.73). This complex of the enzyme results in hydrolysis of xylan into its different monomeric units (Poutanen et al. 1987; Gomez et al. 2008; Juturu and Wu 2014; Walia et al. 2017; Romero-Fernandez et al. 2018; Bhardwaj et al. 2019). The structure of xylan showing the target sites for different xylanolytic enzymes is shown in Fig. 16.2 (Kalim et al. 2015). Endo-1, 4-β-D-xylanase is the main chain cleaving enzyme and rest others in the complex are responsible for cleavage of the side chain. They were first identified in 1955 and termed as pentosanases. In 1961, the International Union of Biochemistry and Molecular Biology (IUBMB) assigned it the code EC 3.2.1.8. Xylanases are widely distributed in different groups of microorganisms, as depicted in Fig. 16.3 (Selvarajan and Veena 2017).

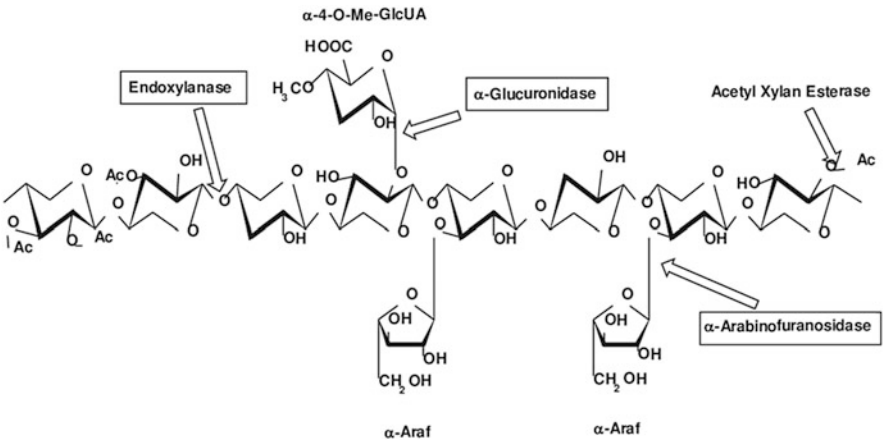

Fig. 16.2 Structure of xylan showing target sites for xylanolytic enzymes (Kalim et al. 2015)

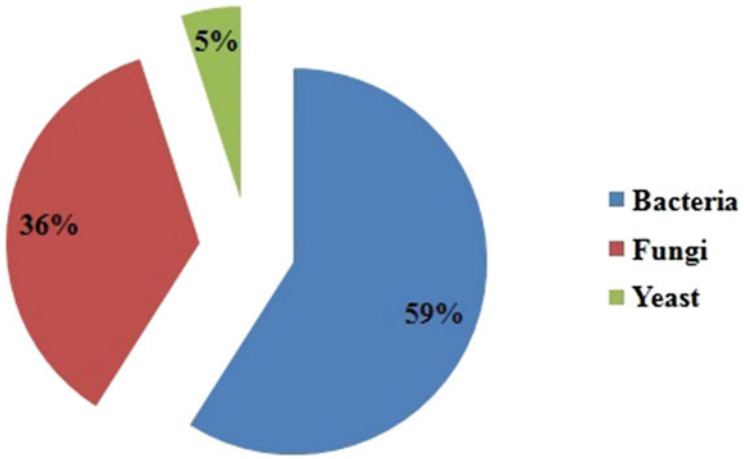

Fig. 16.3 Pie chart showing microbial production of Xylanase (Selvarajan and Veena 2017)

16.2.1 Classification of Xylanase

The following parameters are used to classify Xylanases into different types (Wong et al. 1988; Jeffries 1996; Biely et al. 1997; Juturu and Wu 2012; Liu and Kokare 2017; Bhardwaj et al. 2019; Collins et al. 2002, 2005):

1. Molecular mass and isoelectric point: Xylanases can be either high-molecular weight (>30 kDa) with low isoelectric (acidic) point (HMWLI) or low-molecular weight (<30 kDa) with high isoelectric (basic) point (LMWHI).

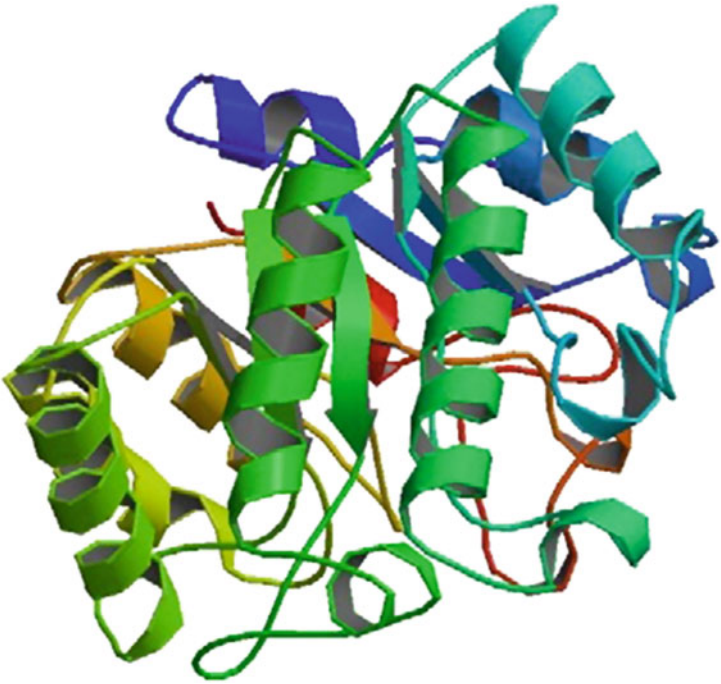

Fig. 16.4 Structure of GH 10 family xylanase showing the (α/β) 8 TIM-barrel fold (Natesh et al. 1999; Juturu and Wu 2012)

2. Crystal structure and kinetic or catalytic property: Xylanases are classified into different glycoside hydrolase (GH) families—GH10, GH11 and other GH families' like—5, 7, 8, 9, 12, 16, 26, 30, 43, 44, 51, and 62.

Xylanases have been included in GH10 and GH11 based on their hydrophobic cluster analysis and amino acid sequences (Verma and Satyanarayana 2012a, b). Endo-1, 4-*β*-D-xylanases are included in family 10 (GH 10). The Xylanase of family 10 has a molecular weight of greater than 30 kDa and a TIM-Barrel structure having 8 alpha-1-beta chains (Fig. 16.4) (Juturu and Wu 2012). GH family 11 Xylanases are monospecific and have high pI, low molecular weight (<30 kDa), and a β-sheets having a jelly-roll structure with two twisted sheets stacked face to face (Fig. 16.5) (Collins et al. 2005; Paes et al. 2012).

16.3 Microbial Diversity for Xylanase Production

Xylanases are highly diverse in nature as they are present in prokaryotic organisms, eukaryotic organisms, marine as well as terrestrial bacteria, rumen bacteria, fungi, and marine algae, to name a few. Most of the xylanases known to date are from bacterial and fungal sources. They have been exploited as microbial factories for the

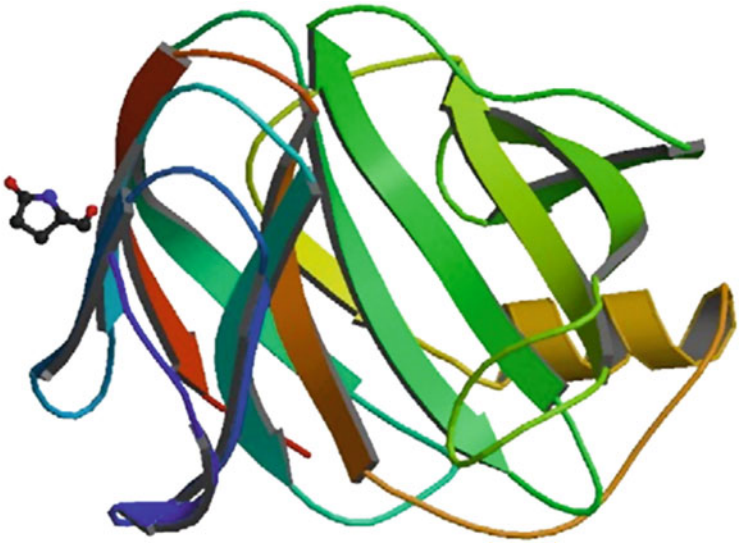

Fig. 16.5 Structure of GH11 family xylanases with two β-sheets forming a β-jelly roll (Juturu and Wu 2012; Gruber et al. 1998)

production of enzymes. Microbial xylanases have preferentially been exploited as catalysts for xylan hydrolysis due to their high specificity and negligible substrate loss and side product generation. Bacterial, fungal, and yeast species have been exploited for the production of Xylanase (Table 16.2).

16.3.1 Thermostable Xylanase

Thermophilic microorganisms (optimum temperature ranges 50–80 °C) and hyperthermophilic microorganisms (optimum temperature more than 80 °C) have been identified and exploited for xylanase production (Kumar et al. 2018) (Table 16.3). Thermostable xylanase has been mostly categorized and placed in GH-10 and GH-11 family. Hot pools, thermal springs, terrestrial and marine self-solfataric fields, and heating decaying organic debris have been the standard source for isolation of thermostable xylanase (Singh et al. 2003; Vieille and Zeikus 2001).

