Anoop Singh
Shaili Srivastava
Dheeraj Rathore
Deepak Pant *Editors*

Environmental Microbiology and Biotechnology

Volume 1: Biovalorization of Solid Wastes and Wastewater Treatment

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Editors
Anoop Singh
Department of Scientific
and Industrial Research (DSIR)
Ministry of Science and Technology
Government of India
New Delhi, Delhi, India

Shaili Srivastava
Amity School of Earth
and Environmental Sciences
Amity University Haryana
Gurugram, Haryana, India

Dheeraj Rathore
School of Environment
and Sustainable Development
Central University of Gujarat
Gandhinagar, Gujarat, India

Deepak Pant
Separation and Conversion Technology
Flemish Institute for Technological
Research (VITO)
Mol, Antwerpen, Belgium

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Foreword

Dr Hemant Purohit

Dr Shekhar C. Mande

We are witnessing exhilarating times in technological shifts, wherein the resource management processes are seeking "Green options" with almost no waste generation. For any country, to address quality of life, it should have an ecosystem sustainability plan with effective resource management that can only be achieved, if it is energy-efficient, biodiversity-conscious, with no-waste attitude. Waste management is an essential step towards achieving the sustainability goals.

This biotransformation capacities of microbes and its possible exploitation have given rise to a lot of new environmental applications such as in situ bioremediation for recalcitrant pollutants like petroleum contaminants (polycyclic aromatic hydrocarbons), metal ion precipitation through transformations, energy recovery from wastewaters by designing microbial fuel cells, or electricity-driven bio-production using microbial systems. This book is targeted at a broad audience, mainly researchers, environment specialists, academicians, entrepreneurs, industrialists, policymakers, and others who wish to know the latest development and future perspectives of microbial and biotechnological approaches for greener and cleaner environment of the future and also discusses the bottlenecks of the various technologies that current status of the scale-up and commercialization.

Waste management mostly involves a series of balanced bioprocesses, which ensures resource recovery in terms of materials or energy in relatively cleaner ways. The postprocess waste inventory and its utilization at every level, from daily life,

industry to ecosystem is dealt in this book. As an example, according to the World Bank's What a Waste 2.0 report, the world generates 2.01 billion tonnes of municipal solid waste annually that can be a resource through proper segregation and valorization to generate value-added products. There are also parallel documents which are suggesting utilization and valorization of wastewater and such issues are scientifically deliberated in this book.

The other issue linked with resource management is linked to the waste to energy conversion. The use of renewable energy sources has achieved a significant role in resource management, wherein bioprocesses have been designed for utilization of organic waste to biomethanation, bioethanol, and bio-hydrogen which took a lot of scientific attention in recent days and these bioprocesses have been seen as future clean energy options.

Overall the book addresses all the technical aspects related to resource management and recommends the application of microbial capacities for waste management as the only option for sustainability.

Chief Scientist, CSIR-NEERI
Nagpur, Maharashtra, India

Hemant Purohit

Secretary to Government of India,
DSIR and Director General, CSIR
New Delhi, India

Shekhar C. Mande

Preface

Environmental microbiology and biotechnology are essential in the modern lifestyle as they are directly linked with environment, human health, economy and serve as a foundation to produce novel bio-products and energy. The main areas of utilization of these technologies for human welfare include bioremediation, waste management, waste valorization, energy production, health, hygiene, biological control, biofertilizers and natural product discovery. Environmental microbiology and biotechnology will continue to engage future generations of scientists, engineers, entrepreneurs and policymakers for the well-being of human and maintaining the natural resources.

Microorganisms are ubiquitous and inhabit soil, water, air and sediments as well as animals and plants living there. Prokaryotic microorganisms represent by far the largest reservoir of genetic diversity on Earth. They are much older than protests, metazoans and plants. They outnumber all other organisms, have a larger biomass and make the planet liveable by managing its biogeochemistry, cycling nutrients and breaking down wastes, natural or anthropogenic. Microbes possess the highest potential for the production of bioactive products, enzymes, polymers and most of the tools used in biotechnology. A diverse range of these microbes play a major role in our life and are key players in several environmental processes. Most of these are natural processes such as agriculture (nitrogen fixation), waste degradation (bioremediation, anaerobic digestion) and maintaining the natural recycling processes. This ability of microbes in transforming one substrate to another through their metabolism has given rise to a lot of new environmental applications such as in situ bioremediation for recalcitrant pollutants such as petroleum contaminants (polycyclic aromatic hydrocarbons), energy recovery from wastewaters in microbial fuel cells (MFCs) and electricity-driven bioproduction using microbial electrosynthesis. Bioremediation utilizes the application of microorganisms (bacteria, fungi, yeast and algae), to clean up contaminated sites. In this process, hazardous organic compounds are broken down, which serve as food for the microorganisms. Traditionally, the identification and characterization of microbial communities in contaminated soil and water have been limited to those microorganisms that are culturable. However, through the use of culture-independent molecular techniques, new insights into the composition of uncultivated microbial communities have been gained.

The uncultured microorganism could be exploited by the use of biotechnological tools for the benefit of environmental restoration. Biotechnology is a valuable tool which promotes and helps to regulate the biological system for efficient use of microorganism and plant. Biotechnology applications including genomics and proteomics analysis, bioinformatics, sequencing and imaging are all techniques to generate vast amounts of information, which can be utilized for the betterment of human well-being. Biotechnological applications on microbes could be more efficiently utilized for environmental protection by utilizing them for the purpose of wastewater treatment, degradation of toxic compounds, food purpose, sustainable agriculture, climate mitigation and industrial applications.

The present book is an effort to provide an up-to-date information and knowledge on the state-of-the-art applications of biotechnological and microbiological tools for the upliftment of environment by the internationally recognized experts and subject peers in different areas of biotechnology, microbiology and environment. It is a comprehensive collection of chapters related to possible applications of biotechnological and microbiological techniques in solid waste management, wastewater treatment, agriculture, energy and environmental health. The book is divided into two volumes, volume I comprises three sections, viz. Solid Waste Management, Water Treatment Technology and Agricultural Utilization and volume II comprises two sections, viz. Bioenergy and Environmental Health. An introductory chapter is also included in volume I, which gives a general background of current biotechnological and microbiological techniques available for the treatment of various waste and development of value-added products, their utilization for various purposes, socio-economic relevance of these technologies and their associated risks. Volume I covers three sections: Section I is focused on solid waste management and covers waste valorization, green polymer, management of different wastes, their industrial perspective, challenges and opportunities, etc. Section II deals with the utilization of microbial and biotechnological approaches for the management of agricultural waste, production of biofertilizers, biological pest control and future perspective of these technologies in agriculture. Section III covers the treatment of wastewater through microbial biotechnology, its electrochemistry, biotransformation of pollutants, applications and their constraints and opportunities.

New developments in the applications of biotechnical and microbiological methodology to reduce the wastage by converting them into valuable products and reducing the environmental pollution due to their disposal, from all over the world have been discussed and, wherever possible, complemented with real-life examples by the renowned experts in the field. Integration of all the recent developments into a new, consistent methodology for each type of biotechnological and microbiological system is the main aim of this book. A major advantage of this book is that it also provides advice on which procedures should be followed to achieve adequate, relevant and acceptable results. We sincerely hope that this book will contribute to the necessary transition to environmentally benign and sustainable utilization of various waste products. Though we have tried to be very objective in our choice of topics to be covered in this book, some not so common themes might have been

missed out but which may become important in the future, we will try to cover them in the second edition of the book.

This book is aimed at a broad audience, mainly researchers, environment specialists, academicians, entrepreneurs, industrialists, policymakers and others who wish to know the latest development and future perspectives of microbial and biotechnological approaches for the upliftment of the environment and also discusses the bottlenecks of the various technologies that currently limit the scale-up and commercialization. This book is intended to have three roles and to serve three associated audiences, namely the students and research community who will benefit from the lucid explanation of the possible applications of biotechnology and microbiology for the betterment of environment, the policymakers who will find it easier to identify the pros and cons of biotechnological and microbiological systems for the welfare of environment and human being and finally the industries involved as it will give them a feeling about the current loopholes and ways to fix them. Each chapter begins with a fundamental explanation for general readers and ends with in-depth scientific details suitable for expert readers. The text in all the chapters is supported by numerous clear, illustrative and informative diagrams, flowcharts and comprehensive tables detailing the scientific advancements, providing an opportunity to understand the process thoroughly and meticulously. Written in a lucid style, the book comprehensively covers each point to give the reader a holistic picture about environmental microbiology and biotechnology and its future perspective. The book may even be adopted as a textbook for university courses that deal with environmental microbiology and biotechnology.

Despite the best efforts of authors and editors along with extensive checks conducted by many experts in the field of environmental microbiology and biotechnology, mistakes might have crept in inadvertently. We would appreciate if the readers could highlight these and make comments or suggestions to improve and update the book contents for future editions.

New Delhi, India — Anoop Singh
Gurugram, Haryana, India — Shaili Srivastava
Gandhinagar, Gujarat, India — Dheeraj Rathore
Mol, Belgium — Deepak Pant

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Editors and Contributors

About the Editors

Anoop Singh has a PhD in Botany (2004). He is a Scientist at Department of Scientific and Industrial Research (DSIR), Ministry of Science and Technology, Government of India. Before joining DSIR, he worked at Technical University of Denmark, Denmark; University College Cork, Ireland; The Energy and Resources Institute (TERI), New Delhi, India; Indian Agricultural Research Institute (IARI), New Delhi, India; Banaras Hindu University, Varanasi, India and VBS Purvanchal University, Jaunpur, India. He has visited several European countries and participated in several international conferences. He has published more than 50 research articles in scientific journals (h-index 27, citation 7187) and also published three edited books with Springer and CRC Press. He is a member of several scientific communities and serving as Editorial board member for a number of journals besides being a reviewer. His research interests are focused on sustainable agriculture, waste management through agriculture, the utilization of industrial, agricultural and household waste for eco-friendly energy production, renewable energy and their life cycle assessment (LCA). anoop.s@nic.in

Shaili Srivastava is an Associate Professor in the Amity School of Earth and Environmental Sciences, Amity University Haryana. Dr Srivastava has done masters and Ph.D. from G.B. Pant University of Agriculture and Technology with specialization in Microbiology and Biotechnology. Before joining Amity, she worked as a lecturer in college and a postdoctoral fellow in Jawaharlal Nehru University, New Delhi. Her research focuses on waste water treatment, degradation of PAH, remediation of heavy metal, microbial sequestration of CO2, biofuel and biosurfactant production, genomics and proteomics involve in CO2 sequestration and value added product formed by microorganisms. She had visited Murdoch University, Perth, Australia, as a visiting research fellow and attended various international conferences as a speaker. Dr Shaili had published twenty-eight research papers in reputed refereed international journals. She had also published one book and 8 book chapters. She is a life member of various scientific communities and a reviewer of various international journals. She had completed three research projects sponsored from CSIR, UGC, and DST. She had eighteen years of research and teaching experience. She had a Google Scholar profile of h-index 15 and total citations are 1300. ssnigam@ggn.amity.edu

Dheeraj Rathore is an Assistant Professor in the School of Environment and Sustainable Development at the Central University of Gujarat (CUG), Gandhinagar, India. He obtained his Ph.D. in Botany from AAI-DU, Allahabad, India and his M.Phil. in Environment Management from Vikram University, Ujjain, India. Before Joining CUG, he worked at The University of Dodoma, Tanzania. He has 2 books and 40 peer-reviewed articles (h-index 13) to his credit. He is a member of several scientific communities besides being a reviewer. His research interest focused on ecology, environmental physiology, biofuel production, and waste utilization. dheeraj.rathore@cug.ac.in

Deepak Pant is a Senior Scientist at the Flemish Institute for Technological Research (VITO), Belgium. His research focuses on electrosynthesis and bioenergy, specifically, combining electrochemical and biological conversion systems with a focus on CO2 conversion and their combination with separation systems. He has 5 books (on Springer, CRC Press and Elsevier), 6 Patents, 130 peer-reviewed publications with 9900 citations (h-index 56) and 30 book chapters to his credit. He is the member of scientific communities like ISMET, ISE, BES, BRSI, IFIBiop and AMI. He is serving as Editor of one scientific journal and is on the Editorial Board of 6 other journals.