Mutational studies, crystal structure analyses, and sequence alignment studies shown that the main reason for higher thermostability of xylanase is due to modifications in protein structure (Kumar et al. 2018). Thermostable xylanases have increased the number of hydrogen bonds and salt bridges, charged surface residues, improved internal packing and disulphide bridges specifically at the N- or C-termini or in the α-helix regions. The thermostable enzymes offer several advantages such as higher mass transfer rates, and low viscosity that increases the solubility of reactants and products; lower risk of contamination from mesophilic microbes, improved hydrolysis performance due to long half-lives at high

Table 16.2 Microbial diversity for xylanase production

Species of Microorganism	References
Bacteria	
Acidobacterium capsulatum	Inagaki et al. (1998)
Actinomadura geliboluensis	Adiguzel and Tuncer (2017)
Bacillus circulans WL-12	Joshi et al. (2008)
Bacillus stearothermophilus T-6	Khasin et al. (1993)
Bacillus polymyxa CECT 153	Morales et al. (1995)
Bacillus sp. strain K-1	Ratanakhanokchai et al. (1999)
Bacillus sp. NG-27	Gupta et al. (1992)
Bacillus subtilis ADI1	Nawawi et al. (2017)
Bacillus brevis	Goswami et al. (2014)
Cellulomonas fimi	Khanna (1993)
Cellulomonas sp. N.C.I.M. 2353	Chaudhary and Deobagkar (1997)
Cellulomonas flavigena	Lisov et al. (2017)
Clostridium papyrosolvens	Heinze et al. (2017)
Kitasatospora	Rahmani et al. (2019)
Pediococcus acidilactici	Adiguzel et al. (2019)
Pseudomonas aeruginosa	Iloduba et al. (2016); Lee et al. (2018)
Serratia marcescens HK2	Sharma et al. (2020a)
Staphylococcus sp. SG-13	Gupta et al. (2000)
Staphylococcus aureus	Iloduba et al. (2016)
Streptomyces sp B-12-2	Elegir et al. (1995)
Streptomyces thermoviolaceus OPC-520	Tsujibo et al. (1992)
Streptomyces viridisporus T7A	Magnuson and Crawford (1997)
Streptomyces sp. QG-11-3	Beg et al. (2000)
Thermoanaerobacterium sp. JW/SL-YS485	Shao et al. (1995)
Thermoactinomyces vulgaris	Selim (2016)
Thermotoga maritima MSB8	Winterhalter and Liebel (1995)
Thermomonospora curvata	Stutzenberger and Bodine (2008)
Marinifilaceae SPP2	Han et al. (2019)
Micrococcus sp.	Mmango-Kaseke et al. (2016)
Fungi	
Aspergillus niger ANL-301	Okafor et al. (2010)
Aspergillus kawachii IFO 4308	Ito et al. (1992)
Aspergillus sojae	Kimura et al. (1995)
Aspergillus sydowii MG 49	Ghosh and Nanda (1994)
Aspergillus Flavus MTCC 9390	Bhushan et al. (2012)
Cephalosporium sp.	Bansod et al. (1993)
Fusarium oxysporum	Christakopolous et al. (1996)
Fusarium roseum	Gascoigne and Gascoigne (2019)
Geotrichum candidum	Radionova et al. (2000)
Humicola insolens	Shi et al. (2015)
Penicillium purpurogenum	Belancic et al. (1995)
Rhizomucor pusillus	Huttner et al. (2018)

(continued)

Table 16.2 (continued)

Species of Microorganism	References
Thermoascus aurantiacus var. levisporus	Chanwicha et al. (2015)
Thermomyces lanuginosus DSM 5826	Cesar and Mrsa (1996)
Thielavia terrestaris	Thanh et al. (2019)
Trichoderma harzianum	Ahmed et al. (2011)
Trichoderma reesei	Tenkanen et al. (1992)
Tricoderma reesei NRRL 6156	Cekmecelioglu and Demirci (2020)
Tricoderma piluliferum, *T. viride*	da Costa et al. (2019)
Tricoderma asperellum	Ezeilo et al. (2019)
Isoptericola variabilis strain UD-6	Patel and Dudhagara (2020)
Yeast	
Aureobasidium pullulans Y-2311-1	Li et al. (1993)
Cryptococcus albidus	Morosoli et al. (1986)
Trichosporon cutaneum SL409	Liu et al. (1998)

Table 16.3 Microorganisms having thermostable xylanase enzyme activity

Microorganism species producing thermostable xylanase	Reference
Clostridium Thermocellum	Lo Leggio et al. (1999)
Caldicellulosiruptor sp.	Zverlov et al. (1996)
Bacillus vallismortis RSPP-15	Gaur et al. (2015)
Bacillus stearothermophilus	Abou-Hachem et al. (2002)
Rhodothermus marinus	Luthi et al. (1990)
Thermoascus aurantiacus	Khasin et al. (1993)
Thermotoga sp.	Winterhalter et al. (1995)
Thermotoga sp. strain FjSS3-B.1	Simpson et al. (1991)
Caldicoprobacter algeriensis TH7C1	Amel et al. (2016)
Chaetomium thermophilum	Hakulinin et al. (2003)
Nonomuraea flexuosa	Fan et al. (2014)
Malbranchea cinnamomea S168	Kumar et al. (2000)
Paecilomyces variotii	Shrivastava et al. (2016)
Thermomyces lanuginosus *Dictyoglomus thermophilum* *Nonomuraea flexuosa*	Andrade et al. (1999)
Thermoanaerobacterium sp.	Zarafeta et al. (2020)
Geobacillus stearothermophilus	Shulami et al. (2014)
Geobacillus thermodenitrificans	Daas et al. (2017)
Geobacillus sp. strain WSUCF1	Bhalla et al. (2015)
Geobacillus thermoleovorans	Verma and Satyanarayana (2012a, b)
Archaeal microorganisms	
Pyrodictium abyssi *Thermofilum* strains	Andrade et al. (1999)
Pyrococcus furiosus Thermococcus *zilligii*	Cady et al. (2001)
Sulfolobus solfataricus	Cannio et al. (2004)

temperatures, and structural and functional stability at higher temperatures (Bhalla et al. 2013; Shi et al. 2013). Moreover, thermostable Xylanase can tolerate high temperature, which is required during various industrial processes for their isolation (Bhalla et al. 2013; Wang et al. 2019).

16.3.2 Xylanase Production

Strain improvement for overproduction and overexpression of Xylanase has been possible and exploited due to advancements in recombinant DNA technology and developments of new techniques—gene editing, gene modification protein engineering, and metabolomics (Tseng et al. 2002; Thomas et al. 2013). Figure 16.6 (adopted from Tseng et al. 2002) shows a flowchart showing the molecular mechanism of Xylanase production and its industrial applications. The optimization of growth conditions—carbon and nitrogen source, temperature, pH, and other physical and chemical parameters enhances the production of Xylanase. The concentration of xylanase enzyme is achieved by ammonium sulphate or acetone precipitation method and dialysis tubing against the polyethylene glycol or by cationic exchange chromatography (Khasin et al. 1993).

Solid-state fermentation (SSF) or submerged state fermentation (SMF) is the most common method for xylanase production. The choice of the method employed depends upon the choice of organism and substrate. For example, SSF is used for thermophilic xylanase preparation. Moreover, SSF have certain advantages over SMF like the requirement of a lesser amount of liquid for product recovery,

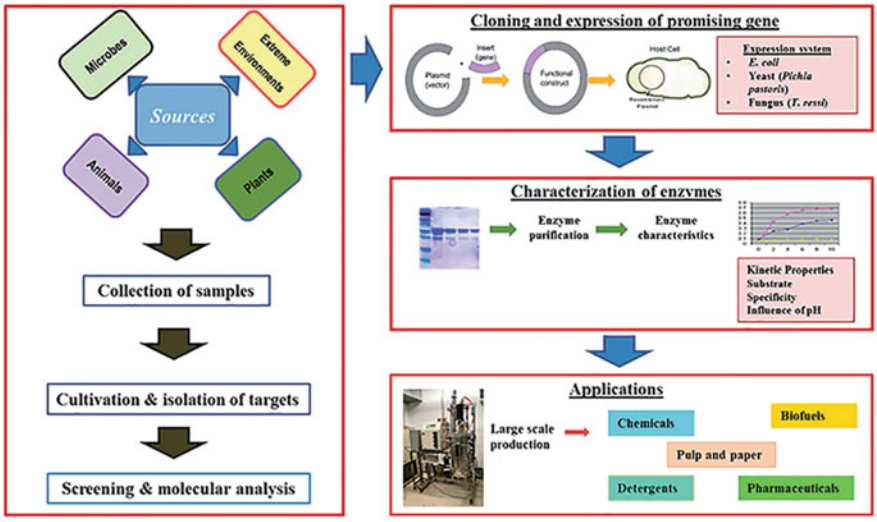

Fig. 16.6 A flowchart showing the molecular mechanism of Xylanase production and its industrial applications (Basit et al. 2018)

inexpensive substrate, cultivation cost is low, enzyme yield is high, and risk of contamination is low. The most common substrates exploited for xylanase production under SSF are wheat bran (Gautam et al. 2015), Birchwood xylan, Nicotiana tobacco leaf dust (Acharya and Shilpkar 2016), Corncob, Carboxy methyl cellulose (Pandey et al. 2014), and Eucalyptus kraft pulp (Stutzenberger and Bodine 2008). The oat spelt xylan has been used as a carbon source for the production of extracellular Xylanase in *Penicillium sclerotiorum* (Knob and Carmona 2008). The filamentous fungi—*A. niger* and *T. reesei* have also been exploited for the production of xylanases at industrial level (Deshpande et al. 2008).

Agri waste has been an essential source of xylanase enzyme. Various agri-waste products have been screened for potential xylanase enzyme activity. Thite et al. (2020) had shown that wheat bran peels induce xylanase enzyme activity in *Bacillus safensis* M35 and *Bacillus altitudinis* R31 (Thite et al. 2020; Thite and Nerurkar 2015).

E. coli has been exploited as the expression host system for heterologous expression of xylanase (Jun et al. 2009; Zafar et al. 2016; Elgharbi et al. 2015; Panbangred et al. 1985; Goswami et al. 2014). But due to some shortcomings like lower specific activity and stability (Chang et al. 2017), alternative expression host systems have been developed like Gram-positive bacteria—*Lactobacillus* species and *B. subtilis* (Liu et al. 2005; Verma and Satyanarayana 2013).

Apart from bacterial systems, yeasts have also been a right choice for expression of Xylanase due to their ability of very high cell growth densities and protein secretion in the fermentation media. Glycosylation and polyhistidine tags in the yeast system increase the thermostability of Xylanase.

16.3.3 Xylanase Assay

There are various methods to assay xylanase activity—colourimetric, turbidometric, and viscometric methods. The colourimetric method is the most common method to assay xylanase activity. This method measures the increase in the concentration of reducing sugars due to enzymatic hydrolysis of xylan dinitro salicylic acid, popularly known as DNS method (Miller, 1959), Nelson Somogyi's method (Nelson 1944; Somogyi 1952), or Bernfeld's method (Bernfeld 1955). The absorbance of the product formed (amount of reducing sugars) is measured at 540 nm. The substrates which are used commonly for xylanase assay are beechwood xylan, birchwood xylan, carboxymethyl xylan, Larchwood xylan, oatspelt xylan, aspen wood xylan, rice straw arabinoxylan, and wheat straw glucuroxylan. The other substrates which are used in xylanase assay are 4-O-methylglucuronoxylan covalently conjugated with dye-Remazol Brilliant Blue (RBB xylan) (Juturu and Wu 2012) or the Congo-Red (Basit et al. 2018).

The activity of the Xylanase can also be estimated by the Zymogram analysis. In this method, PAGE is run with a gel containing 0.1% xylan. The Congo-Red dye is used to stain the gel to observe the bands after gel electrophoresis (Basit et al. 2018). Carboxymethyl cellulase assay has also been used for assaying the activity of Xylanase (Tseng et al. 2002).

Nowadays, kit-based methods are more familiar to assay xylanase activity. A fluorescence-based method like EnzChek® Ultra Xylanase Assay Kit (Invitrogen, Carlsbad, CA) or the Xylazyme tablet (Megazyme, Bray, Ireland) are used in routine practice to assay xylanase activity. In this method, azurine-crosslinked arabinoxylan (AZCL Arabinoxylan) acts as substrate which is hydrolyzed by Xylanase to form water-soluble fragments of the dye.

16.4 Industrial Applications of Xylanases

Due to their industrial application microbial, Xylanases have drawn considerable attention. Some important commercial xylanases and their suppliers are given in Table 16.4. Potential applications of xylanases include bioconversion of lignocellulosic material to fermentative products. Some of the major industrial applications of Xylanases are given below.

16.4.1 Bioenergy Production: Biofuel Industry

Depletion of non-renewable sources of energy has occurred at an alarming pace indicating a threat to the energy crisis. The renewable source of energy as an alternate has been in demand, which includes biofuel production. For this, agro wastes are the perfect choice of being cheap and readily available (Sharma et al. 2020b; Garg 2016). The biological conversion of lignocelluloses for production of ethanol has recently attracted the attention of researchers and is a promising area of research (Huang et al. 2011). Lignin, cellulose, protein, and pectin in different proportions make up lignocelluloses (Behera et al. 2014). Hydrolysis of lignocellulose biomass is performed either by acid treatment at high temperature or by enzymatic action. Due to high energy consumption and harsh chemical requirement in acid hydrolysis, enzymatic hydrolysis being eco-friendly is a more favoured method.

Lignocellulosic mass is converted to simple sugars, which is further converted to ethanol. Delignification of lignocellulosic biomass liberates hemicellulose and cellulose from their complex with lignin. This is followed by saccharification of the carbohydrate polymers to produce simple mono sugars. These simple sugars (hexose and pentose sugars) undergo fermentation to produce ethanol (Tiwari et al. 2016). Significant work is done by Saini et al. (2020), using paper waste resulted in the production of 40.85% bioethanol. Nishimura et al. (2017) used mixture of waste paper, food waste, and kitchen waste which resulted in 46.6 g/L ethanol after 96 h of fermentation. Multiple enzymes—endoglucanases, (EC 3.2.1.4), β-glucosidases (EC 3.2.1.21), endo-1, 4-β-xylanases (EC 3.2.1.8), and β-xylosidases (EC 3.2.1.37) are required for complete hydrolysis of lignocelluloses—a complex biomolecule (Bajpai 2012). The hydrolytic action of cellulolytic enzymes on cellulose is shielded by xylan. The swelling of cellulosic fibre and porosity increases in the presence of Xylanase, which accelerates its hydrolysis (Paridah et al. 2011).

Table 16.4 Commercial Xylanases and their suppliers (Polizeli et al. 2005; Nagar et al. 2013; Mathur and Pathak 2017; Goswami and Pathak 2013)

S. no.	Name of enzyme	Commercial supplier
1.	Allzym	PT Alltech
2.	Amano 90	Amano pharmaceutical
3.	Bio feed plus	Novo Nordisk
4.	Bleachzyme	Biocon India
5.	EcopulpX200	Primalco
6.	Ecosane	Biotec.
7.	Grindazym GP eGV. Irgazyme 40 Nalco	Danisco ingredients
8.	Multifect XL	Genencor
9.	Pulpzyme	Novozymes, Denmark
10.	Solvay pentonase T	Solvay enzymes
11.	Sternzym HC46	Stern Enzym
12.	Sumizyme X	Shin Nihon
13.	Xylanase	Seikagaku
14.	Xylanase	Granotec do Brazil
15.	Xylanase GS35	Iogen
16.	Nutrizyme XY45	Sunson, China
17.	Veron @ 292, Veron @ 393, Veron @ special	AB enzyme
18.	Pentopan Mono BG	Novozyme
19.	XBK-B300	Leveking, China
20.	Ecopulp	Alko Rajamaki, Finland
21.	Cartazyme	Sandoz,Charlotte, N.C. and Basel,
22.	Cartazyme HS 10, Cartazyme SR 10 Cartazyme PS10, Cartazyme 9407, Cartazyme NS10	Clariant, UK
23.	Irgazyme 40-4X/Albazyme 40-4X, Irgazyme-10A, Albazyme-10A	Genercor, Finland; Ciba Giegy, Switzerland
24.	VAI Xylanase	Voest alpine, Austria
25.	Pulpzyme HA, HB and HC	Novo Nordisk, Denmark
26.	Ecopulp X-100,200, 200/4,TX-100,TX200 and Ecopulp XM	Rohm enzyme 0Y; Primalco, Finland
27.	Ecozyme	Thomas swan, UK
28.	GS-35, HS70	Iogen, Canada
29.	Sanzyme X,PX and Alpelase F	Sankyo, Japan
30.	Enzeko xylanase	Enzyme development, USA

Xylanase is used for degumming of bast fibres such as flax, hemp, jute, and ramie in the presence of pectinases (Paridah et al. 2011). Thermostable Xylanase plays an essential role in assisting the saccharification process during bioethanol production as it is performed at high temperature and pressure in the biofuel industry.

Fig. 16.7 General flow-diagram of biobleaching process (Kumar et al. 2016)

16.4.2 Pulp and Paper Industry

Bleaching is the process of removal of colour to increase the whiteness of the product. Lignin has to be removed from the pulps to get brightness in the paper. Both chemical and biological processes are used to achieve this. Chemical bleaching is done in the presence of some chlorine-containing chemicals like ClO_2, Cl_2, and hypochlorite, which results in the removal of lignin from the pulp. It requires cooking the pulp at 170 °C. The use of chlorine and other chlorine derivatives for bleaching process releases toxins and mutagens as byproducts which causes environmental pollution (Bajpai 2012). An alternative to chemical bleaching is biobleaching. In bioleaching, microorganisms or enzymes are used in place of harsh chemicals for bleaching the pulp in the paper industry (Fig. 16.7). Hence it is considered as an eco-friendly method (Kumar et al. 2016).

Xylanase is one of the enzymes which have attracted the attention of the scientific community for bioleaching of pulp in the paper industry (Sharma et al. 2015; Bajpai 2004). Role of xylanase enzyme in pulp and paper industry for the process of bleaching was first reported by Viikari in 1986 (Viikari et al. 1986). Xylanases improve pulp fibrillation and water retention time, reduction of beating duration, restoration of bonding, and increases freeness in recycled fibres, etc. It is used to increase the brightness of the pulp as xylanase enzyme reduces lignin content (Sunna and Antranikian 1997).

16.4.3 Food Industry

Xyalanse finds applications in various domains of food industries—bread baking, juice, beer, papad industries (Polizeli et al. 2005; Butt et al. 2008; Harris and Ramalingam 2010; Goswami and Rawat 2015). Due to maximum activity at acidic pH and high stability, enzyme xylanase finds wide application in food industries. Water-insoluble hemicellulose is converted into its soluble forms with the help of Xylanase. The use of chemical additives—bromates has been decreased due to the same functional role of xylanases in the baking industry (Kulkarni et al. 1999). Xylanase, along with amylase, helps in volume retention and resistance potential of bread against fermentation, improving the bread production (Butt et al. 2008). Elasticity improves handling and stability of the dough. The hydrolysis of hemicelluloses of wheat flour in the presence of Xylanase results in redistribution of water content, causing softness of dough (Harris and Ramalingam 2010). The volume of the bread loaf increases nearly by 10% on addition of xylanases to wheat flour (Garg et al. 2010).

Juice yield from fruits and vegetables have also been shown to be high in the presence of Endo-1, 4-β-xylanase. The polysaccharides—starch, pectin, cellulose, and hemicelluloses present in fruit extracts cause turbidity and viscosity of fruit extracts (Lee et al. 2006). Xylanases in combination with amylases, pectinases, and cellulases result in liquefaction of vegetables and fruits; stabilization of the fruit pulp; increase recovery of aromas, essential vitamins, oils, mineral salts, pigments, edible dyes, etc., and help in reducing the viscosity of the juices (Bailey 1985; Kalim et al. 2015).

Endo-1, 4-β-xylanase also finds application in beer production. It helps in extracting more fermentable sugars from barley. It improves filterability by reducing the viscosity of the brewing liquid (Garg et al. 2010).

16.4.4 Animal Feedstocks

Growth of animals has been stimulated by supplementing the animal feed with endo-1, 4-β-xylanase (Lee et al. 2006; Damiano et al. 2003). Polysaccharides—arabinoxylans, β-glucans, celluloses, mannans, and galactans constitute a major portion in endosperm cell walls of different cereal grains (Longland et al. 1995). Different cereal grains are enriched with different forms of polysaccharides (Bonnin et al. 1998; Beer et al. 1997; Cui et al. 2000). The domestic animals cannot digest the polysaccharides due to their viscousness property. So, Xylanase is included in cereal diets which increase the availability of polysaccharides to the feed animals (Amnison 1992; Bedford and Classen 1993; Salih et al. 1991). The internal β-1, 4-linkages of 1, 4-β-D xylan and 1, 4-β-D-glucosidic bonds in β-glucans are the target sites for endo-1, 4-β-xylanase and 1, 3–1, 4-β-glucanase (Collins et al. 2005; Planas 2000). It helps in increasing the absorption of nutrient and diffusion of pancreatic enzymes by thinning of gut contents. The chickens obtain sufficient

energy from hemicellulose of cell wall of feed by converting it into sugars in the presence of Endo-1, 4-β-xylanase (Garg et al. 2010).