Contributors

Madhoolika Agrawal Laboratory of Air Pollution and Global Climate Change, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

S. B. Agrawal Laboratory of Air Pollution and Global Climate Change, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

Nabin Aryal Biological and Chemical Engineering, Aarhus University, Aarhus N, Denmark

Somvir Bajar Department of Environmental Sciences, J.C. Bose University of Science and Technology, Faridabad, Haryana, India

Suman Bajracharya Biochemical Process Engineering, Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, Luleå, Sweden

Ashutosh Kumar Choudhary Himalayan School of Science and Technology, Swami Rama Himalayan University, Dehradun, Uttarakhand, India

Jairam Choudhary ICAR-Indian Institute of Farming Systems Research, Meerut, Uttar Pradesh, India

Sumistha Das Amity Institute of Biotechnology, Amity University Haryana, Gurgaon, India

Nitai Debnath Amity Institute of Biotechnology, Amity University Haryana, Gurgaon, India

Tenzin Dolker Department of Environmental Sciences, Central University of Himachal Pradesh, Dharamshala, Himachal Pradesh, India

Ahmed ElMekawy Genetic Engineering and Biotechnology Research Institute, University of Sadat City (USC), Sadat City, Egypt

Pooja Ghosh Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi, India

Milena Ginic-Markovic Future Industries Institute, University of South Australia, Adelaide, SA, Australia

Hanaa M. Hegab Department of Chemical Engineering, Monash University, Clayton, VIC, Australia

Bhavna Jaiswal Laboratory of Air Pollution and Global Climate Change, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

Tanveer Khan Centro de Tecnologia, Canavieira, Piracicaba, SP, Brazil

Suman Kharel Environmental Chemistry and Toxicology, Aarhus University, Roskilde, Denmark

Nicholas E. Korres Global Change Photosynthesis Research, USDA-ARS, Urbana, IL, USA

Manish Kumar Department of Microbiology, Central University of Rajasthan, Ajmer, Rajasthan, India

Parveen Kumar School of Basic and Applied Sciences, G. D. Goenka University, Gurugram, Haryana, India

S. Senthil Kumar Geobiotechnology Laboratory, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India

PG and Research Department of Botany, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India

Sandeep Kumar Centre for Environmental Sciences and Climate Resilient Agriculture, ICAR-Indian Agricultural Research Institute, New Delhi, India

Deepak Pant Separation and Conversion Technology, Flemish Institute for Technological Research (VITO), Mol, Belgium

Deepak Pant Department of Chemical Sciences, Central University of Haryana, Mahendergarh, Haryana, India

Nidhi Pareek Department of Microbiology, Central University of Rajasthan, Ajmer, Rajasthan, India

Shiv Prasad Centre for Environmental Sciences and Climate Resilient Agriculture, ICAR-Indian Agricultural Research Institute, New Delhi, India

K. Preeti Department of Energy and Environment, TERI SAS, TERI University, New Delhi, India

Ranwen Qu Department of Chemical Engineering, Monash University, Clayton, VIC, Australia

M. K. Ramkumar Geobiotechnology Laboratory, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India

National Institute for Plant Biotechnology, New Delhi, India

Dheeraj Rathore School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

P. S. Renjith ICAR-Central Arid Zone Research Institute (CAZRI), Regional Research Station, Bikaner, Rajasthan, India

Shivali Sahota Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi, India

Christopher P. Saint Future Industries Institute, University of South Australia, Adelaide, SA, Australia

Deepak Sehgal ESALQ, University of Sao Paulo, Piracicaba, SP, Brazil

Goldy Shah Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi, India

Maulin P. Shah Industrial Waste Water Research Lab, Division of Applied & Environmental Microbiology Lab Enviro Technology Limited, Ankleshwar, Gujarat, India

Shalik Ram Sharma Section for Sustainable Biotechnology, Department of Chemistry and Bioscience, Aalborg University, Copenhagen, Denmark

K. R. Sheetal ICAR-Central Arid Zone Research Institute (CAZRI), Regional Research Station, Bikaner, Rajasthan, India

Mohd Arshad Siddiqui School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

Anita Singh Department of Environmental Sciences, Central University of Jammu, Jammu, Jammu and Kashmir, India

Anoop Singh Department of Scientific and Industrial Research (DSIR), Ministry of Science and Technology, Government of India, New Delhi, Delhi, India

Dhruv Singh Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi, India

Pratibha Singh Department of Chemistry, JSS Academy of Technical Education, Noida, Uttar Pradesh, India

Suruchi Singh Laboratory of Air Pollution and Global Climate Change, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

Jayesh M. Sonawane Department of Chemical Engineering and Applied Chemistry, Centre for Global Engineering, University of Toronto, Toronto, ON, Canada

Shaili Srivastava Amity School of Earth and Environmental Sciences, Amity University Haryana, Gurugram, Haryana, India

K. Varuna PG and Research Department of Biotechnology and Microbiology, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India

Roli Verma Department of Chemistry, JSS Academy of Technical Education, Noida, Uttar Pradesh, India

Vivekanand Vivekanand Centre for Energy and Environment, Malaviya National Institute of Technology, Jaipur, Rajasthan, India

R. Hiranmai Yadav School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

Linda Zou Natural and Built Environments Research Centre, University of South Australia, Adelaide, SA, Australia

Department of Chemical and Environmental Engineering, Masdar Institute of Science and Technology, Abu Dhabi, United Arab Emirates

Perspectives of Environmental Microbiology and Biotechnology

1

Anoop Singh, Dheeraj Rathore, Deepak Pant, and Shaili Srivastava

Abstract

Microbes are the integral parts of the nature and played an important role in natural recycling of materials. Various natural, synthetic materials and xenobiotics are being added in the environment due to developmental activity, industrial growth, and modern life style. These materials are complex and recalcitrant in nature and cause serious threat to the environmental sustainability. Recent advancement of research in microbiology and molecular tools showed positive response to reduce the environmental problem and suggested that the microbes can play an important role to resolve environmental problem. The waste generated during the production process arise the new synthetic materials which exert higher pressure on the microorganisms. This may cause natural evolution and genetic changes in environmental microbes. In view of above current scenario is to identify potential microbes from the contaminated environment and use of genetically engineering to improve the efficiency of microbes for production and degradation process.

A. Singh
Department of Scientific and Industrial Research (DSIR), Ministry of Science and Technology, Government of India, New Delhi, Delhi, India

D. Rathore
School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

D. Pant
Separation and Conversion Technology, Flemish Institute for Technological Research (VITO), Mol, Belgium
e-mail: deepak.pant@vito.be

S. Srivastava (✉)
Amity School of Earth and Environmental Sciences, Amity University Haryana, Gurugram, Haryana, India

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1.1 Introduction

Microbes are integral part of the nature and essential for maintaining the ecological nutrient balance. They play important role not only in the natural production process but also for the degradation and cleaning of its own product and also other product. The use of microbes for the benefit of human being is not new; however, the use of microbial process or product has increased since the industrial revolution for fulfilling the need of increasing global population. Industrial revolution not only benefited the human race but also developed several problems for the environment. Changing life style and development of several human supporting complex products exert a potential pressure on the nature and natural degradation process. Development of new complex products for human use does not have natural cleaners. Therefore, identifying new microbes and developing its process or product is inevitable and need of the hour. Danso et al. (2019) support this view and argue the requirement of novel biocatalytic product or organism that allows rapid degradation, recycling, or converts waste into value added product.

Air, water, and soil pollution due to rapid industrialization, urban proliferation, and other human inputs posed a threat to human population and ecological balance. Environmental microbiology and biotechnology deals with application of microbes or its modified strains, process, and product for the benefit of nature and natural processes (Singh et al. 2016b; Krzmarzick et al. 2018; Danso et al. 2019). Microbial processes and biotechnology offer several promising techniques and approaches enable to resolve these problems in many countries (Singh et al. 2016b; Krzmarzick et al. 2018; Bodor et al. 2020). Microbes are the fundamental organism for development of biotechnology (Singh et al. 2016b); however, 99% of these potential resources are not known (Locey and Lennon 2016) mainly due to limited microbial availability by cultivation ((Epstein 2013). Scientific approaches and biotechnological tools such as denaturing and temperature gradient gel electrophoresis (DGGE/TGGE), terminal restriction fragment length polymorphism (T-RFLP), 16S rDNA clone library preparation, fatty acid methyl esters (FAME) analysis, flow cytometry (FCM) and next generation sequencing technology (Cavigelli et al. 1995; Marzorati et al. 2008; Smets et al. 2016; Coggins et al. 2020) of culture free screening and isolation processes of various microbes need to be explored for their specific utilization and to solve the environmental problems.

The present chapter is an overview of the microbial and biotechnological approaches for the benefit of environmental health, and the associated socio-economic aspect and risk involved.

1.2 Waste Management

In recent year, rapid increase of population has caused exponential increase in waste generation (Rastogi et al. 2020). Waste was generated from various sources including industrial, domestic, and agriculture (Asefi et al. 2019; Symonds et al. 2019). Drzyzga and Prieto (2019) reported that, globally about 335 million ton of plastic

waste was generated in 2016 caused significant damage to ecology and economy. Similarly, China annually generated 600 million ton of agricultural waste (MOA 2011). Combustion and incineration is the commonly employed method for solid waste management, while, specific effluent treatment plants are used for liquid waste.

Waste is considered as the burden on the earth and generated lots of problem for the civilization and environment. However, the waste is potential resource and can be converted into value added products using microorganism. Composting is the most employed method for conversion of agricultural waste into environment friendly soil conditioner, fertilizer, and remediation agent using microbes (Zhang et al. 2015). Similarly, conversion of agricultural or other organic waste including domestic and organic fraction of industrial waste can be converted into energy rich gaseous (biohydrogen, biogas, Singh et al. 2016a; Miandad et al. 2017; Prasad et al. 2017), or liquid (bioethanol, biodiesel, Rathore et al. 2016, 2019) fuel product and bioelectricity (Moqsud et al. 2015), through a set of microbial processing, and provide organic manure as by-product (Prasad et al. 2017), which improve soil health (Prasad et al. 2020).

However, municipal and industrial wastes are heterogeneous in nature and included plastic and other toxic organic and inorganic substances. Microorganism and their product are also used for such waste. Annadurai et al. (2002) has demonstrated significant degradation of phenol, a commonly found pollutant in industrial effluent, using mixed liquors of *Pseudomonas putida* and activated sludge. Singh et al. (2018) explained that soil micronutrient availability can be increased by adding industrial effluent using biosurfactant, a microbial product. Singh and Rathore (2019) demonstrated that the lipopeptide biosurfactant could be an effective biological tool to reduce the toxic effect of azulene (a dye compound) and chromium (a heavy metal) in soil, thus maintaining soil health and sustainable agriculture. Drzyzga and Prieto (2019) discuss the role of microbes and engineered microbial enzymes for plastic degradation. While, Yoshida et al. (2016) isolated novel bacteria *Ideonella sakaiensis* which can successfully utilize polyethylene terephthalate (PET) as carbon source.

The role of various microbes and biotechnological approach in waste management is elaborated in various sections of this book.

1.3 Bioremediation

The industrialization and developmental activity cause releasing enormous amounts of chemical and hazardous material in our environment. Most of the chemicals are xenobiotics and recalcitrant in nature. Bioremediation method is a useful and sustainable way of management of environmental pollutants and xenobiotics compound. In bioremediation technology toxic, recalcitrant, man-made pollutants can be degraded by using living microorganisms, algae, fungi, and plants, or by their enzymes.

Bioremediation technology can be classified into in situ and ex situ. In situ technology involves treatment of waste material or contaminants at the site while

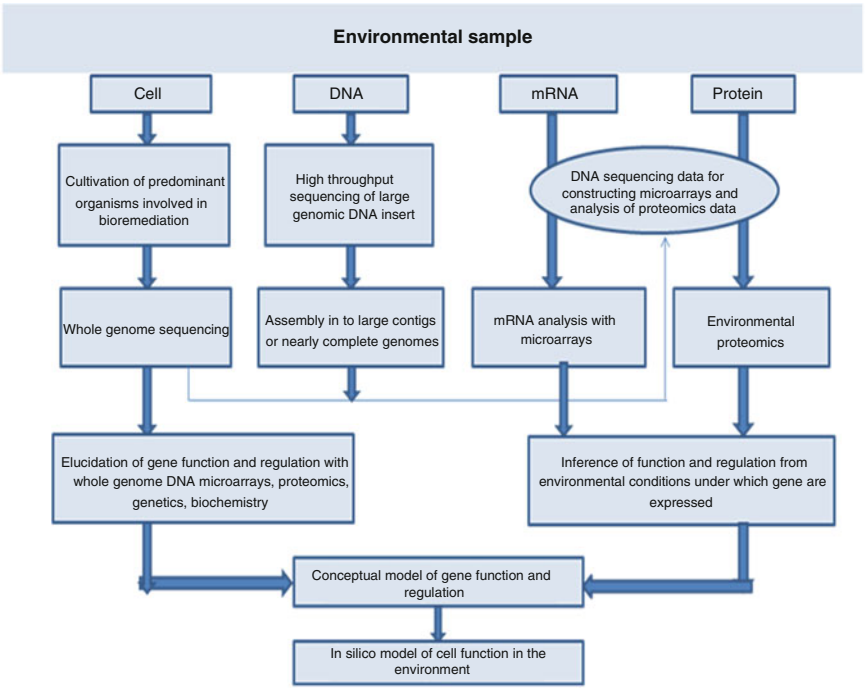

Fig. 1.1 Genome-enabled techniques contribute to the development of models of how microorganisms function in contaminated environments. (Adapted from: Derek R. Lovley 2003 Nature Reviews)

ex situ involves remediation of contaminated material or waste to be treated elsewhere. A bioremediation technology includes bioventing, land farming bioreactor, bioaugmentation, rhizofiltration, and biostimulation. The remediation or removal of toxic heavy metal is only possible by biosorption or bioaccumulation of heavy metals on living cell surface or accumulation or sorption of metals inside the active or passive cells. Phytoremediation is also possible for heavy metals (Srivastava 2012).