16.5 Future Scope

The production of biofuel/bioenergy is the need of the hour as it is a renewable source of energy. The enzyme xylanase has been a raw material for the production of bioenergy. So, in future, the concerted efforts of the biotechnologists, microbiologists, synthetic biologists, and process scientists along with the advances in technologies like genome and metagenome analysis will provide opportunities in identifying novel microorganism/s having xylanase activity to be exploited for industrial applications. The enzyme xylanase can be modulated to develop a technology for the production of 'green fuel' based on further research. With the use of recombinant DNA technology, the expression of Xylanase can be regulated for any desired property as per the need and requirement of the industry. Also, different techniques for easy and quick production of an enzyme to fulfil the demand of the industries can be a promising opportunity in this research field.

16.6 Conclusions

Industrially important enzymes have been under the scanner for mass production of the protein. Microbial xylanases specifically thermophilic xylanases have been exploited for different applications in various industrial sectors and expressed in both prokaryotic and eukaryotic expression system using recombinant DNA technology. With rapid advancements in biology—genome editing, synthetic biology, xylanase enzyme has been further modified for increasing its the production at a mass level.

Conflict of Interest The authors have no conflict of interest.

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Organic Acid Production from Agricultural Waste

17

Neha Sharma, Param Pal Sahota, and Mohini Prabha Singh

Abstract

The escalating population has prompted the focus to meet the demand of food materials by increasing crop yield. Out of the total farm output, India is the second largest producer of agricultural crops. Consequently, the lack of congruence between crop production and the disposal of agricultural wastes is a matter of serious environmental concern. An opportunity, therefore, prevails to switch the view of these agricultural residues from microbial bioprocessing of waste to valuable products which can be a huge market.

The global organic acid market stood at $6.94 billion in 2016 and is projected to reach $12.54 billion by the end of 2026. They constitute a significant portion of the fermentation market in the world, and microbiological production is an economic alternative using pre-treated agricultural wastes. The production of organic acid covers two aspects: first, the metabolic pathways involved in the biosynthesis, and second, the industrial process strategy adopted. Two groups of acids are defined, those with a "long" biosynthetic path from glucose, involving the glycolytic pathway or tricarboxylic acid (TCA) cycle, viz. citric acid (CA) and succinic acid (SA), and those acids with a "short pathway", essentially biotransformation of glucose such as gluconic acid (GA) and kojic acid (KA). Consequently, the huge problem of agricultural waste disposal can be used as a bioresource for the production of high valued products, i.e. organic acids along with the exploitation of natural producers.

Keywords

Agriculture waste · Citric acid · Succinic acid · Gluconic acid · Kojic acid

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17.1 Introduction

17.1.1 Agricultural Waste: A Historical Juncture

The rapid development of agriculture resulted in generating an increasing quantity of agricultural wastes across the globe. Agricultural wastes are defined as the residues resulted from the agricultural product comprised mainly of straw, residual stalks, husk, bagasse, shells, etc. Recent Food and Agriculture Organization (FAO) reports estimated that every year as much as 30% of the agricultural produce is wasted in harvest, storage, and processing stages with an average of 23.7 million tons per day worldwide (Food and Agriculture Organization of the United Nations (FAO) 2017). Moreover, in India, it has been estimated that among the total agricultural field residues generated, 75% of it is utilized as fodder and other agriculture and household purposes such as mulching, composting, fuel, thatch, etc., while finding the proper disposal technology for the remaining 25% of the waste causing an issue of concern (Cardoen et al. 2015). Consequently, the lack of congruence between the crop production and the disposal of agricultural wastes is a matter of serious environmental concern. Henceforth, need of the hour is the utilization of the available feedstocks in the sustainable innovative technologies for bio-based industries for the benefit of humankind while curtailing their unwanted consequences without the formation of toxic and unsafe by-products (European Commission 2017; Swain 2018). Agricultural residues, a promising sustainable carbon source, could serve as an excellent raw material for bioprocessing in value-added products. The nutrient rich biomass supports the growth of microorganisms by providing suitable conditions and moisture which open up their possibilities for industrial processes.

In this chapter, we will explore the advances in the organic acids production. Furthermore, we will focus on the metabolism routing of organic acids to get the desired metabolite. However, the chapter will be restricted its study to four organic acids, viz., citric, succinic, gluconic, and kojic acid. Emphasis on commercially successful microbial production processes has been laid down with the special focus on the filamentous fungi. Several researches have been published covering the various aspects of organic acid production with recent developments (Ciriminna et al. 2017; Liu et al. 2017; Morgunov et al. 2018; Pal et al. 2016; Rokem 2020; Saxena et al. 2017; Saeedi et al. 2019).

17.1.2 The Panoply of Building Blocks: Organic Acids

Organic acids are defined as the polyfunctional acids that contain one or more carboxyl groups, hydroxyl, thiol groups, etc., which encompasses the central metabolic pathway. Although these carboxylates had less visible impact on the humans in the past, however, at the same time organic acids are among the most promising future products of industrial microbiology, owing to their wide scale of applications in the cosmetics, detergent, food, pharmaceutical, polymer and textile industries (Liu

et al. 2017; Yang et al. 2017). These functional groups have made organic acids as key building block chemicals, making them the versatile due to their three main properties, i.e. are water soluble, hygroscopic in nature, have buffering and chelation properties. Most of the organic acids are produced as metabolites or intermediate products in the major metabolic pathways of microorganisms. Depending on their metabolic origin, organic acids can be readily placed into two main groups:

1. Acids with a "long" biosynthetic pathway from glucose, involving the glycolytic pathway and TCA cycle, viz. citric acid (CA) and succinic acid (SA).
2. Acids with a "short pathway", essentially a biotransformation of glucose such as gluconic acid (GA) and kojic acid (KA).

Recently, a surge in the demand for organic acids is due to their wide applications in the food industry as additives and as chemical feedstocks. The global organic acid market stood at $19.91 billion in 2018 and is projected to reach $36.86 billion by the end of 2026 (Globe Newswire, 2019, Internet). The market of organic acid is witnessing a comparative leniency in the regulatory and approval norms pushing the market growth forward. Perhaps a greater understanding to produce organic acids along with the exploitation of agricultural wastes will break the cycle of resource utilization for the manufacturing industries in an economic way.

17.2 Microbial Organic Acid Production

Organic acids are a promising approach for obtaining building block chemicals from renewable carbon sources. Moreover, an incentive to the environment is by replacing the chemical production means with the microbial based organic acid production. These developments have the potential to accelerate the novel, efficient, economically feasible, and environment responsible fermentations. Therefore, it is evident that the quickest way to establish any industrial process is the exploitation of natural producers. These acids get accumulated as a metabolite (either due to catabolism or anabolism), so with the knowledge of the production processes involved in recent years, large-scale production is still an imperative task.

17.2.1 Citric Acid: From Lemons to Filamentous Fungi

17.2.1.1 Brief History

Citric acid (CA) is one of the high volume-low value organic acid having the annual production of approximately 2 million tons. The acid is naturally present in the citrus fruits (lemons, lime, oranges, grapefruit, tangerine) and animals (blood, bone, thyroid, and mammary glands). This tricarboxylic acid plays a central role and is involved in the metabolism of all aerobic organisms. It was first isolated by C.W. Scheele in 1784 from lemon juice and named accordingly as "citric acid". The journey of the industrial production of oldest microbial process was initially

begun in the mid-1800s and relied on the extraction from Italian lemons until it was discovered by Wehmer in 1893, that a filamentous fungi *Penicillium* species can accumulate this acid in high amounts under certain conditions (Thauer 1988; Apelblat 2011). However, this fungus did not succeed for the industrial production as it gets easily contaminated. The issue has been resolved by the Currie in 1916 with the introduction of *Aspergillus niger*, which has been found to have the ability to accumulate high concentrations of CA using high sugars at the pH of 2.5–3.5 while also suppressing the accumulation of by-products, i.e. oxalic and gluconic acids. The first CA fermentation was carried out in surface cultures in Belgium in 1919 and then by the Pfizer company in the USA in 1923. With the introduction of submerged culture in the 1940s, the microbial fermentation was carried out in the liquid depth cultures. Owing to its remarkable physicochemical properties and environmentally benign nature, the consumption of CA across several industrial sectors increased rapidly throughout the nineteenth century. Today, China is the key supplier of CA with a global market share of 59% and holds 74% of the world exports. Nonetheless, most of the CA production traditional plants have been closed down in France, Ireland, UK, USA, and even in India. The reasons might be due to the lower current price as compared to earlier mainly due to the large Chinese production capacity (Cavallo et al. 2017; Ciriminna et al. 2017).

17.2.1.2 Attributes with Applicability

CA ($C_6H_8O_7$), also called as 2-hydroxy-1,2,3-propanetricarboxylic acid, is water soluble with three pK_a values of 3.15, 4.77, and 6.39 (Table 17.1). When dissolved in water, it shows weak acidity but imparts strongly acid taste which provides a tangy flavour for which it is widely used as flavouring agent in the food and beverage

Table 17.1 Characteristics outline of organic acids

Organic acid	Chemical formula	IUPAC name	Molecular weight	Solubility in water (g L^{-1})	Production methods (chemical/ biological processes)
Citric acid	$C_6H_8O_7$	2-Hydroxypropane-1,2,3-tricarboxylic acid	192.12	592	–/+
Succinic acid	$C_4H_6O_4$	Butanedioic acid	118.09	83.2	+/+
Gluconic acid	$C_6H_{12}O_7$	2,3,4,5,6-Pentahydroxyhexanoic acid	196.16	316	+/+
Kojic acid	$C_6H_6O_4$	5-Hydroxy-2-hydroxymethyl-4-pyrone	142.11	_	+/+

https://pubchem.ncbi.nlm.nih.gov/compound/citric_acid
https://pubchem.ncbi.nlm.nih.gov/compound/succinic_acid
https://pubchem.ncbi.nlm.nih.gov/compound/gluconic_acid
https://pubchem.ncbi.nlm.nih.gov/compound/kojic_acid

Table 17.2 Bioproduct quantity, production method involved along with its applications

Organic acids	Annual production (tons)	Production methods	Applicability
Citric	2,000,000[a]	Fermentative bioprocess involving *Aspergillus niger*	Acidulant, adhesive, anticoagulant, antioxidant, flavouring agent, preservative, sequestrant, stabilizer
Succinic	40,000[b]	Both by chemical synthesis and bioprocess methods by bacterial, yeast and fungal fermentations	Absorbent, acidulant, adhesive, antiscaling agent, bio-solvents, buffering agent, Deicer solution, flavouring agent, neutralizer, sequestrant
Gluconic	100,000[c]	Both by chemical method and by using *A. niger*	Antifreeze, antiseptics, buffering agent, chelator, cleaning agent, sequestrant
Kojic	NA[d]	Both by chemical method and fermentative method using *A. oryzae*	Antioxidant, antifungal, antimicrobial, bleaching agent, cosmetics, flavour enhancer

[a]Ciriminna et al. (2017)
[b]Prediction for 2020
[c]Pal et al. (2016)
[d]No data available

industry. Furthermore, the acid has the ability of metal ion sequestration which helps in preserving the flavour and colour loss of fruits by preventing their oxidation. In combination with citrate, the acid also exhibits excellent buffering capacity that makes it ideally suited for food, cosmetic, nutraceutical, and pharmaceutical applications.

The acid has the INS330 food ingredient code in the International Numbering System (INS331 and INS332, respectively, for sodium and potassium citrate) indicating it as a food additive. Moreover, it has been approved to be a GRAS (Generally Recognized as Safe) ingredient.

The main use of CA in the food industry is that it is widely used as a buffering, flavouring, and preservative agent, particularly in soft drinks. It is the most versatile and widely used acidulant owing to its pleasant taste and its property of enhancing flavours. Recently, there is the surge in its demand and production due to the extended use of trisodium citrate as a substitute for phosphates, allowing for use of ecologically friendlier detergents. Moreover, citric acid is biodegradable and ideal for increased use without negative impact on the environment. It is also used as a multipurpose chemical for cleaning, sequestering, buffering, and wetting. CA has become a prominent chemical in the emerging bioeconomy, with applications beyond conventional usage in the food, pharmaceutical, and cosmetic industries (Table 17.2).

17.2.1.3 Production Conditions

A number of microorganisms including bacteria, yeast, and fungi such as *Arthrobacter paraffinens*, *Bacillus licheniformis*, *Candida citroformans*, *C. guilliermondii*, *C. tropicalis*, *C. oleophila*, *Corynebacterium* species, *Yarrowia lipolytica*, *Aspergillus aculeatus*, *A. awamori*, *A. carbonarius*, *A. foetidus*, *A. fonsecaeus*, *A. niger*, *A. phoenicis*, and *Penicillium janthinellum*, have been employed for CA production (Grewal and Kalra 1995; Pandey et al. 2001; Lu et al. 2019). Most of them, however, are not able to produce commercially acceptable yields due to the fact that CA is a metabolite of energy metabolism and it accumulates in appreciable amounts only under drastic imbalance conditions (Fig. 17.1).

Initially, the yeast *Yarrowia lipolytica* was used at an industrial scale in the 1960s (Coelho et al. 2010; Cavallo et al. 2017; Morgunov et al. 2018; Timoumi et al. 2018) but later on almost double yield was achieved with that of the *A. niger* (Kurtzman 2011; Levinson et al. 2007). The cultivation of yeast is advantageous due to its higher productivities (shorter time for fermentation) and easier cultivation (non-filamentous growth), resistance to high substrate concentrations, and tolerance to metals; however, the simultaneous accumulation of isocitric acid is very distractive in the purification for pure citric acid (Timoumi et al. 2018).

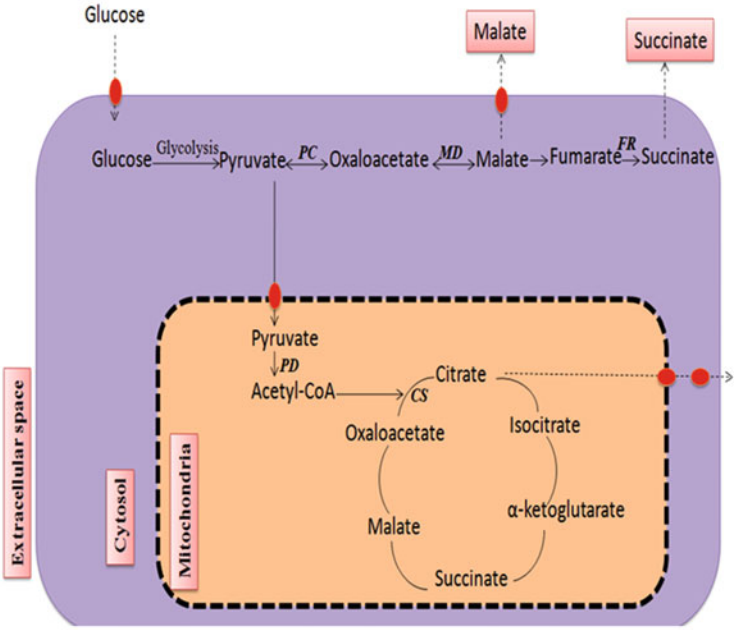

Fig. 17.1 The TCA cycle representing the synthesis of citric acid (CA) and succinic acid (SA). *PC* pyruvate carboxylase, *MD* malate dehydrogenase, *FR* fumarate reductase, *PD* pyruvate dehydrogenase, *CS* citrate synthase enzymes. The cytosolic pathway for SA production represents the reductive branch of TCA, not the complete cycle

In general, CA is produced commercially by submerged microbial fermentation of molasses using *A. niger* due to its ease of handling, its ability to ferment a variety of cheap raw materials, and high yields. Although chemical synthesis of CA has also been well developed, but microbial fermentation is preferred and has become the ultimate choice. The crucial parameters for the efficient production of CA by *A. niger* have been determined empirically and include high carbon source concentration, limiting concentration of nitrogen and certain trace metals, thorough maintenance of high dissolved oxygen (DO) and low pH. The nitrogen sources (ammonium sulphate, ammonium nitrate) decrease the pH and restrain the accumulation of undesirable by-products (mainly oxalic acid). In addition, phosphate and trace metal ions (Mn^{2+}) should be suboptimal (Karaffa and Kubicek 2003; Röhr et al. 1996).

The production medium is inoculated with a spore suspension (~1 mm diameter) of *A. niger* with continuous mixing, aeration at a temperature of 25–28 °C for 5–8 days to achieve high CA concentrations (Papagianni and Mattey 2006). Recovery is customarily as the calcium salt by precipitation with calcium hydroxide followed by acidification with sulphuric acid resulting in equimolar amounts of $CaSO_4$ (gypsum), a problematic high volume side or waste product. In pilot scales, surface fermentation is still preferred as this requires simple installation, less energy and thereby lowers the cost (Vandenberghe et al. 2000; Krishna 2005; Darouneh et al. 2009).

17.2.2 Succinic Acid: Amber Acid

17.2.2.1 Brief History

The succinic acid (SA), produced as an intermediate of the tricarboxylic acid (TCA) cycle, is placed among the top 12 high-value based chemicals according to the U.S. Department of Energy (Saxena et al. 2017). This is on account of increasing its importance for the synthesis of industrially important chemicals related to agricultural, food, and pharmaceutical industries (Song and Lee 2006). Traditionally, an antibiotic and curative agent "Amber" has been used by the Europeans for centuries without knowing about the chemical compound present within. By the dry distillation, Georgius Agricola in 1546 was the first to extract this acid from amber, and, therefore, named it as succinic acid, derived from the word "*succinum*", i.e. amber (Smyth et al. 1951). Plants, animals, and microorganisms are known to accumulate SA naturally, but its optimum formation is by anaerobic (*Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*, *Bacteroides fragilis*, *Mannheimia succiniciproducens*) growth of microorganisms (Putri et al. 2020).

The commercial process for SA production was relied on the petroleum as raw material, obtained from n-butane through maleic anhydride. However, the process was cost effective, thereby limiting it to become the market competitive. Recently, the introduction of microbial based SA production process by several companies is considered to be an economically viable and eco-friendly venture (theoretically 1 mole CO_2 per mole SA produced. "BioAmber", "Reverdia", "Succinity" are

some of the companies involved in the production of biosuccinic acid has been detailed by the Rokem (2020).

17.2.2.2 Attributes with Applicability

SA ($C_4H_6O_4$) is a water soluble, dicarboxylic acid with pK_a at pH of 4.3 and 5.6. It is a versatile building block chemical as is shown in Table 17.1 and is used in the production of more than 30 commercially important products. The potential market for bio-based SA is estimated at 2.5 billion euros, with potential uses in products such as 1,4-butandieol, coolants, deicer solutions, flavours, fragrances, plasticizers, pigments, polybutyrate succinate (PBS), polyesters, solvents, etc. (Table 17.2). The largest market for SA is in the manufacturing of surfactants and detergents or foaming agents. SA and its derivatives are widely used as an acidulants, flavouring agents to enhance the sensorial properties of beverages, relishes, and sausages. Moreover, with the increasing demand for high performance elastomers, cements and coating applications have escalated the use of this carboxylate. Na, K, and Ca salts of succinates have medicinal properties and so are generally used as sedatives, antispasmodics, antirheoters, and contraceptive drugs. In addition to this, an esterified SA product "dimethylsuccinate" is widely used as coolant in vehicles. Furthermore, recent applications of SA have been extended in the fields of polymer synthesis such as biodegradable PBS, polyamides (Bionolle), and various green solvents (Corona-Gonzalez et al. 2010; Song and Lee 2006; Willke and Vorlop 2004).

Some of the global vital players include BioAmber, Nippon Shokubai, Reverdia, Succinity. In 2016, Reverdia promotes Biosuccinium based microcellular polyurethane foams to be used as soles for footwear. Recently, Nippon Shokubai expanding its core business under "Reborn Nippon Shokubai 2020", the novel platform technology targeting the reduction in the consumption of solvents and harmful chemicals.