The goal of bioremediation is to stimulate living microorganisms with nutrients and other chemicals that will enhance them to degrade the contaminants. The bioremediation systems rely on microorganisms native to the contaminated sites, enhancing living cell potency by supplying with the optimum levels of nutrients and other chemicals essential for microbes metabolism. Researchers are investigating ways to augment polluted sites with non-native microbes including genetically engineered microorganisms specially suited to degrading the contaminants of concern at particular sites (Bodor et al. 2020). Application of genomics to bioremediation is the important tool for cleanup of contaminant site. The key features for application of genomics are study of pure culture, environmental samples, and availability of whole genome sequences in gene database for several microbes (Fig. 1.1).

1.4 Xenobiotics

Xenobiotic compounds are man-made chemicals that persist in environment at very high concentrations. The xenobiotic compounds are not produced naturally. Xenobiotic compound produced at very low concentration naturally as compare to man made. Microorganism has the capability of degrading all naturally occurring compounds.

Microorganisms are also able to degrade many of the xenobiotic compounds, but many compounds are refractile to biodegradation. The compounds that resist biodegradation and persist in the environment very long time are called recalcitrant (Godheja et al. 2016). The xenobiotic compounds are recalcitrant due to following reasons: (1) They are not recognized as substrate by the existing degradative enzymes, (2) They are highly stable and inert due to the presence of substitution groups like halogens, nitro-, sulfonate, amino-, methoxy-, and carbamyl groups, (3) They are not easily soluble in water, or are adsorbed to external matrices like soil, (4) They are highly toxic and produce toxic by-products or cometabolites due to microbial activity, (5) Their large molecular size prevents entry into microbial cells, and (6) Inability of the compounds to induce the synthesis of degrading enzymes.

The recalcitrant xenobiotic compounds can be grouped into the following types: (1) Halocarbons, (2) Polychlorinated biphenyls, (3) Synthetic polymers, (4) Alkyl benzyl sulfonates, (5) oil mixtures.

The structural features like presence of halogens in the place of hydrogen make these compounds resistant to microbial degradation. The carbon–halogen bond is highly stable and its cleavage requires energy. Xenobiotics are often refractory to biodegradation. The main cause is they cannot be recognized by naturally present organisms and therefore do not enter common metabolic pathways. The physical and chemical nature of the xenobiotics, as well as environmental factors, may influence their biodegradability. Some compounds may be transformed only in the presence of another compound which appears as a carbon and energy source. Better understanding of metabolic pathways for the biodegradation of specific organic compounds as well as thorough knowledge of degrading microorganisms will make purposeful application of biodegradation (Rozgaj 1994).

The main degraders in nature are microorganisms, which include mostly bacteria and some fungi. These organisms have nature of rapid rates of multiplication and high metabolic potential which make microorganism to adapt to new substrates. Selection of degradative potent microorganisms and their successive adaptation to a naturally persistent compound might be a powerful means for environmental detoxification. There are many xenobiotic compounds which are reported in literatures for microbial degradation. The mechanisms of microbial degradation of xenobiotics are due to some enzymes present in bacteria and fungi. The cometabolism mechanism for xenobiotics is also feasible for degradation of such molecules. The principal role of enzymes that helps in bioconversion of xenobiotics such as lyases and oxidoreductases, specifically hydrolases, oxygenases and various dehalogenation enzymes.

Some white rot fungi such as *Penicillium* sp. and *Arthrobacter* sp. reported for degradation of xenobiotics. They have enzymes such as lignin peroxidase (LiP),

manganese peroxidase (MnP), versatile peroxidase (VP), and laccase which help in mineralization of xenobiotics (Lamar 1992; Srivastava and Kumar 2019). Bacteria such as *Acinetobacter*, *E. Coli*, *Veillonella alkalescens*, *Arthrobacter*, *Rhodococcus*, *Flavobacterium*, and *Burkholderia*, *Pseudomonas* have reported for enzymes which are responsible for degradation of xenobiotics compounds. Bacterial dehalogenases catalyze the cleavage of carbon–halogen bonds, which is a key step in aerobic mineralization pathways of many halogenated compounds that occur as environmental pollutants (Janssen et al. 2005).

1.5 Health and Hygiene

A healthy life is a dream of each individual. World Health Organization (WHO) declared April 7 as World Health Day. This is celebrated with the goal of creating awareness among each individual. For being healthy, we need to know the actual meaning of health and hygiene. According to WHO, health is a complete state of physical, mental, and social well-being, and not merely the absence of disease or infirmity. As per the definition, a person cannot be said to be healthy merely by a disease-free condition. He/she should be physically and mentally healthy. This demands a hygienic condition. Health and hygiene are two correlated terms. Environment directly influences the health of human being. Hygiene can be defined as the practice of certain habits to maintain a good health. It can be at the personal level (personal hygiene) and at the community level (social hygiene).

Environmental health is the science of practice for preventing human being from illness by identifying environmental sources and hazardous agents. The main objective of environmental health is limiting the exposure of hazardous physical, chemical, and biological agents' exposure to air, water, soil, and other environmental components that may cause adverse health effect on human being. The environment in which, we live is a major determinant of our health and well-being. We depend on the environment for energy and the materials needed to sustain life, such as clean air and water, safe nutritive food, and safe place to live (Resnik and Portier 2008).

Environmental exposure such as natural and manmade both impact human health in many aspects. Environmental hazard may increase the diseases like cancer, asthma, bronchitis, and heart disease. It is important that we interpret health issues in the wider context of our environment, where we live (Barton and Grant 2006) (Fig. 1.2).

1.6 Biological Control

Biological pest controls are increasingly used for improving plant disease control for sustainable agriculture. Plant pathogens cause the loss of 10–20% of agricultural production worldwide each year. This is despite controlling them with synthetic chemicals costing several billion dollars. With time, there is an increasing awareness about integrated pest management (IPM) (or bio-based pest management) strategies

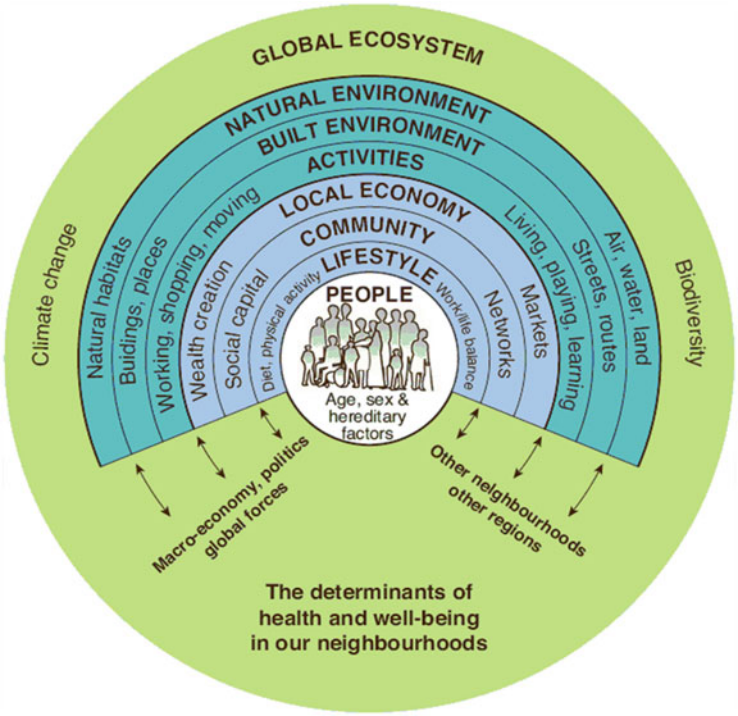

Fig. 1.2 Determinants of health and well-being in our neighborhoods (Adapted from Barton and Grant 2006)

which provide environmentally sustainable and economically viable alternatives for traditional agriculture. As a result, biocontrol of plant pathogens has become an integral components of IPM approach. In recent years, there has been a renewed interest in biological control based on application of populations of antagonistic microorganisms. Fungi, nematodes, and oomycete pathogens of field, nursery, and glasshouse crops have been the primary targets of such control strategies.

According to Eilenberg (2006), "biological control or biocontrol is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be." The main difference between biological control and other control methods is the use of living populations of beneficial organisms, having several modes of action and thus avoiding the risk of rapid resistance development in the target population. Despite several products currently being available on the market, the sales of such products represent only a small fraction of the total pesticides sold for controlling plant pathogens. In general, biocontrol approaches are based on two main methods: classical biocontrol which involves introduction of exotic enemies against either exotic or native pests and conservation biocontrol involving protection and

enhancement of biocontrol agents already present in the agroecosystem (Escobar-Ramírez et al. 2019).

The biocontrol strains described further come from microbial genera that are both well studied and widely distributed in nature. Biological control agents (BCAs) are most often used as epiphytic colonists, but may also colonize internal tissues. In every approach whether via competitive exclusion, biochemical antagonism, or induction of host defenses, BCAs must be well adapted for survival and functional activity in the phytosphere (Siddiqui 2006).

Biological control is a critical focus area within the discipline of Plant Pathology and in recent years has emerged as a major research discipline for both basic and/or applied research. This increased awareness and research efforts have led to the publication of thousands of articles in scientific literature during the past decades. There is increasing interest among conventional and organic producers in developing effective biorational controls. Due to the development of new diseases and the shifting trends of management and regulation, much research still focuses on the isolation and testing of new biocontrol agents (OARDC 2006).

Some of the most common biocontrol agents are discussed here some of which has already reached a commercialized state. *Bacillus cereus* strain UW85 is an effective biocontrol agent of damping-off and root-rot diseases of soybeans and alfalfa under diverse field conditions. This species is quite common in the field soils (OARDC 2006). *B. cereus* is a member of the "*Bacillus cereus* group," comprising of *B. cereus*, *B. thuringiensis*, and *B. anthracis*. (Ash et al. 1991; Kazmar et al. 2000; Osburn et al. 1995; Stabb et al. 1994).

Another ubiquitous bacterial species found in agricultural systems is *Bacillus subtilis*. These bacteria can be readily cultured from soils, food stuffs, plant and animal tissues and they are known to be genotypically and phenotypically diverse. Numerous strains of this species have been shown to express activities that suppress plant pathogens and promote plant growth. Several *Bacillus*-containing products are available to growers, who are interested in using biological seed treatments or drench applications as part of their integrated pest management programs. While the utility of these products has been validated in a number of studies, not much is known about the genetic basis for their beneficial effects on plant health. Mechanistic descriptions have been offered including direct antagonism of pathogens (i.e. through antibiosis and competition for resources) and indirect stimulation of plant growth (i.e. by promoting nodulation and secreting growth factors). All of these activities have been observed with applications of *B. subtilis* GB03. This strain is the active ingredient in one of the most widely distributed biofungicides (Kodiak, Gustafson LLC) which is applied in millions of acres of cotton and other field crops every year. The commercial success of this strain is a testament to the phenotypic characteristics of the strain that make it a successful biological control agent. The strain is known to effectively colonize plant roots, produce antifungal compounds, and secrete volatile substances that can directly stimulate plant growth. (Brannen and Kenney 1997; Kokalis-Burelle et al. 2003; Mahaffee and Backman 1993; Ryu et al. 2003; Wipat and Harwood 1999).

The *Burkholderia cepacia* complex comprises a group of closely associated, gram-negative, non-fluorescent bacteria including strains beneficial for biological control and bioremediation, besides having strains that are plant pathogens or opportunistic pathogens of humans with cystic fibrosis. (Parke and Gurian-Sherman 2001).

Pseudomonas fluorescens is a common colonist of the phytosphere. Elaborate studies both in lab and field have shown this strain to provide excellent biocontrol of fire blight of pear and apple, as well as frost damage to a variety of plant species. Thus *P. fluorescens* strain A506 has been registered by the EPA as a biological pesticide and is commercially available as a freeze-dried preparation. Blightban A506® is among the most successful and widely used biological control agents, being used on over 25% of pear and apple acreage in the USA (Lindow et al. 1996; Wilson and Lindow 1993; Stiner and Halverson 2002; Lowder et al. 2000).

Trichoderma spp. are highly diverse and ecologically successful fungi. Members of the genera have been known for a long time to act as biocontrol agents of plant pathogens. Of late, these fungi have been used in quite significant amounts in commercial agriculture (e.g. about 30% of the total soil fungicide used in the greenhouse industry in the USA are products based on *T. harzianum* strain T22) (Harman 2000; Yedidia et al. 1999, 2000).

1.7 Biofertilizer

Biofertilizers are defined as a product containing carrier based (solid or liquid) living microorganisms, which are agriculturally useful in terms of nitrogen fixation, phosphorus solubilization, or nutrient mobilization, so as to increase the productivity of the soil and/or the crop (The Gazette of India: Extraordinary 2006). These broadly include the nitrogen fixers (symbiotic and non-symbiotic bacteria), phosphate solubilizing fungi and bacteria, and the mycorrhizal fungi that are capable of mobilizing nonlabile nutrients from soil and transporting them to and across plant roots. A recent addition is the plant growth promoting rhizobacteria (PGPR), specifically the fluorescent Pseudomonads, which stimulate plant growth and repress root diseases by a variety of mechanisms (Adholeya and Pant 2007).

One of the most common types of biofertilizers is mycorrhizae, which literally means “fungus-roots” and defines the close mutually beneficial relationship between specialized soil fungi (Mycorrhizal fungi) and plant roots. It was first applied to fungus-tree associations described in 1885 by the German forest pathologist A.B. Frank (Frank 2005). About 95% of the world’s land plants form the mycorrhizal relationship in their native habitats. It is estimated that mycorrhizal fungal filaments explore hundreds to thousands more soil volume compared to roots alone. These specialized fungi colonize plant roots and extend far into the soil resource. Mycorrhizal fungal filaments in the soil are extensions of root systems and more effective in nutrient and water absorption than the root themselves. Mycorrhizae are important in structuring plant communities and in determining the rate of succession. In general, the use of mycorrhizal fertilizer helps in improved

nutrient and water uptake, root growth, plant growth and yield, disease resistance, reduced transplant shock, and drought stress. In today's man-made environments, plants can be greatly stressed and the relationship between fungus and root is critical. Many nursery and agricultural soils lack mycorrhizae due to excessive and long-term use of chemical fertilizers and pesticides.

Plant establishment and growth benefits greatly enhanced by associating with mycorrhizal fungi and these are involved with a wide range of activities. For instance, the extensive network of fungal filaments is important in water uptake and storage. Under non-irrigated conditions, mycorrhizal plants are grow well under much lower drought stress as compared to non-mycorrhizal plants. Disease and pathogen suppression is another benefit for a mycorrhizal plant. Mycorrhizal fungi attack pathogen or disease organisms entering the root zone and also improve the soil structure. The mycorrhizal filaments produce humid compounds and organic "glues" (extra cellular polysaccharides) which bind soils into aggregates and improve soil porosity. Soil porosity and soil structure positively influence the growth of plants by promoting aeration, water movement into soil, root growth, and distribution. In sandy or compact soils the ability of mycorrhizal fungi to promote the improvement of soil structure is as important as seeking out nutrients.

The most common is Arbuscular (named for internal structures called arbuscles) or Vesicular-Arbuscular (arbuscles and another structure called vesicles), abbreviated as AM and is the most widespread of the mycorrhizae. These fungi actually reside inside the cells of the plant roots. This is an endomycorrhiza, which means the fungus enters the cells of the roots. AM are found on grasses, most crop plants, many trees, shrubs, flowers, and in fact about 80% or so of the world's plant species.

Mycorrhizal fungi interact with a wide assortment of organisms in the rhizosphere resulting in positive, neutral, or negative on the Mycorrhizal association or a particular component of the rhizosphere. The interaction between rhizobia and AM fungi has received considerable attention because of the relatively high phosphorus demand of nitrogen fixation. The two symbioses typically act synergistically, resulting in greater nitrogen and phosphorus content in combination than when each is inoculated onto the legume alone. Legumes are typically coarse-rooted and therefore inefficient in extracting phosphorus from the soil. The AM fungi associated with legumes are an essential link for adequate phosphorus nutrition, leading to enhanced nitrogenase activity that in turn promotes root and Mycorrhizal growth (Siddiqui 2006).

Apart from mycorrhiza-based biofertilizers, there are several other types which are used in sustainable agriculture currently. Some of these are symbiotic nitrogen fixing biofertilizers (such as *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium, Sinorhizobium,* and *Rhizobium),* Free living nitrogen fixing biofertilizers (such as *Azotobacter and Cyanobacteria*), Associative symbiotic nitrogen fixing biofertilizers (such as *Azospirillum*), Phosphorus solubilizing microbes (such as various species of *Bacillus* and *Pseudomonas*), and Potassium solubilizing microbes (such as *Aminobacter*, *Acidithiobacillus ferrooxidans*) (Kour et al. 2019) (Table 1.1).

Table 1.1 Different types of microorganisms involved in soil productivity enhancement (Uzoh and Babalola 2018)

Factor of productivity	Microorganisms involved	References
Growth hormones	*Pseudomonas spp., Enterobacter sakazakii,* and *Klebsiella oxytoca*	Compant et al. (2010)
Biocontrol	*Mycorrhizae, Rhizobium spp., Bacillus, Pseudomonas spp., Enterobacter sakazakii,* and *Klebsiella oxytoca*	Liu et al. (2007), Nasrin and Rahman (2007), Yuttavanichakul et al. (2012)
Adaptation to drought/increased water uptake	Mycorrhiza	Selosse et al. (2007)

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The success of microbiological control requires a sufficient understanding of the modes of action of the antagonist and also of its interactions with the plant, the pathogen, and the rest of the microbiota. It takes time to achieve such understanding, and most of the BCAs already on the market were studied for >20 year before registration. However, the use of registered BCAs is limited, mostly because of the lack of efficacy and consistency of biological control (Eilenberg 2006).

1.8 Socio-Economic Relevance

Biotechnology and bioindustries are becoming an integral part of the knowledge-based economy, as they are closely associated with progress in the life sciences and in applied sciences and technologies linked to them (Chekol and Gebreyohannes 2018). Microbial products and biotechnology involved in process can achieve the sustainable development goal for food security and healthy world (Lokko et al. 2017, Singh et al. 2017). However, it also raised ethical issues (Lokko et al. 2017). Public concern and acceptability of genetically modified products is still need to be discussed and clarified. Criteria of Cartagena protocol consider socio-economic issue as one of the measure for decision-making process for biosafety (Ratledge and Kristiansen 2006). The risks associated with microbiomes range from regulatory (e.g. screening stool for fecal microbiota transplants (FMT)) to social justice concerns (e.g. ownership related to human microbiomes), and public perceptions (Yeo et al., 2019). Lokko et al. (2017) advocated the use of biotechnology in manufacturing and process chain for economical stability of developing countries. They also suggest to include ethical, environmental, economic, legal and social implications alongside of product and resource biotechnology.

1.9 Risk Assessment

Technology development has made revolutionary changes for existing technologies and created several opportunities for the policy maker and product developer. However, application of such technology can simultaneously create new risk to the environment and society (Trump et al. 2018). Risk can be expressed as the uncertainty in expected outcome. Beside the several benefits of microbial and biotechnological approaches for environmental benefit, application of microbes or its products, or process also involve risk not only for human health but also for ecological resources or social welfare (Beacham et al. 2017; Vallero, 2010). Risks involved the changes in ecological structure and invasion, and entry of harmful toxin into the society (Campbell, 2011). Barzkar et al. (2018) discuss the risk associated with the microbial protease enzyme and suggested to consider safety profile before industrial application. Many of the risks to human health and the environment associated with GM will be specific to the types of traits and genes selected and the type of modifications performed (Beacham et al. 2017). Kaushik et al. (2019) reported the risk associated with the antibiotic resistant gene through horizontal gene transfer a evolutionary and adaptive bacterial mechanism can develop a microbe with multidrug resistant and become a threat to human survival.

Quantitative microbial risk assessment (QMRA) includes hazard identification, exposure assessment, hazard characterization, and risk characterization (Codex, 1999) and is widely used as a tool to describe the microbial risk (Nauta, 2000). Risk assessment model of Bromfeild et al. (2018) and life cycle assessment (LCA) could also be used as a useful tool to assess the risk associated with new traits in biocontrol. Trump et al. (2018) concludes that the risk assessment and governance will continue to refine and develop in the coming years; a quantitative framework that builds from existing practice is one potentially beneficial option for risk assessors that must contend with the technology's limited hazard characterization or exposure assessment considerations in the near term.

1.10 Conclusion

Microbes are basic and key component of the nature. Microbes play an important role for the degradation, decomposition, and recycling of the material. Natural or genetically modified microbes are proven to be successful for the pollution abatement. Researchers has identified or developed potential microbes for waste management including xenobiotics, health and hygiene, agricultural sustainability, and biotic control. Socio-economic benefit of environmental microbiology and biotechnology was established for useful microbial products and management of sustainable environment. The chapter concluded that microorganisms are the potential solution for environmental pollution, although risk may be involved in the process and should be checked before field application.

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Part I

Solid Waste Management

Towards Waste Valorization: A Promising and Sustainable Approach of Waste Management

2

Goldy Shah, Dhruv Singh, Shivali Sahota, and Pooja Ghosh

Abstract

Increasing urbanization and waste generation along with rapidly depleting fossil fuel reserves and the need for energy security are some of the major global issues the world is facing today. This has led to growing interest in transforming wastes to energy and other value-added products because of its potential of solving the dual issues of waste management as well as energy security. Thus, waste is now being looked upon as a raw material for production of numerous value-added products having the potential to supplement the petroleum feedstock to a large extent, provided it is properly utilized. The book chapter deals with providing an overview of waste as a resource for production of different energy sources—Bioethanol, Biogas and Biohydrogen. It gives an insight into the future when fossil-based economy will be replaced with a sustainable waste-derived circular economy where energy and value-added products will be recovered from discarded materials.

2.1 Introduction

The major challenges that the world is facing today includes energy security, waste management and climate change. According to the estimates, around 7–10 billion tonnes of wastes were generated worldwide in 2010 (Wilson et al. 2015). Though half of this waste was generated by the developed countries of the world, but it is in the developing countries particularly that improper waste management is leading to health and environmental hazards. In developing countries like India, most of the waste is dumped in the un-engineered landfill sites leading to environmental pollution and effects on human health (Ghosh et al. 2014). Hence, there is an urgent need

G. Shah · D. Singh · S. Sahota · P. Ghosh (✉)
Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi, India

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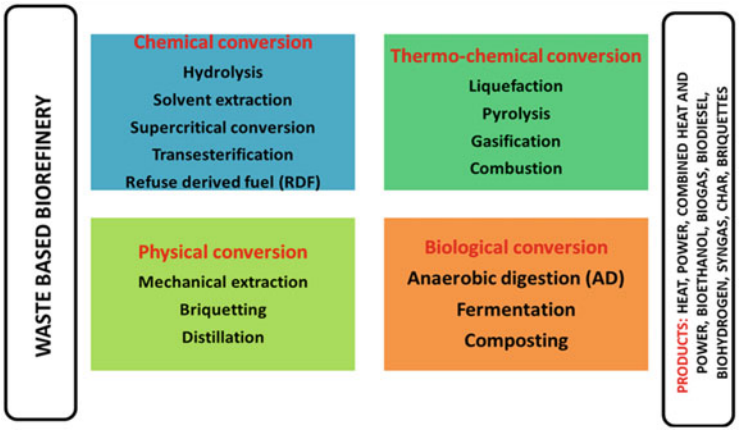

Fig. 2.1 Conversion strategies for waste to energy and other value-added products

to follow a sustainable waste management strategy involving utilization of the waste as a raw material for the production of numerous value-added products (Allesch and Brunner 2014).

It has been predicted that the world fossil fuel reserves (oil and gas) will get exhausted in the next 20–30 years due to the increasing utilization of non-renewable fuels (Shafiee and Topal 2009). Also, there is an increasing concern about emission of greenhouse gases (GHGs) due to burning of fossil fuels leading to the problem of climate change. Developing waste-derived economy can help in addressing all the problems of waste management, energy security and environmental pollution simultaneously. If properly utilized, the enormous amounts of wastes available can even replace the fossil resources. For doing this, different technological tools are increasingly being used for recovering value-added products from wastes and the concept of sustainability and circular economy is gaining attention.

Waste biorefinery includes different technologies which can be broadly classified into thermo-chemical, physicochemical and biochemical processes (Fig. 2.1). Chemical conversion technologies include processes such as hydrolysis, solvent extraction, transesterification which involve the use of chemicals to convert feedstocks into liquid fuels and bioproducts. Thermo-chemical technologies include pyrolysis, gasification and incineration and involve use of high temperatures to convert waste into fuels, electricity, heat and other value-added products. In physical conversion of waste, the waste is mechanically processed to produce a more usable and durable forms like the pellets and briquettes. Biological technologies include processes such as anaerobic digestion (AD), fermentation and composting which make use of biological agents to transform different types of feedstocks into fuels and bioproducts (Nizami et al. 2017).

This book chapter aims to provide an insight into the potential of wastes as a resource to be used for production of different energy sources—Bioethanol, Biogas and Biohydrogen (Fig. 2.2) to solve the issues of energy security, waste management and climate change in an effective way.