17.2.2.3 Production Conditions

The first microbial approach for the production of SA was the engineering of the mixed acid fermentation of *Escherichia coli* (Chatterjee et al. 2001). Later, it was discovered that several anaerobic rumen and facultative bacteria can naturally produce large amounts of SA. The best known producers among them are *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens*, *Ruminococcus flavefaciens*, however, SA is seldom detected in measurable amounts as it rapidly converted itself to propionic acid by the species of *Propionibacterium*, *Pectinatus*, and *Bacteroides*. Among them, *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens* are known to be the efficient producing strains using a wider spectrum of carbohydrates. The four key enzymes, namely phosphoenolpyruvate carboxykinase, malate dehydrogenase, fumarase, and fumarate dehydrogenase are known to regulate their metabolism, thereby giving higher yield by limiting the by-products. However, the cultivation of such bacteria requires expensive and complex nutrient sources that remain to be solved despite (Lee et al. 2005; Isar et al. 2006; Andersson et al. 2008; Li et al. 2010). One another strategy using *Mannheimia succiniciproducens*, a rumen microorganism, remained

unsuccessful due to the lower yield of resulting strain than that of the natural producers. Many researchers have made tremendous efforts to develop a bioprocess for succinic acid production by employing fungi and yeast, *Aspergillus fumigatus*, *A. niger*, *Lentinus degener*, *Penicillium viniferum*, *Paecilomyces variotii*, *Saccharomyces cerevisiae.* Finally, filamentous fungi, including *Penicillium simplicissimum* have been shown to accumulate succinic acid naturally (Gallmetzer et al. 2002) with the fumarate reductase as the main enzyme involved. Although the titers and yields are also not comparable with bacterial hosts, the cultivation of the fungus is easier. To date, no industrial process for microbial SA production has been established; however, calculations show that such a process can be competitive provided that formation of by-products such as acetic, formic, lactic acids and its recovery can be resolved.

17.2.3 Gluconic Acid: Platform Chemical

17.2.3.1 Brief History

Gluconic acid (GA), a multifunctional organic acid, has been identified as one of the top 30 "building block" compounds that can be produced from biomass sources in future biorefineries (Werpy and Petersen 2004). According to a new report, its global market is poised to cross USD 80 million by 2024. With its increasing demand, the interest spurred in different industries for the development of an effective and economically viable system for GA production (Singh and Kumar 2007).

GA has a long history when it was discovered by Hlasiwetz and Habermann in 1870. Later on, Molliard (1922) detected GA in the culture of fungal species known as *Aspergillus niger*. In the subsequent years, GA producing bacterial species like *Acetobacter*, *Gluconobacter*, *Pseudomonas*, and various other fungal species were discovered. Studies of Bernhauer in 1924 showed that *A. niger* produced high yields of GA when it was neutralized by $CaCO_3$ and the production was found to be highly pH dependent. Using *Penicillium luteum* and *A. niger* Currie et al. filed a patent in 1931 employing submerged culture giving GA yields up to 90% in 48–60 h. Later, Moyer et al. (1937) used *A. niger* in pilot plant studies and achieved 95% of theoretical yields with glucose solution of 150–200 g L^{-1} in 24 h. Currently, sodium gluconate is commercially produced involving fed-batch type submerged fermentation at 34°C temperature with pH 6.0–6.5 using *A. niger*, developed by Blom et al. (1952). Some of the major manufacturers of GA and its salt in the USA are Pfizer Inc., New York, Premier Malt Products Inc., Wisconsin, Roquette Frères, Benckiser, Fujisawa, and Kyowa Hakko. Calcium gluconate is also an important product among the derivatives of gluconic acid and it is available as tablets, powder, and liquid for dietary supplements (Röhr et al. 1983; Ramachandran et al. 2006).

17.2.3.2 Attributes with Applicability

GA ($C_6H_{12}O_7$), a weak, non-volatile, non-toxic acid, results from the specific oxidation of the aldehyde to carboxyl group at C1 in D-glucose by chemical, catalytic, or through biotransformation process (Ramachandran et al. 2006). Its

properties have been highlighted in Table 17.1. This six carbon sugar has both the features of acid and alcohol, thus favoured by the 1,5 intramolecular esterification leading to the formation of an acyclic ester, glucono-γ-lactone by the loss of a water molecule. In aqueous solutions, the acid is in equilibrium with its lactones, i.e. why it is commercially available as a 50% aqueous solution with a pH of 1.82 and 1.23 g/cm^3 density. However, with the addition of a base to the ester rapidly forms an open-chain gluconate salt by cleaving the aldonic ring. This salt can form highly stable chemical compounds by chelating metals in the presence of strong oxidants, thereby yielding a mixture of 2-keto and 5-keto-D-gluconate (2-KGA and 5-KGA, respectively) and, under extreme conditions, 2,5-diketo-D-gluconate (in variable proportions) (Hustede et al. 2012). Among all the derivatives, sodium gluconate is widely marketed accounting for >80% of the world production (Roehr et al. 2001). Below 30 °C gluconic acid can be isolated; between 36 °C and 70 °C the β-lactone is predominated and above 70 °C, γ-lactone is obtained, because the various equilibria change slowly.

The gluconic acid and its derivatives are mainly used as additives by food, pharmaceutical, hygiene, and building industries. For example, they are commonly added to dairy products and soft drinks to preserve and/or enhance their sensory properties by imparting a bitter but refreshing taste (Rogers et al. 2006; Ramachandran et al. 2006). GA derivatives are acidity regulators (INS574–INS580) with raising, sequestering, hardening, and flavour enhancing properties. In addition, the metal binding capacity helps to prevent cloudiness by binding trace metals present in drinks, such as Ca and Fe in fruit juices. GA is also used as a food preservative for pickled foods, and glucono-γ-lactone is used as a preservative for cured meat-based sausages. In addition, some food processing plants use GA derivatives as cleaning agents for their industrial facilities (Milsom and Meers 1985). Thus, alkaline solutions of sodium gluconate are used to clean glassware, but GA is preferred for metal (steel, alloyed) components. For example, the dairy industry uses GA derivatives to prevent the precipitation of calcium salts in processing equipment and glass storage vessels. Furthermore, in bakery industry, glucono-γ-lactone is used as an acidifier and chemical baker's yeast. The pharmaceutical industry uses Ca^{2+}, Mg^{2+}, and Fe^{2+} gluconates as mineral supplements to treat hypocalcaemia, hypomagnesaemia, and anaemia, respectively (Sawyer 1964). The uses of GA, thus, continuing to expand. The excellent chelating properties of GA with Fe^{2+} and Fe^{3+} have been used to remove, at a neutral pH, hazardous chlorinated substances such as 2,4,6-trichlorophenol (TCP) and trichloroethylene from ground water through the Fenton reaction (Ahuja et al. 2007). Additionally, GA has been used for recycling Mo, Ni, and Al catalysts with recoveries of 99%, 46%, and 14%, respectively, under optimal bioleaching conditions. Finally, GA is used as a substrate for the production of derivatives such as 2-KGA and 5-KGA, through regio-selective oxidation by the action of dehydrogenases such as in *Gluconobacter oxydans* strains. 2-KGA is also used as a building block for chemical synthesis, as in its chemical conversion to isoascorbic acid or the synthesis of heterocycles (Stottmeister et al. 2005). 5-KGA is useful for the production of tartaric acid (Herrmann et al. 2004), xylaric acid, the savoury flavoured compound

4-hydroxy-5-methyl-2,3-dihydrofuranone-3 as well as for vitamin C by the Gray method (Saichana et al. 2015; Bremus et al. 2006).

17.2.3.3 Production Conditions

The GA is usually produced by microbial oxidation of glucose. Enzymatic conversion of glucose into GA can be achieved through a simple dehydrogenation reaction using glucose oxidase. Ever since Isbell's general method for the electrochemical synthesis of calcium salts of aldonic acids by reacting sugars with calcium carbonate and bromide ion was reported (Isbell et al. 1932), a number of authors have promoted the development of chemical (electrolytic or catalytic) oxidation methods for the production of GA from D-glucose. However, the high cost, low yield, and deactivation of the catalyst strongly favoured microbial production (Pal et al. 2016). Although a number of organisms have been described to accumulate the acid naturally like *Aspergillus niger*, *Penicillium luteum*, *Gluconobacter*, *Pseudomonas*, and *Acetobacter* (Ramachandran et al. 2006). However, the industrial bioprocess of GA, nowadays, has been employed *A. niger* for its production. Interestingly, unlike the CA process, where glucose is taken up by the organism, converted and exported in the extracellular medium, GA is directly produced extracellularly in a two-step reaction by glucose oxidase action has been shown in Figs. 17.1 and 17.2, respectively.

The first step is catalyzed by glucose oxidase, which oxidizes β-D-g1ucopyranose to D-glucono-1,5-lactone. The hydrolysis of the lactone to form GA occurs spontaneously in aqueous solutions, but the rate is six orders of magnitude greater with the enzyme gluconolactonase (Ogawa et al. 2002). The enzyme exists as a dimer of identical subunits containing one FAD per subunit. The FAD is reduced in the course of oxidizing glucose to GA, and the subsequent oxidation of the reduced FAD by molecular oxygen generates hydrogen peroxide (Gibson et al. 1964). Both glucose oxidase and gluconolactonase are located outside the plasma membrane. The hydrogen peroxide generated by glucose oxidase inactivates the enzyme,

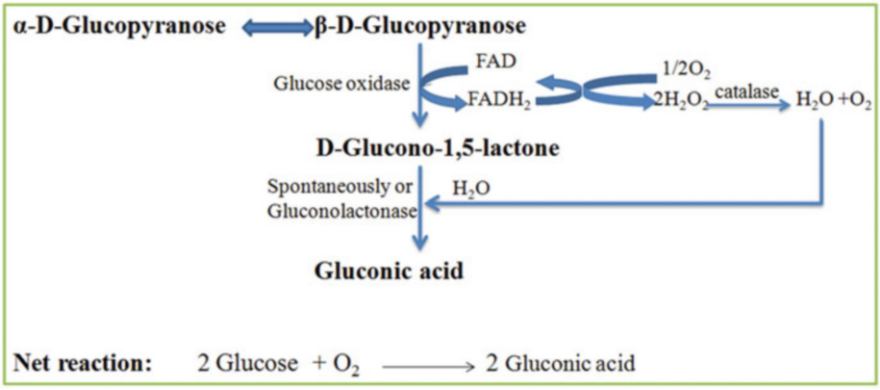

Fig. 17.2 Biosynthetic gluconic acid pathway of *Aspergillus niger*

probably through the oxidation of methionine residues (Kleppe 1966). This emphasizes the need for catalase, which catalyzes the disproportionation of the cytotoxic hydrogen peroxide, formed by the action of glucose oxidase, into water and molecular oxygen (Vroemen and Beverini 1999).