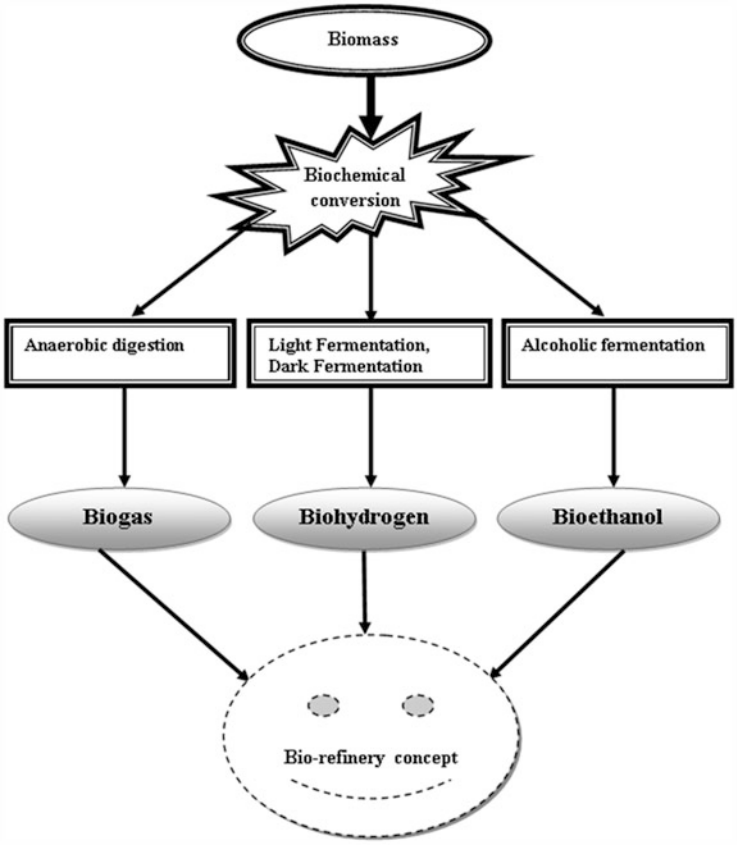

Fig. 2.2 Different strategies of waste to energy conversion

2.2 Potential of Waste as a Resource

With the generation of hundreds of megatonnes of waste across the globe, waste has an enormous potential as a resource. Lignocellulosic residue constitutes as the largest available waste (181.5 billion tonnes) source globally (Kumar et al. 2008). It includes agricultural residues (crop residues), wood and grass which are promising sources of alternative energy (Hu and Ragauskas 2012).

Amongst the lignocellulosic residues, the two residues which are produced in maximum amounts are rice husk and sugarcane bagasse. It has been estimated that for every 4 tonne of rice that is harvested, 1 tonne of rice husk is produced which adds up to 120 metric tonnes of rice husk generated per year, of which only 20 metric tonnes is used in Cambodia (Shackley et al. 2012). For every tonne of sugarcane, 130 kg of bagasse is generated which adds up to 220 metric tonnes per year (Demirbas 2011). Municipal solid waste (MSW) is another potential waste stream

whose management is a huge problem in the developing countries (Ghosh et al. 2019). It has been estimated that by incineration of 1 tonne of MSW compared to landfilling without recovering energy, approximately 1.3 tonnes of CO_2 equivalent emissions to the environment can be avoided (Kumar and Samadder 2017). Other commonly available waste includes animal manure which is highly suitable for AD to produce biogas having methane as the major component (Svanberg et al. 2018). Another category of waste that is increasingly being produced includes sludge from the wastewater treatment plants. Dry sludge has been mainly found to comprise fatty acids particularly in the range of C10 to C18, making it an excellent feedstock for production of biodiesel (Kumar et al. 2016). Food that is lost from the industrial processing facilities can be targeted for the production of unique high-value products, for example, potato peels can be fermented to produce lactic acid and can help in recovering around 5,600 million USD per year, or can be digested anaerobically for biogas production resulting in a revenue of 900 million USD per year (RedCorn et al. 2018). Thus, it is quite evident that different types of wastes are available globally in huge amounts and majorly their potential for recovering energy and other value-added products remain untapped. Consequently, waste valorization seems to be a prospective strategy for solving the issues related to energy security, waste management, and environmental pollution.

2.3 Waste Valorization to Produce Biofuels

2.3.1 Bioethanol

Bioethanol is a high-octane number and economically entice biofuel having the potential to provide energy security and cut the dependency of fossil fuels. There is large quantity of biomass available and use as a feedstock for the production of bioethanol can also reduce greenhouse gas emissions. Also, ethanol is fewer toxic in nature and quickly biodegradable in comparison with petroleum-based fuels and it produces lower amounts of pollutants. It is an environment friendly oxygenated fuel as it comprises 35% O2, although there is no oxygen present in gasoline. Due to the presence of oxygen, bioethanol has 15% higher combustion efficiency than gasoline. However, the major challenge associated with production of ethanol is inconsistency in the availability of feed material such as sugar molasses. Though, lignocellulosic biomass is abundantly available, but their recalcitrant and complex structure remains a main economic and technical hindrance to produce biofuels (Limayem and Ricke 2012).

The first-generation bioethanol is obtained typically from starch and sugar material, while second-generation bioethanol from lignocellulosic biomass and used industrial by-products like glycerol. However, third-generation bioethanol is mainly produced from algal biomass.

Second-generation bioethanol is used as a substitute of first generation and is too highly suggested from the sustainability point of view (Ozdingis and Kocar 2018). The invention of third-generation bioethanol from algal biomass is still in an

underdeveloped stage and limited just to the laboratories, although more variety of biomass has already revealed their potential as a bioethanol feedstock on a marketable scale (Zabed et al. 2017). Nevertheless, bioethanol generation technology is improving with every generation, and we are also continuously escalating the production potential (Niphadkar et al. 2018).

In the USA, the most common feedstock used for ethanol production are the starchy biomass such as wheat, corn and potatoes. The most common feedstock in Brazil has been sucrose or sugar-based biomass, and in Europe, the sugar beet. Moreover, bioethanol production can also be through the utilization of lignocellulosic materials, starches, agricultural wastes, sugars, and more recently the algal biomass are also being used as primary raw materials for ethanol production. Amidst all these raw materials, the lignocellulosic feedstock is abundantly achievable at a low cost which makes them a good enough and potentially inexpensive feedstock for production of ethanol sustainably. However, the major key challenge associated with the production of bioethanol is that despite the range of available feedstocks, the availability of raw material differs significantly from over the period of the year. (Xu et al. 2015). However, the second-generation bioethanol production technology is more complex in comparison to the first generation and that is the reason they must further development. Cellulosic biomass includes wood, grasses and crop residues. There are problems associated with cellulosic residues as conversion of cellulose into glucose is more difficult than starch and sugars. The use of wastes generated from agricultural and other sectors would lead to reduction in the current bioethanol cost to a competing price in the global market. With regard to ethanol production, different group of biomass have different potential as an ethanol feedstock. Kreith and Krumdieck (2013) reported that the potential ethanol yield from agricultural residues is 235–450 L/ton of biomass, whereas from MSW, bioethanol production is around 152 L/ton (Li et al. 2012).

The major steps for bioethanol production are shown in Fig. 2.3. The production of bioethanol consists of four phases i.e. biomass pre-treatment, hydrolysis of pre-treated biomass to produce cellulose, conversion of sugars into ethanol by fermentation, and finally distillation/ethanol recovery (Limayem and Ricke 2012). The first step involves the lignocellulosic biomass pre-treatment. There are several advantageous factors for pre-treatment of biomass like to increase the surface area of biomass, to reduce the crystallinity of cellulose and also to break the lignin seal and remove hemicellulose. Pre-treatment helps cellulose to be more accessible to enzymes so that conversion of carbohydrate polymers into fermentable sugars can be achieved faster and also increasing the yield of the system. Pre-treatment technique may be classified into physical, chemical, physicochemical and biological. It is important to take into consideration the compatibility of the pre-treatment method with the hydrolysis and fermentation steps. Pre-treatment step is followed by hydrolysis, the most commonly used being enzymatic hydrolysis and the concentrated acid hydrolysis. Enzymatic hydrolysis is a cost-effective technology of producing ethanol from biomass while the acid hydrolysis is the most progressive one. The concept behind two stages hydrolysis is that it allows better fulfilment of carbohydrates in lignocellulosic materials. During first stage, the hemicellulose

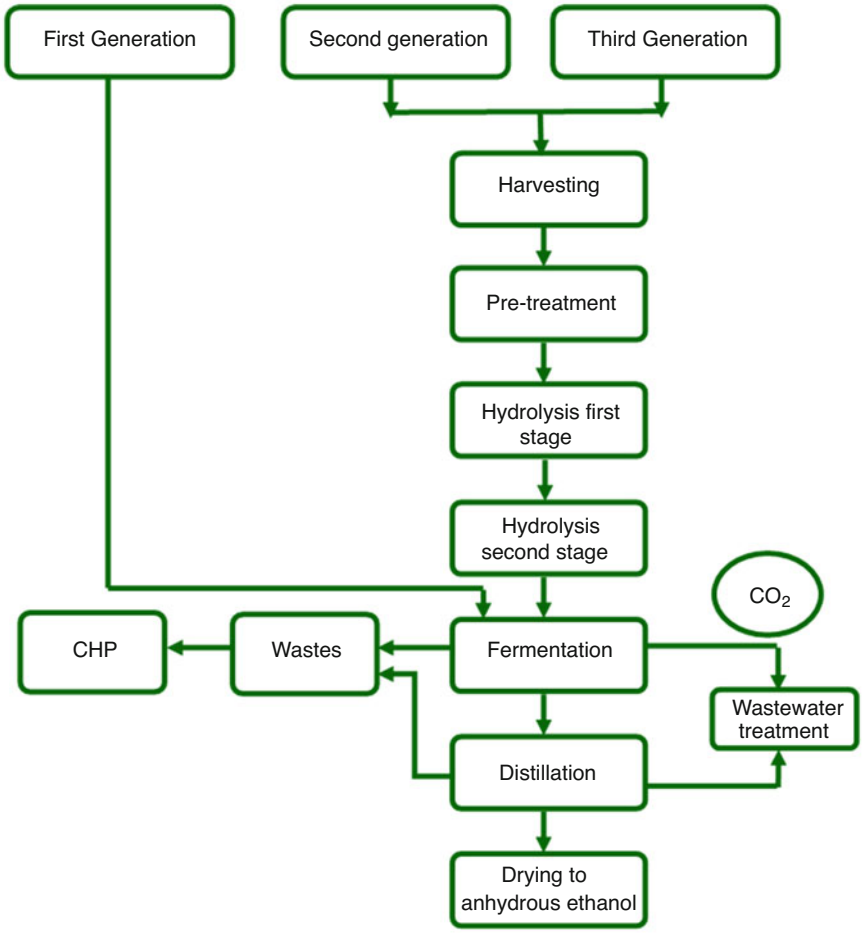

Fig. 2.3 Different strategies of waste to energy conversion

hydrolysis is prioritized and in the second stage, conversion of cellulose to glucose is done. Though, for acid hydrolysis process, both nitric and sulphuric acid have been used while acid is most commonly used (Mussatto et al. 2010). Enzymatic hydrolysis is carried out by the cellulolytic enzymes that degrade cellulose to sugars. This process is carried out in milder surroundings in comparison to acid hydrolysis. Furthermore, enzymes have the benefits of producing larger amounts of sugars. After hydrolysis step, the sugars can be transformed into ethanol by microorganisms. Hexoses are readily converted by the conventional yeast Saccharomyces cerevisiae. For feedstocks rich in pentoses, microorganisms capable of converting pentoses to ethanol, such as yeasts of the genus Candida and Pichia, or genetically modified S. cerevisiae need to be used (Agbogbo and Coward-Kelly 2008). The last stage involves the separation and concentration of the ethanol.

In the coming 20 years, bioethanol produced from renewable biomass is expected to be one of the leading renewable biofuels in the transport sector. Ethanol being a renewable and locally produced biofuel can help in reducing our dependency on oil exported from other countries. It is also beneficial to the environment as it has lower carbon dioxide emissions. According to a study by the U.S. Agriculture Department based on life cycle assessment, by the year 2022, the greenhouse gas emissions from corn ethanol will be 43% lower than the baseline emissions from gasoline. Cellulosic-based biofuel is a probable substitute to food derived bioethanol originating mainly from starch, corn and sugarcane provided by the world's largest producers the USA and Brazil, respectively. Pre-treatment, the costliest step and is of particular concern due to the high recalcitrance of lignocellulosic raw materials. There is a huge potential of bioethanol production from sugarcane crop residues as well as by-products like bagasse, molasses and vinasse. Utilization of these residues could improve the sustainability of the bioenergy chain and reduce the negative environmental impacts related to inappropriate disposal (Sindhu et al. 2016).

2.3.2 Biohydrogen

Biohydrogen is a clean fuel with a potential alternatives of fossil fuel economy as it forms only water with a high energy yield between 120 and 142 MJ/kg (Basak and Das 2007; Ghimire et al. 2015). Also, H2 has 2.75-fold more energy efficiency than other forms of hydrocarbon containing fuels and feedstocks for the biohydrogen production are also renewable resources. Despite this, at present nearly 90% of H_2 is produced from steam reformation of natural gas. Notably H_2 production from renewable sources, especially biomass resources has gained a great interest in recent years as an environment friendly and sustainable approach (Mohan and Sarkar 2017). Various countries have placed emphasis on biohydrogen production as a fuel and Japan is the pioneer in the world outset research on hydrogen energy (Solomon and Banerjee 2006).