17.2.4 Kojic Acid: From Shoyu to Cosmetics, Koji's Journey of a Century

17.2.4.1 Brief History

Kojic acid (KA), a promising carboxylate, is well known for its wide applications in various fields of agriculture, cosmetics, environment, food, and pharmaceuticals. Since antiquity, soya beans were converted to food additives such as soybean paste, soy sauce, and fermented whole beans using the yellow-green fungus as starter culture. In the seventh century, Buddhist priests apparently brought their methods from China to Japan, where the fungal inoculum, often *Aspergillus oryzae*, became known as koji. After fungal growth on steamed rice, the resulting koji was used as a starter in brewing a rice wine, soy sauce, miso (paste from soy beans). One hundred years ago, the koji process in Japan was discovered by K. Saito in 1907 and so named "koji", i.e. a fungus or starter inoculum used in oriental foods (Terabayashi et al. 2010; Chaudhary et al. 2014). In 1912, T. Yabuta gave the name koji acid to the steamed rice product and discovered few other KA producing strains of *Aspergillus* species (*A. albus*, *A. candidus*, *A. nidulans*). KA was first marketed in 1955 by the Charles Pfizer and Company, USA. Some of the currently leading companies are Hubei Xinxinjiali Bio-tech, Triveni Interchem, Chengdu Jinkai, Syder, Xi'an Hao-xuan Bio-tech Co, Hubei Hongjing, Hubei Xiangxi Chemical, Sichuan Huamai Technology, Hubei Artec Biotechnology Co, Sansho Seiyaku.

17.2.4.2 Attributes with Applicability

KA ($C_6H_6O_4$) is water soluble γ-pyrone ring, reactive at every position on the ring with weakly acidic properties (Table 17.1). Due to its reactivity, a number of products with industrial value, such as azo dyes, ethers, mannich base, metal chelates, pyridones, pyridines can be formed (Wilson 1971). The hydroxyl group at C-5 position acts as a weak acid, capable to form salts with Na, Zn, Cu, Ca, Ni, Cd (Crueger and Crueger 1984). Its natural origin provides it a striking feature for the formulations of biologically active derived compounds. KA has antibiotic, antioxidant properties and also used as food additive to prevent browning in foods (Beelik 1956; Bentley 2006; Nohynek et al. 2004). Probably, the most widely used application of kojic acid is as a skin-whitening agent in cosmetics as it has the ability to act as the ultra violet protector by chelating copper ions required for tyrosinase activity, thereby, curtailing the melanin formation in human skin (Noh et al. 2009).

KA and its derivatives (azidometalkojates) possess antibacterial and antifungal effects on several species of *Bacillus*, *Staphylococcus*, *Saccharomyces*, *Aspergillus*, *Rhizopus*, and *Fusarium*. Besides its antibiotic functions, KA also shows a certain insecticidal activity against *Heliothis zea* and *Spodoptera frugiperda* insects

(Table 17.2). In addition to this, KA also acts as a precursor for flavour enhancer (i.e. maltol and ethyl maltol), used as flavour enhancers, and as an active ingredient in perfumes and flavours (Ichimoto et al. 1965; Kashyap et al. 2019; Uchino et al. 1988). Besides, it also acts as an antibrowning agent for apples, mushrooms, potatoes, crustaceans such as grass prawns, white shrimps, and Florida spiny lobsters (Chen et al. 1991; Saruno et al. 1978).

17.2.4.3 Production Conditions

KA is an organic acid produced by several fungi including *A. oryzae*, *A. flavus*, and some *Penicillium* species. Generally, the formation of secondary metabolites is restrained to a limited number of organisms. However, KA can be produced in aerobic conditions by an array of microorganisms including *Aspergillus* species, *Bacterium xylinoides*, *Penicillium* species, *Gluconoacetobacter opacus mobilis*, and *Gyrinium roseum* (Wilson 1971). The majority of the industrial production of KA belongs to yellow-green species of *Aspergillus*. High yields (0.456 g g^{-1} glucose) have been obtained with *A. flavus* (Ariff et al. 1997; Rosfarizan and Ariff 2007). However, *A. oryzae* has been used for production and has received Generally Recognized As Safe (GRAS) approval by the U.S. Food and Drug Administration (FDA) (Machida et al. 2008). Kojic acid was unambiguously confirmed by single-crystal X-ray studies, was isolated for the first time from the African plant *Kigelia africana*, and is a possible intermediate in the shikimic acid pathway. This is a key pathway in the biosynthesis of quinones, suggesting that KA is a possible taxonomic marker in the biogenesis of the quinone skeleton. This pathway involves the condensation of 3-carbon phosphoenolpyruvate with a tetrose sugar (D-erythrose-4-phosphate) to yield Di-hydroxyacetone phosphate (DAHP), a seven-carbon sugar. This DAHP further undergoes intramolecular condensation and subsequent dehydration to yield the compound. The gene cluster responsible for KA biosynthesis is composed of three genes, *kojA*, *kojT*, *kojR*. The anticipated functions of *kojA* and *kojT* are a FAD-dependent oxidoreductase and a major facilitator superfamily transporter, respectively (Terabayashi et al. 2010). The *kojR* gene encodes a transcription factor that specifically activates *kojA* and *kojT* genes.

The concentration of glucose has a big impact on the KA production. The highest titer (24.2 g L^{-1}, yield 0.24 g g^{-1} glucose) was obtained using 100 g L^{-1} glucose. Using less glucose, the carbon source was entirely used for biomass formation, while more glucose does not increase its titer. Nitrogen limiting conditions are required for growth, of which organic N is preferred over inorganic N sources. In fact, a C/N ratio of about 100 was found to be optimal. More carbon did not increase the KA titer. Less nitrogen leads to much less production (Rosfarizan et al. 2010). Low pH appears to be a requirement for efficient KA production. About 83 g L^{-1} KA was produced from two rounds of repeated cultivation for 100 days using *A. oryzae* immobilized in Ca-alginate beads (Kwak and Rhee 1992). In another study, pilot-scale production of this acid using an improved strain of *A. oryzae* in repeated batch fermentation with cell retention has been achieved with the productivity of 5.3 g L^{-1} day^{-1} (Wan et al. 2005).

17.3 Filamentous Fungi as Cell Factories: Commercial Successes

Aspergillus niger is one of the most important industrial filamentous fungal species, non-toxic, safe for the production and offers several advantages such as higher productivity, yields, and lower contamination risk. Here, we summarizes the recent developments exemplified by four products: CA and GA, which have been in the market and manufactured via large-scale bioprocesses, SA and KA, which (despite the fact that a feasible industrial bioprocess has not yet been developed) have huge potential as building block chemicals. Even though, a few other organic acids have also been explored for the development of novel processes, however, till date the largest commercial quantities of fungal organic acids are primarily CA and GA which are prepared by fermentative bioprocess involving glucose or sucrose by *A. niger.* Another *Aspergillus* species, *A. oryzae*, is used to make KA. A significant commercial source of SA at the time of this writing is a bioprocess employing the *Aspergillus saccharolyticus* and *Penicillium simplicissimum.*

The fungi have the ability to accumulate large amount of a particular organic acid which confers it a competitive advantage. First, at acidic pH, the solubility of most metal compounds increases which leads to the enhancement in the chelating properties of organic acids, in cases where metals are present at very low concentrations or in their insoluble state. Secondly, metabolism in signal transduction pathway is dependent on the ambient pH as rise in acidification brings inhibition of rapidly growing bacterial and fungal species at pH below 3 (Andersson et al. 2008). The three filamentous fungi follow different strategies to produce an array of organic acids, such as, unusual ability of many strains of *A. niger* to oxidize the glucose into gluconic acid extracellularly in low pH using the enzyme glucose oxidase. Side-by-side other strains of *A. niger* has the ability to produce CA inside the cell and exporting it out. *A. oryzae*, another strain of *Aspergilli*, acidifies the environment by producing kojic acid. Kojic acid is not a primary metabolite, so both the anabolism and catabolism of this acid are relatively rare metabolic attributes. Once again, acidification of the environment with kojic acid will inhibit the growth of many microorganisms. Subsequently, the relatively unusual nature of kojic acid would permit *A. oryzae* and only a few other species to catabolize the acid. Henceforth, the commercial success of fungal bioprocesses is ultimately based on the rapid and economical efficiency of sugars to acid.

17.3.1 Mechanism of Citric Acid Production with *Aspergillus niger*

The accumulation of CA is a very complex process which onsets with the utilization of glucose in the glycolysis. Earlier studies on the regulation of CA laid down the emphasis of enzyme citrate synthase along with the cofactors required for the enzyme activation. Peculiarly, citric acid is in principle a potent inhibitor of glycolysis. It has been shown that citric acid effectively inhibits phosphofructokinase I (*pfk I*), which is the most important enzyme controlling the flux through glycolysis. Initially, it was believed that a certain intracellular ammonium concentration would

relieve this inhibition (Papagianni 2007). However, Papagianni et al. (2005) showed that the intracellular ammonium concentration is low throughout the citric acid production process. Legiša and Mattey (2007) describe post-translational modifications of *pfk I* triggered by a drop in the intracellular pH and a cAMP (cyclic adenosine monophosphate) peak before the onset of citric acid accumulation. The enzyme is cleaved and phosphorylated leading to a fragment that is highly active and highly inducible by ammonium, but less susceptible to citrate inhibition, which explains why glycolysis is not repressed. However, this connects the high intracellular tricarboxylic acid concentration at the beginning of the process with a drop in intracellular pH and the onset of CA production.

The bioprocess involved for citric acid fermentation is batch wise of submerged type using 15–22% C (molasses as source of sucrose, starch hydrolysates as glucose source) carried out at 30 °C for 5–10 days. Using hexoses as C source, one molecule of ATP and three molecules of NADH are formed via substrate phosphorylation (so there is no need to generate ATP by oxidative phosphorylation, which would decrease citrate yield).