Hydrogen produced by microalgae, bacteria, or biomass is known as biohydrogen. Biohydrogen production is produced via carbon neutral approach in which CO_2 is taken from the environment by photosynthesis of plants (Rai and Singh 2016). Biohydrogen production is a form of clean energy by utilizing various available biomass resources, is a promising approach to fulfil the increasing energy demands of fossil fuels (Mohan and Sarkar 2017). Moreover, lignocellulosic wastes, agricultural crops and their waste by-products, animal wastes, aquatic plants like algae and water weeds, and organic fraction of solid wastes from industrial or municipal are recommended as biomass sources. Furthermore, biomass resources for biohydrogen production can be divided into three broader groups: First-generation, second-generation and third-generation biomasses (Argun et al. 2017). Agricultural products containing sugars and starch such as sweet sorghum, sugar beet, potato, wheat, pumpkin, etc. and their residues are known as first-generation biomasses. Sugar beet is found to be favourable feedstock as it contains high sucrose and water content. In spite of higher yields of hydrogen production obtained from

first-generation biomasses, using these sources as feedstock leads to competition of utilization of arable land to produce energy crops or food crops which are the biggest drawback of this technology. As a result, it leads to severe food shortages and overuse of water and fertilizers (Mussatto et al. 2010). For this reason, biohydrogen production has shifted from first-generation biomass to second-generation biomass. Second-generation biomass includes mainly lignocellulosic materials, agricultural waste of corn stover, cornstalk, corncob, wheat straw, wheat straw, wood chopsticks, rice straw, sugarcane bagasse, water hyacinth and sugarcane leaves, non-edible crop residues and forestry wastes, wastes from food processing organic fractions of industrial and municipal wastes (Argunet al. 2017; Sharma and Arya 2017). Utilization of such biomass is allowing efficient waste management. Although lignocellulosic biomass has better yield of biohydrogen, but due to high lignin content, there is an additional step of delignification of biomass for efficient hydrogen production (Wang and Wan 2009). Requirement of pre-treatment for second-generation biomass on a large scale is a major challenge for biohydrogen production as it requires high costs as well. Third-generation biomass includes algae which contain rich carbohydrate content due the fact, algae are a suitable feedstock for biohydrogen production. Chlorella sorokiniana, Chlorella vulgaris, Scenedesmus obliquus, Laminaria japonica are the major species of algae which are commonly used for biohydrogen production (Sambusiti et al. 2015). Cultivation of algae can be low cost with no requirement for expensive substrate and high energy inputs (Sharma and Arya 2017).

The biohydrogen production potential of different biomass feedstocks varies based on its composition. Organic fraction of municipal solid waste (OFMSW), rice slurry and waste generated from food show high biohydrogen production potential compared to feedstocks such as pig slurry, rice straw and wheat straw (Ghimire et al. 2015).

For an efficient biohydrogen production, pre-treatment of algal biomass is must to do step in hydrogen production due to embedded carbohydrate within the cell which has to be broken into simpler compounds properly. Therefore, for efficient hydrogen production from the first-, second- and third-generation biomasses, various pre-treatment methods have to be applied like enzymatic, heat, sonication, alkaline, and acid-heat treatments as shown in Fig. 2.4 (Wang and Wan 2009).

Though, hydrogen energy can be considered as one of the best choices in renewable energy production, but the selection of production process is very important. H_2 production can be done viz. biological and thermo-chemical (Fig. 2.5).

Biohydrogen is produced through two main pathways: photosynthetic and fermentation (Sharma and Arya 2017). The specific hydrogen production from fermentative processes is much higher compared to photosynthetic processes (Yoshida et al. 2007). Photosynthesis is a light-dependent process, comprising of direct biophotolysis and indirect biophotolysis whereas fermentation is of two types, either photo-fermentation or dark fermentation. Direct photolysis involves dissociation of water into hydrogen and oxygen in the presence of light. Green microalgae possess chlorophyll a and the photosynthetic systems to carry out photosynthesis in the presence of light. Green microalgae hold special property of 10X more solar

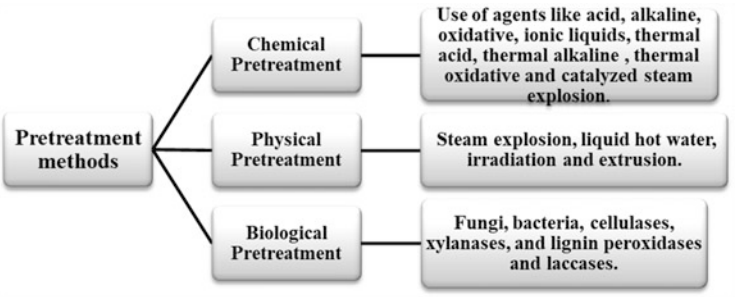

Fig. 2.4 Different pre-treatment methods (Adapted from Wang and Wan 2009)

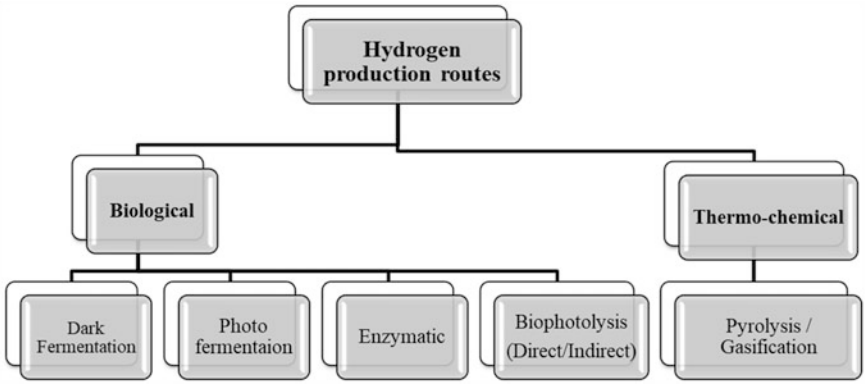

Fig. 2.5 Flowchart showing various routes of hydrogen production

conversion which makes process more efficient. Apart from the advantages, the process exhibits some drawbacks i.e. the hydrogenase enzyme is very sensitive to oxygen and very less amount of oxygen in the system can inhibit the activity of enzyme hydrogenase leading to inhibition of hydrogen production. Another drawback is requirement of high intensity of light for optimum production (Sharma and Arya 2017). Indirect photolysis is a two step process via splitting of water in the presence of sunlight and oxygen with the formation of protons and oxygen (Levin et al. 2004). Furthermore, carbon dioxide fixation occurs and carbohydrate is produced, followed by the production of hydrogen gas by enzyme hydrogenase. Blue-green algae (cyanobacteria) are the best proven feedstock for this process. Advantages are that hydrogen production is independent of oxygen evolution which leads to relatively higher hydrogen yields. However, the main hurdle of the process is that the enzyme nitrogenase requires significant amount of adenosine triphosphate (ATP). Also, it is difficult to provide continuous source of light at large scale processes (Sharma and Arya 2017).

Dark fermentation (anaerobic) is a light-independent catabolic process. Hydrogen production via dark fermentation involves multistep metabolic biochemical

reactions viz. hydrolysis, acidogenesis, acetogenesis and methanogenesis in a dark environment without the presence of sunlight, water and oxygen (Mohan et al. 2010). Dark fermentation is similar to methanogenic anaerobic digestion process as it shares many common features of the process. Eventually, dark fermentation has gained importance due to its feasibility of utilizing wastewater as a fermentative substrate and mixed cultures as biocatalysts (Ghimire et al. 2015). The process simplicity and efficiency are strong features. The complex organic polymers are converted into simple monomers via hydrolysis by fermentative microorganisms that are further converted to a mixture of lower molecular weight organic acids and alcohols by hydrogen producing bacteria (Mohan et al. 2010). The biggest benefit of dark fermentation over other H_2 production processes is that it can use variety of organic sources as a feedstock with the production of valuable by-products like butyric acid, lactic acid and acetic acid (Elbeshbishy et al. 2017). Photo-fermentation is the process of conversion of organic substrate into hydrogen and carbon dioxide in the presence of sunlight as energy source. In the presence of light, organic acid substrates are oxidized using the tricarboxylic acid (TCA) cycle, producing electrons, protons and carbon dioxide. Purple non-sulphur bacteria (PNS) are found to be promising as they lead to higher yields of hydrogen generation in photo-fermentation process (Basak and Das 2007). Sometimes, pre-treatment of industrial effluent may be the problem as it produces toxic compounds as well as it needs nitrogen limiting conditions.

Biohydrogen production from carbon-containing materials is attained by two major biological processes i.e. dark fermentation and photo-fermentation (Mohan et al. 2010). Most of the biological H_2 production processes are less energy intensive as they are operated at ambient temperature and pressure. (Elbeshbishy et al. 2017). The two key enzymes, hydrogenase and nitrogenase act as mediator for hydrogen production (Mohan et al. 2010). Both the enzymes are activated at specific process conditions, for instance, nitrogenase is activated in the presence of light, and absence of nitrogen and hydrogenase is activated at high light intensity and pH. Amongst various biological routes, dark fermentation offers an excellent potential for treatment of organic wastes. However, optimization of process at commercial scale depends on several factors like bioprocess design, type of feedstock, temperature, pH, HRT, substrate loading rate and biocatalyst along with an understanding of the structure of biohydrogen producing communities and their improvement (Mohan et al. 2010; Khanna and Das 2013).

Hydrogen being an important energy carrier affirms its significant applications in synthesis of nitrogenated fertilizers, production of ammonia, Hydrogen Sulphide removal, chemical based plants, preparation of food based products, formation of methanol, ethanol, dimethyl ether (DME), alternative fuels synthesis by Fischer–Tropsch (FT) synthesis, gas to liquid (GTL) synthesis technology, rocket fuel, IC engine fuel, high temperature industrial furnaces fuel, etc. (Wang et al. 2018). Hydrogen energy is also being utilized in electricity generation, vehicular applications, jet planes, cooking, microbial fuel cell, and power generation (Mohan and Sarkar 2017). The major future markets for hydrogen depend primarily upon the aspects like reducing the future cost of hydrogen production and stepping

towards some integrated technologies (Mohan et al. 2010). Integrated strategies include biohythane hybrid dark–photo-fermentation microbial electrolysis (Rai and Singh 2016; Nikhil et al. 2017).

2.3.3 Biogas

With the increasing demand for bioenergy and environmental sustainability, biogas technology via anaerobic digestion can be an attractive solution. Anaerobic digestion involves the action of microorganisms for degradation of organic matter into biogas, which is the form of renewable energy that could be used to produce electricity, heat or as vehicle fuel (Weiland 2010). The climate change policies and energy policies in the EU have helped in promoting the utilization of renewable resources for biogas production for generating energy.

In developing countries, the application of biogas is very limited for cooking and lighting purposes. In comparison to the developed countries, the use of biogas application is extended to electricity generation and vehicular application at larger scale. Along with a source of energy, additional benefits include production of by-products such as high quality biofertilizer, complete waste recycling and environmental protection from pollutants.

Particularly, in contrast to Europe, the global biogas production reached 1.35×107 t in 2014 with Germany; with approximately 25% installed capacity due to the strong development of agricultural biogas plants on farms (Achinas et al. 2017). So many countries have already shown concern about the development of new routes of biogas production from biomass and organic fractions of different wastes. Several European countries have developed the favourable conditions for electricity generation from biogas. It is notable that the agricultural waste accessible for biogas production is as high as 1.5×109 t in Europe (Wagner 2015). Like European countries, the USA, India and China are also developing alternative technologies for biogas production from lignocellulosic wastes and are more likely to be future competitors in biogas production (Lebuhn et al. 2014).

All biomasses that contain carbohydrate, proteins, fats, cellulose and hemicelluloses can be used as a substrate for biogas production. However, the composition of biogas and methane content varies based on the feedstock type, the digestion system, and the retention time of the system (Ghosh et al. 2020). So, the choice of the substrate is a very important factor for determining the quantity of biogas generated. If the substrate contains high fat content, then 80% of biomass can be converted into biogas. On the other hand, if the substrate has high protein content, then it can produce biogas containing up to 70% methane, while carbohydrate rich substrate will lead to production of around 60% methane content. Thus, any type of waste which contains main constituent as organic fraction has ability to produce biogas. Agricultural waste, cattle manure, organic fractions of industrial and municipal waste and sewage sludge and any waste with high organic matter are the best suited substrate for biogas production. For efficient anaerobic digestion process, it is necessary to feed the substrate in a proper manner.

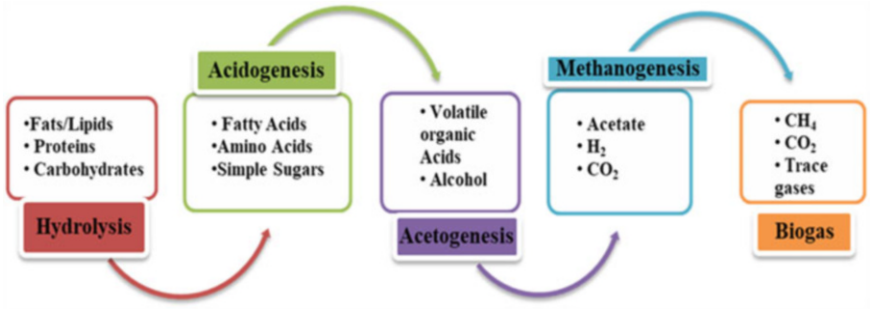

Fig. 2.6 Steps of anaerobic digestion

The use of cattle manure for biogas production is being carried out for the past 50 years due to the intensive livestock farming in the developing countries. The abundance of the cattle manure exceeds its demands as fertilizer and results in adverse impact on both environment and humans and the best solution is biogas production from the available cattle manure. Manures and slurries from different varieties of animals such as cows, buffaloes, horses, minks and poultry animals have been used for long as a substrate for biogas production (Mao et al. 2015). Animal manures and slurries have some limitations as a substrate for biogas production which includes low dry matter content (<10%) leading to low methane yield per unit volume of digested feedstock. Municipal solid waste (MSW) mainly contains yard waste, paper, peel of fruit and vegetable waste, leaf waste, waste, leaf litter, among which the food waste accounts for majorly of the organic content of MSW (Yong et al. 2015). But the major challenges for production of biogas from MSW is lack of awareness among the people about segregation of organic and inorganic fraction of MSW. Sewage sludge from wastewater treatment plant is also another type of resource for AD processing (Popescu and Jurcoane 2015).

The biogas composition depends on the nature of feedstock and operational conditions of the digester. Ideally, biogas contains 50–75% of CH4, 25–50% of CO2, along with trace elements of H2S (300–30,000 ppm), ammonia and water vapour. Anaerobic digestion process can be divided into four steps, named as hydrolysis, acidogenesis, acetogenesis/dehydrogenation and methanogenesis (Amon et al. 2007). The five physiologically distinct groups of microorganisms are responsible for individual degradation steps which leads to conversion of complex molecules to simple molecules via intermediates such as volatile fatty acids, acetic acid, carbon dioxide, hydrogen to final CO2 and CH4 (Fig. 2.6). Firstly, hydrolytic bacteria excrete hydrolytic enzymes (cellulose, lipase, protease, etc.) which are responsible for hydrolysis of complex organic polymers to monomers such as amino acids, simple sugars and fatty acids. Furthermore, some facultative anaerobes such as Streptococci and Enterobacteriaceae help to convert hydrolysed organic material into volatile organic acids and alcohols via acidogenesis process. Apart from facultative anaerobes, most of the bacterial species are strict anaerobes in acidogenesis phase (El-Mashad and Zhang 2010). During acetogenesis, alcohols and

VFAs are anaerobically oxidized by acetogenic bacteria. These acetogenic bacteria, typically homoacetogenic bacteria *Acetobacterium woodii* and *Clostridium aceticum* convert into acetate and some amount of hydrogen and carbon dioxide (Surendra et al. 2014). In the final stage hydrogenotrophic and acetotrophic methanogens lead to the conversion of the above into methane and carbon dioxide as major products with trace elements of hydrogen sulphide, ammonia, siloxanes and water vapour.

The composition of biogas and its production is influenced by several factors such as pH, temperature, retention time, C/N ratio, organic loading rate and type of feedstock too. The digestion process can take place in a mesophilic (35–42 °C) condition as well as in a thermophilic (45–60 °C) temperature conditions. It is important to control optimum temperature as per requirement for anaerobic digestion process due the fact that fluctuation in the temperature will affect biogas production negatively. The pH of the anaerobic digestion system is an important parameter that directly affects the activity of bacteria. The methanogenic bacteria are highly sensitive to a quick change in pH of the system. So, the general operating pH for anaerobic digestion should be maintained between 6.5 and 8.5 with an optimum interval between 7.0 and 8.0. The C/N ratio should be in the range of 15 and 30 ratio to avoid process failure by ammonia accumulation. Organic loading rate (OLR) is the amount of volatile solid fed into the digester per day in a continuous feeding manner. With the increasing OLR, rate of biogas production increases but sometimes it leads to disturbances in the equilibrium of the digester as well as the productivity process which is greatly hampered. Therefore, for a digester with a specific feedstock, the OLR has to be optimized (Mao et al. 2015).

To increase the biogas production from biomass, pre-treatment is necessary step. The rationale behind the pre-treatment is to enhance the accessible surface area and pore size with the decrease in cellulose crystallinity (Taherzadeh and Karimi 2008). Anaerobic co-digestion (AcoD) has been extensively used to increase biogas production (Mata-Alvarez et al. 2000). A number of studies have been carried out on co-digestion of livestock manure with lignocellulosic biomass to enhance the biogas production rates (Astals et al. 2013). The AD co-digestion leads to increase in digester gas production, enhanced AD performance to an integrated waste-to-energy process with mixed substrates available locally (Shen et al. 2015). However, economical, technological, market and regulatory barriers have prevented the dissemination of biogas technologies.

2.4 Conclusion

Depleting global fossil reserves and rapidly increasing problem of waste management have led to shifting of interest towards valorization of wastes to produce value-added products. This type of waste-derived bioeconomy has a huge potential for solving the dual issues of efficient waste management and providing a source of bioenergy. Adopting waste-derived bioeconomy will also help in addressing the Sustainable Development Goals (SDGs). Further, a biorefinery concept involving production of a number of products simultaneously from waste can help in

maximizing its value and make the products economical to compete in the world market.

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Bioplastics: A Green Approach Toward Sustainable Environment

3

Pratibha Singh and Roli Verma

Abstract

Petroleum-based plastics are synthetic compounds which are derived from oil and other fossil materials. Plastics are widely used because of their various superior properties like durability, but this also makes it stubborn. Most of the known plastics are not biodegradable and they persist in the environment for hundreds of years. Plastics are not bad but because they are non-biodegradable and they create harmful effects to animals, human beings, wild animals, and marine life. We are not able to manage them and also unable to find a substitute for same. Some suitable green alternatives are required to reduce plastic pollution. Plastics are referred green if they are from renewable resource, biodegradable or compostable after the end of life, and their processing is environmentally friendly. In recent years, naturally occurring biofibers have attracted increasing interest due to their wide applications in food packaging and in the biomedical sciences. Biodegradable plastics are made from starch, cellulose, chitosan, and protein extracted from renewable biomass. These eco-friendly polymers reduce greenhouse gases which require no petrochemicals. They reduce the use of fossil fuels and reliance on non-renewable resources. Manufacturing process can use up to 65 per cent less energy and generates fewer greenhouse gases than conventional plastic. Some are biodegradable and/or compostable. Therefore, biodegradable plastics should be produced and utilized at a large scale to fulfill demand of increasing population. The present paper summarizes all these content regarding the applications, production, types, challenges, sustainability, and use of eco-friendly and cheap substrates for the production of bioplastics.

P. Singh (✉) · R. Verma
Department of Chemistry, JSS Academy of Technical Education, Noida, Uttar Pradesh, India
e-mail: pratibhasingh@jssaten.ac.in

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3.1 Introduction

Plastics consist of a main chain organic link, pendant molecular groups. They are blends of organic and inorganic additives, plasticizers, fillers etc., to enhance material properties for the final application. Polymers are large molecules made up of many smaller units of molecules, called monomers. Figure 3.1 shows a simplified version of the transition from monomer to polymeric structure.

Plastics have very distinct characteristics, but most plastics have the following general attributes: They are resistant to chemicals and corrosion. They are thermal and electrical insulators. They have very high strength to weight ratio, highly durable, resistant to water, and have low toxicity. Plastics are materials with seemingly limitless range of characteristics and colors and are easy to manufacture.

The production of plastic has been significantly intensified to meet the demand of world's rapidly growing population. Most polymers exhibit unique property combinations (Fig. 3.2).

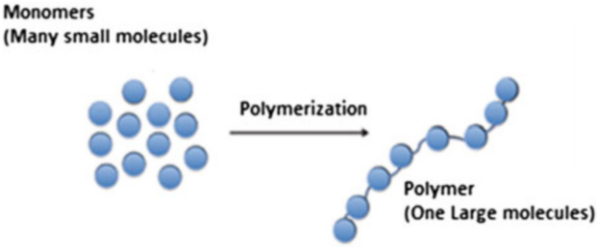

Fig. 3.1 Simplified figure of the transition from many small molecules, monomers to one large molecule, polymer (Saldivar and Vivaldo 2013)

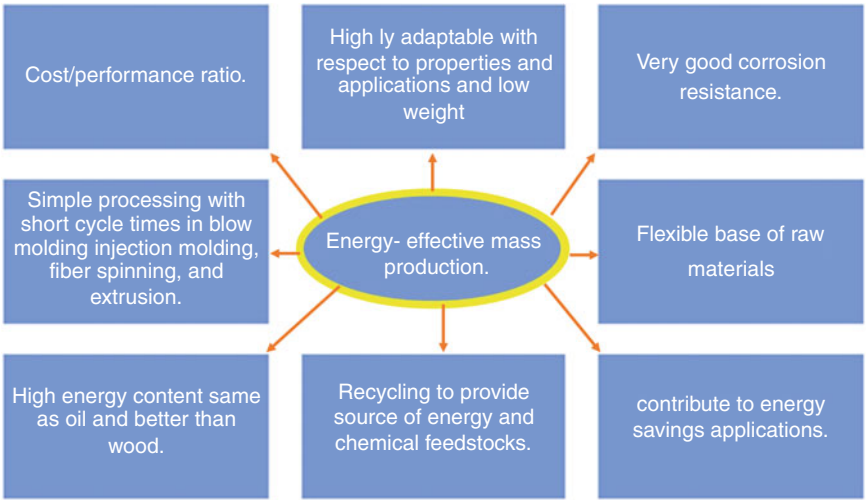

Fig. 3.2 Properties of polymer (Saldivar and Vivaldo 2013)

Chemically synthesized polymers are different in structure from the natural polymers (Saldivar and Vivaldo 2013). Natural polymers were not identified till 1861, when Thomas Graham dissolved organic compounds, such as cellulose, and reported that they could not penetrate through fine filters without leaving residuals on it. They called these materials as colloids. In 1870 John Wesley Hyatt chemically converted cellulose to produce a new material called celluloid. It was not until 1907 that the first completely synthetic polymer, bakelite, was synthesized by Leo Hendrik Baekeland. During and after the Second World War mass production of polymers as plastic materials began and has been growing ever since (Halden 2010). Synthetic fossil-based polymeric materials have been found as the largest application field of petroleum; the annual production in 2013 reached 299 million tons. The low production cost makes them versatile and are used in wide applications (Mekonnen et al. 2013).

Most of the plastics produced in the last century contain toxic additives which are hazardous for the environment and human health (Lithner et al. 2011); due to these additives precautions are required to be taken both during their production and disposal. The greatest disadvantage of plastic is the time they take to decompose—the average plastics take 500 years. The presence of additives shows adverse effects on human health as they are used in manufacturing of plastics. The three most commonly cited plastic additives are:

Bisphenol A Bisphenols are a group of chemicals used to manufacture plastics, epoxy resins, and other products since the 1960s. Bisphenol A (BPA), the most infamous of a group of around 40 chemicals, was initially investigated for pharmaceutical use as synthetic estrogen in the 1930s. It acts as endocrine disruptor in humans, causes thyroid cancer, osteoporosis, hypo and hypertension.

Phthalate or Plasticizer Phthalates are a group of chemicals most commonly used to make plastic more flexible and harder to break. They also act as a binding agent or a solvent. It has effect on reproductive organs, malformation, developmental disorders, causes pulmonary system effects including asthma allergies.

Flame Retardants Several chemicals have been used to stop the spread of fire in a wide range of plastic products. Common flame retardants include: brominated flame, OFRs, TBBPA, HBCD, and OPFRs. Brominated flame retardants belong to the same class of chemicals as PCBs, which were banned by the EPA in 1979. It has impact on immune system, fatal and child development, cancer, neurologic dysfunction.

Plastics can be micro-molecular or macro-molecular compounds depending on their structure. The burning of plastics is also a difficult chemical process. During plastic combustion, different phases take place, such as warming, degradation, flashover, and combustion. Low molecular compounds can be vaporized directly in the air and are able to form a combustible mixture. Macro-molecular plastics have

Table 3.1 Impact of uncontrolled plastic on health and economy (Ying et al. 2014)

Effect on health	Effect on economy
• Increases risk of heart disease • Aggravates respiratory ailments such as asthma and emphysema • Causes skin rashes, nausea, or headaches • Damages the nervous system • Damages the kidneys and liver • Disrupts the reproductive, endocrinal, and development systems • Disrupts the central nervous system	• Loss of income for hotels, restaurants, bathing resorts, other amenities, etc. • Loss of income for clothing manufacture, food industry, general commerce, etc. • Damage to fisheries activities • Damage to the image of the coasts as a recreational resort at both national and international levels • Damage to the local tourist infrastructure • Damage to tourist-dependent activities

to degrade into small molecule compounds to initiate the combustion process. Impacts of uncontrolled plastic burning are listed in Table 3.1. (Ying et al. 2014).