Two different respiratory routes are being employed by *A. niger* based on the oxidation of matrix NADH: proton pumping based NADH-ubiquinone oxidoreductase enzyme and non-proton pumping based alternative oxidase (Prömper et al. 1993; Joseph-Horne et al. 2001). Various studies have shown that the proton pumping based enzyme gets impaired due to sudden interruptions in oxygen supply during CA accumulation, thereby, favouring its route for alternative oxidase activity. The alternative oxidase enzyme necessitates the efficient reoxidation of glycolytically produced nicotinamide adenine dinucleotide (NADH) whose activity gets impaired by short interruptions of oxygen supply (Hattori et al. 2009), without affecting the mycelial growth. The involvement of this enzyme is due to the metabolic imbalance which leads to the reduction in the level of ATP production, thereby, allowing the efficient conversion of glucose to CA. The mechanism involved for the transport of CA from mitochondria to cytosol and further to the extracellular medium is still unknown. However, many co-workers postulated the role of pH gradient for the effluxing of CA.

The trace metals exert cellular morphology and the production rates, of which iron and manganese are considered to be a crucial prerequisite for efficient production (Magnuson and Lasure 2004; Grimm et al. 2005). When the trace metal concentration is not tightly controlled, growth of unproductive filamentous mycelia is promoted instead of pellet formation which leads to reduction in the CA yield (Magnuson and Lasure 2004). The conditions for obtaining such pellets have been determined empirically (Liao et al. 2007; Liu et al. 2008). The significance of the correct fungal morphology for good productivity has stimulated various attempts to manipulate the growth characteristics of the fungi (Teng et al. 2009; Driouch et al. 2010). A final approach is to gain understanding of the biological regulation of the morphology development. By understanding the signalling networks it will be possible in the future to modulate the regulating signals in order to obtain the desired morphology. A first molecular biology approach has been shown by Dai et al. (2004), who proved that antisense expression of a putative amino acid transporter

allows the formation of productive cell pellets even in the presence of normally inhibiting Mn^{2+} concentrations (Grimm et al. 2005; Driouch et al. 2010; Wucherpfennig et al. 2011).

Even though the biochemical mechanisms involved for high concentration of acid accumulation are well known and their conditions are described by Karaffa and Kubicek (2003), Magnuson and Lasure (2004), and (Papagianni 2007) in great detail, still the full mechanism of accumulation is, however, not available. The imbalance of metabolism, as described above, is critical for buildup of the acid, as well as the complexities of transport of the product from both cell compartments and excretion (García and Torres 2011).

Physiological understanding and rational strain design seems to be fundamental for further improvements. A gene homologue of the itaconic acid transporter from *Ustilago maydis*, when overexpressed in *A. niger*, resulted in five times higher citrate formation (109 g L^{-1}) compared to the wild type. The metabolic regulation of citrate biosynthesis is dispersed over several steps with no specific switch point, and it is speculated that at least three different stages control biosynthesis: transport and metabolism of carbon source, the transfer of product out of the mitochondria, and excretion from the cell as shown in Fig. 17.1. The recent capability to perform comparative genomics of different *A. niger* strains (Andersen et al. 2011; Yin et al. 2017) shows that many genes (479) change on the transcriptional level when comparing citrate-producing strains (Yin et al. 2017). However, the molecular mechanism for citrate accumulation is only partially understood, with open questions around citrate transport as well as the role of alternative oxygenases to keep the energy balance. It may be concluded that the control of citric acid accumulation is disseminated over many steps with no distinct and single control location.

17.3.2 Mechanism Involved for Succinic Acid

Succinic acid is one of the intermediates of the TCA cycle, and also is an end product of the mixed acid fermentation of various bacteria. It can, therefore, be produced oxidatively or reductively (Raab and Lang 2011). In fungi, two pathways for succinate accumulation have been suggested. The first pathway is the oxidative route of the TCA cycle from citrate, via isocitrate to α-keto glutarate and succinyl CoA converted by succinyl CoA ligase to succinate. This route for accumulation of organic acid as a product is wasteful, since two CO_2 are formed from the six carbon compound (glucose). From 1 mole of glucose, 1 mole of a four carbon succinate is obtained. An alternative route is the glyoxylate cycle, but is not more efficient, since two carbons of glyoxylate end up in malate when joined to acetyl-coA. Redirecting the carbon flux into the glyoxylate shunt allows the oxidative production of succinic acid. The two decarboxylating steps of the oxidative branch of the TCA cycle are thereby by-passed. However, the maximal theoretical yield amounts to only 1 mol succinic acid mol^{-1} glucose. A third alternative pathway involves the reductive branch of the TCA cycle resulting in higher molar yield than the above two routes which enables a theoretical yield of 2 mol mol^{-1} glucose because CO_2 is fixed

(Fig. 17.1). The one study with filamentous fungi, where the succinate accumulation is shown to be through the reductive pathway, is for *Penicillium simplicissimum* kept at anaerobic conditions (Gallmetzer et al. 2002). In this study the enzymes required to convert fumaric acid to succinic acid, fumarate reductase, was not investigated. The fumarate reductase has, as yet, not been shown to be functional in filamentous fungi.

17.3.3 Gluconic Acid Production Mechanism with *Aspergillus niger*

Gluconic acid production by *A. niger* is an aerobic fermentation with a high oxygen demand. The key enzyme in the process is glucose oxidase, a homodimeric flavoprotein, localized in the mycelial cell wall. Consequently, the conversion takes place entirely extracellularly with a net reaction of 2 glucose + O_2 → 2 gluconic acid (Fig. 17.2). The production parameters are also defined by the enzymatic properties of glucose oxidase. The enzyme gets rapidly inactivated at low pH; therefore, the pH of the culture has to be kept between 4.5 and 6.5 for efficient gluconic acid production. High glucose concentrations activate glucose oxidase. Typically, production process starts with 110–250 g L^{-1} glucose (i.e. 11–25% C) concentration. In fact, the Michaelis constant (*K*m) value of glucose oxidase is in the range of air saturation in water. Hydrogen peroxide, formed by flavin adenine dinucleotide (FAD) recycling is a strong inhibitor of glucose oxidase. Sufficient catalase activity is consequently of utmost importance for an efficient process. Yegin et al. (2020) reported that nitrogen and phosphorus sources have to be kept at a very low level in order to limit growth (=loss of carbon source for biomass production). The process of fungal gluconic acid production is extraordinarily efficient. Yields typically exceed 90% and even up to 98% have also been reported (Singh and Kumar 2007). Fermentation times are short, between 24 and 60 h. It is, therefore, not surprising that strain improvement did not play a significant role up till now. It has been suggested that the quick conversion of glucose to gluconic acid outside the fungal cell leads to an evolutionary advantage.

A. niger lowers the pH of the environment quickly, thereby inhibiting competitive microorganisms. Furthermore, gluconic acid is a poor carbon source for competing microorganisms, but a good one for *A. niger*. A future trend for process improvement could be the conversion of glucose by purified enzymes (Magnuson and Lasure 2004; Wong et al. 2008). This could ease the purification significantly, as all media components, which are only necessary to support growth of the fungus can be omitted. However, till date the production of purified enzymes is still too expensive.

17.3.4 Mechanism Involved for Kojic Acid

The apparently simple structural relationship between the pyranose ring structure of glucose and the pyrone ring structure of kojic acid had caught the attention of early investigators. For a direct conversion of glucose to kojic acid, one oxidation (CHOH

→ CO) and two dehydrations are required. Hence, there came to be two theories; a direct conversion, hexopyranose → kojic acid, or C3 and or C2 compounds → C6 precursor → kojic acid.

A better producer for kojic acid production other than *A. flavus* is *A. oryzae*. The academicians reported highest titer of using 100 g L^{-1} glucose. Using less glucose, the carbon source was entirely used for biomass formation, while more glucose did not convert carbon at all. However, less nitrogen leads to much less production (Rosfarizan and Ariff 2007). About 83 g L^{-1} kojic acid was produced from two rounds of repeated cultivation for 100 days using *A. oryzae* immobilized in Ca-alginate beads (Kwak and Rhee 1992). Pilot-scale production of this acid using an improved strain of *A. oryzae* in repeated batch fermentations with cell retention achieved a productivity of 5.3 g L^{-1} day^{-1} (Wan et al. 2005). It seems surprising that no enzyme system for the formation of kojic acid has been obtained. After all, for the direct synthesis from glucose, at most two or three enzymes are needed. When homogenates of *A. flavus* (culture B) were tested, no synthetic activity was found. Moreover, as already indicated, no possible biosynthetic intermediate has been definitely identified, henceforth, its complete mechanism is still lacking.

17.4 Future Implications

Experiments for renewable, agricultural feedstocks are still on a laboratory scale and there is no information in the public domain as for their use at production scale. There are several means to encourage and obtain the right pellet size and mycelial thickness for each fungal species. The screening for relevant physiological and morphological features should be determined early in process development to avoid experimental work on production scale (Veiter et al. 2018). There is a great potential to metabolically engineer production organisms to approach the theoretical yield of the substrate for a product where the maximal pathway yield is lower. The transport of the acids is another undetermined factor that has great influence on acid accumulation (Vrabl et al. 2012). Attempts to modify by addition of plausible transporters were performed in filamentous fungi but require further investigations.

Efforts are continuing to develop a biological process for organic acid, but so far there is no commercial one step production. As most of the researches investigate using one factor approach responsible for the desired change and thereby understanding of the metabolism is complicated. The complex control of the balance of redox, ATP formation, growth and product formation are still not well understood for the fungi used to obtain excessive formation of the acids. First attempts are done to look at different metabolic levels and to study what change in one level has for consequences in the other levels. In a recent study by Vrabl et al. (2017), the plasma membrane, the energy level, and respiration level were assayed in the fungus *Penicillium ochrochloron* in a chemostat at varying nutrient limitations (C, N, P). All three nutrient levels and the effect on excretion of organic acids were well analyzed. The main conclusion was that organic acid excretion is inversely related to nucleotide concentration.

17.5 Conclusion

The economics for industrial manufacture of organic acid is the main determining factor for use of biological processes. Currently only citric acid is produced using filamentous fungi for the acids discussed. It is recommended that sustainability of the production process should also be included for the appropriate choice of process including substrate(s) and downstream processing. The development of novel genetic tools has no decisive impact on the improvement of organic acid formation by filamentous fungi till now. With the continued development and utilization of both old and novel techniques for strain improvement to construct "super" producers, together with finding the compatible and affordable substrates, fermentation will hopefully be the technique used for all four acids in the not too long future.

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