Recent studies by Jenna et al. (2015) show that plastic waste is entering the ocean and affecting the marine life; according to recent studies, 279 tons of plastic waste was generated in 190 countries and furthermore that 32 tons were mismanaged in the coastal regions of these countries (Jenna et al. 2015). Fossil-based plastics do not degrade completely in nature. The plastic due to the current of water fragmented into small pieces. This process is known as fragmentation and the small pieces are known as micro-plastics. These micro-plastics are consumed by animals and humans on daily basis through breathing and eating. With this awakening, "green" polymers are desirable, the manufacturers start investing into research of degradable polymeric materials made from renewable resource (Lithner et al. 2011). The demand for bioplastics is expected to increase and further advance and innovative research within the field is required (Bastioli and Magistrali 2014). More environmentally friendly plastics are not only obtained by using a biodegradable material from a renewable resource as a raw material, but the final product properties should also be taken into consideration with the help of Life Cycle Assessment (LCA).

3.2 Green Polymer from Solid Waste

Global production of bioplastics will increase significantly in the next years, reaching approximately four million tons in 2020 with respect to the environmental factors caused by synthetic fossil-based plastics (Mekonnen et al. 2013). Plastics can be categorized into four different groups, depending on the raw material they are extracted from (fossil- or bio-based) and if they are biodegradable or not, (Fig. 3.3).

In present scenario biopolymers have received a significant attention due to their environment-friendly nature and sustainability (Mekonnen et al. 2013). Properties such as nontoxicity, hydrophilicity, biodegradability, and biocompatibility contribute in vast range of applications i.e. biomedical field, cosmetic, food, and textile industries. (Petrova and Garner 2014)

Bioplastic is derived from natural raw materials such as biomass and corn starch. They degrade when exposed to environmental conditions such as moisture, naturally

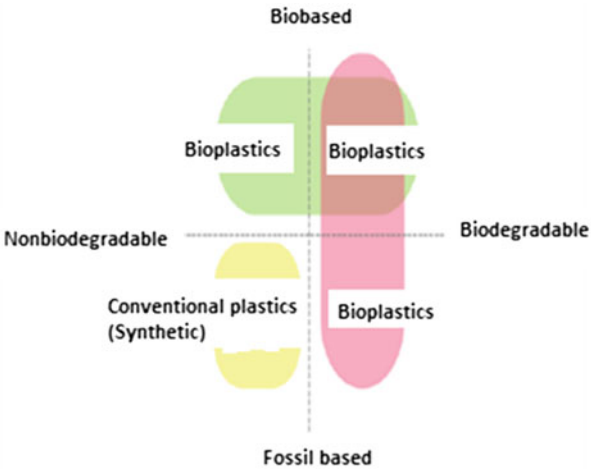

Fig. 3.3 Explanation of different plastics; based on their source and degradability (Jenna et al. 2015)

Table 3.2 Impact of uncontrolled plastic on health and economy (Ying et al. 2014)

Category	Examples
• Polymers directly extracted/removed from natural materials (mainly plants)	• Polysaccharides such as starch and cellulose and proteins such as casein and wheat gluten.
• Polymers produced by "classical" chemical synthesis from renewable bio-derived monomers	• Polyglycolic acid(PGA), Polylactate
• Polymers produced by microorganisms or genetically transformed bacteria	• Polyhydroxyalkanoates (PHAs)

occurring microorganisms such as bacteria, fungi, and algae or in a composting condition.

Bioplastics are plastics in which all carbon is derived from renewable feedstocks. They may or may not be biodegradable. The range of biodegradable plastics available includes:

- Starch-based products including thermoplastic starch, starch and synthetic aliphatic polyester blends, and starch and PVOH blends.
- Water-soluble polymer such as polyvinyl alcohol and ethylene vinyl alcohol.
- Naturally produced polyesters including PVB, PHB, and PHBH.
- Renewable resource polyesters such as PLA.
- Synthetic aliphatic polyesters including PCL and PBS.
- Aliphatic-aromatic (AAC) co-polyesters.
- Hydro-biodegradable polyester such as modified PET.
- Photo-biodegradable plastics.
- Controlled degradation additive master batches.

Bio-based plastics contain both renewable and fossil fuel-based carbon. The percentage of bio-based ingredients and the conditions, under which the bio-based

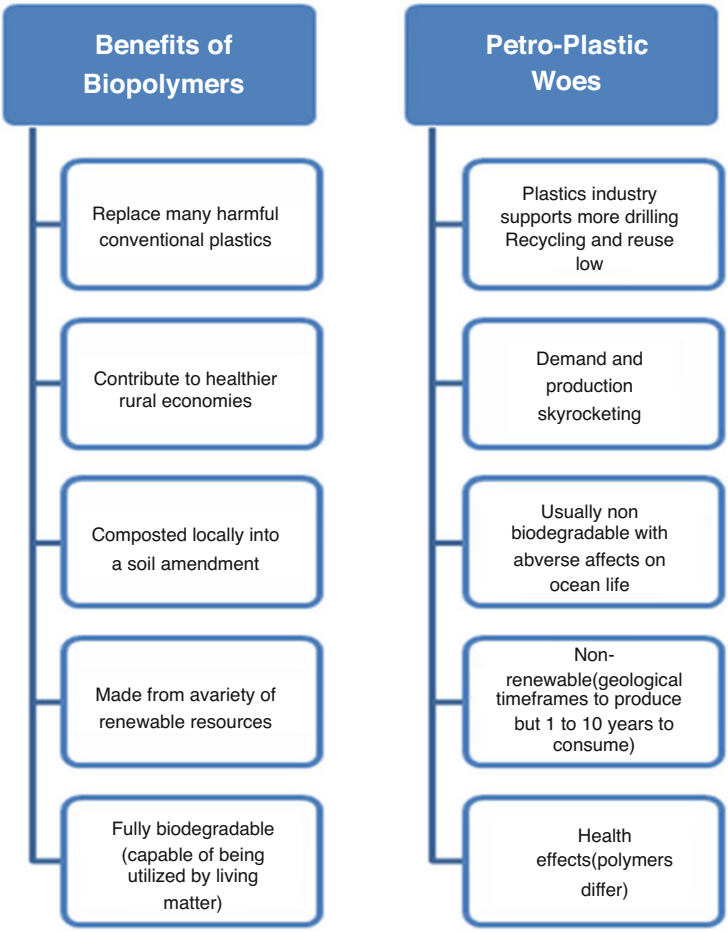

Fig. 3.4 Comparison of bioplastic and petro-plastic waste (Davis and Song 2006)

product may biodegrade, if at all, vary widely. Biodegradable polymers are divided into following category as shown in Table 3.2 (Kong et al. 2010).

These biodegradable polymers have become the recent attraction due to its unique properties. A comparative study of potential benefits of bioplastics, problems with petro-plastics are summarized in Fig. 3.4. These biodegradable polymers have become the recent attraction due to its unique properties. A comparative study of potential benefits of bioplastics, problems with petro-plastics are summarized in Fig. 3.4.

An organic material from which carbon is derived is a renewable resource. Bio-based materials include all plant and animal mass derived from CO2 recently fixed via photosynthesis, per definition of a renewable resource. Life cycle of bioplastic is shown in Fig. 3.5.

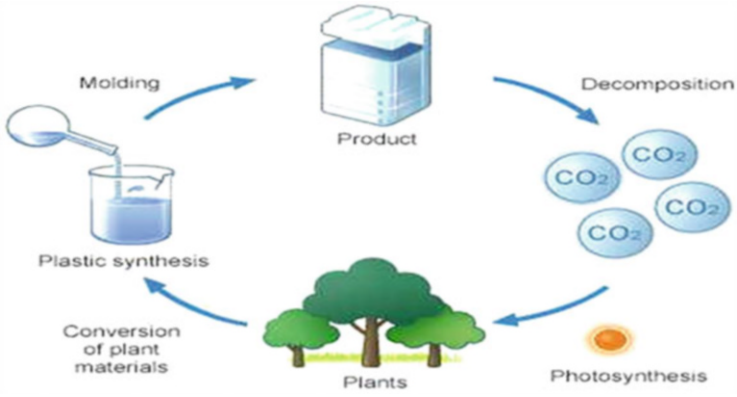

Fig. 3.5 Life cycle of a biodegradable plastic (Mekonnen et al. 2013)

Table 3.3 Starch-based polymers (Source: https://www.greendotbioplastics.com/starch-based Plastics)

S. No.	Type of plastic	Applications
1	Thermoplastic starch products	Food packaging, disposable eating utensils; loose fill, antistatic, and formed protective packaging; compostable films and bags for trash, retail, and agriculture
2	Starch synthetic aliphatic polyester	Blends high-quality sheets and packaging films
3	Starch and PBS/PBSA polyester blends	Thermoformed biscuit trays or film products
4	Starch-PVOH blends	Water-soluble laundry bags, drug control release carrier and bio-membrane, expanded foams as loose fill package

3.2.1 Suitable Solid Waste

Generally used types of bioplastics are based on starch, cellulose, glucose, and bio-oil. The feedstocks are converted into thermoplastic starch, polylactic acid, poly-3-hydroxybutyrate, polyamide 11, and bio-polyethylene by specific techniques.

Starch Thermoplastic starch accounts about 50% of the global bioplastics market according to today's trend and is the most significant and widely used bioplastic. Plasticizer and flexibilizer like sorbitol and glycerine are included to process the starch. Thermoplastic starch usually highlight just one component of starch based bioplastics. Some starch-based polymers and their applications are listed in Table 3.3.

PLA (polylactic acid or polylactide) is a very versatile polymer. Polylactide has been able to replace the conventional petroleum-based thermoplastics because of excellent combination of properties it possesses (Cosimo 2013). It is one of the most promising biopolymers used today and has a large number of applications such as healthcare and medical industry, packaging, automotive applications, also the use of

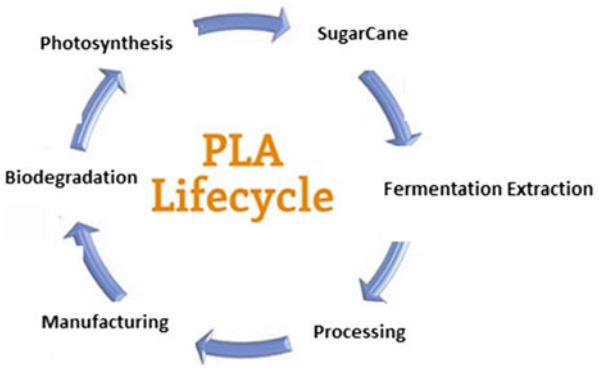

Fig. 3.6 Breakdown of polylactides into nontoxic products during degradation

PLA nanoparticles as drug carrier or MRI contrast agent is currently investigated. Its blends have a wide range of applications including computer and mobile phone casings, biodegradable medical implants, foil, molds, tins, cups, bottles and packaging devices, etc. (Chen and Patel 2012) As compared to other biopolymers, PLA exhibits several benefits such as:

1. Eco-friendly—It is renewably sourced, biodegradable, recyclable, and compostable.
2. Biocompatible—It is nontoxic.
3. Processability—It has better thermal processability compared to poly(hydroxyl alkanoate) (PHA), poly(ethylene glycol) (PEG), and poly(γ-caprolactone) (PCL).
4. High transparency—it has extraordinary stability, as well as high transparency.

Polylactides break down into nontoxic products during degradation and being biodegradable and biocompatible also reduce the amount of plastic waste (Fig. 3.6). PLA is mostly extracted by the fermentation of starch from crops, generally corn, wheat, or sugarcane into lactic acid followed by successive polymerization (Viviana et al. 2014). PLA has some disadvantages:

1. Its glass transition temperature is low (Tg ~ 55 °C.) It is thermally unstable and has poor gas barrier performance.
2. It has low crystallization rate and processing results mainly in amorphous products.
3. Its poor ductility, low impact strength, and brittleness limit its use as compared to other thermoplastics such as ABS.
4. As compared to PET (aromatic polyester), PLA is much more susceptible to chemical and biological hydrolysis.
5. It has low flexibility and requires long mold cycles.
6. It is relatively hydrophobic.
7. It has slow degradation rate.

Table 3.4 Applications of PHB

Area	Application
Biomedical	Part of bones, sutures, engineering of heart valves
Packaging	Food packaging
Environmental	Bags, bottles, disposable items
Agricultural	Encapsulation of fertilizers
Pharmacological	Encapsulation of medicines
Industrial	Synthesis of polymers

Wide application is still restricted due to the high cost of production that is significantly higher. But the cost of crude oil is constantly increasing and better PLA manufacturing methods are developed, the difference in prices may continuously decrease (Goodship 2007).

PHB (Polyhydroxybutyrates) are members from family of polyesters. They accumulate in intercellular granules by gram-positive and gram-negative microorganisms. They are produced in excess carbon source with a shortage of one essential nutrient (Tabone et al. 2010; Nishino et al. 2011). They are known as biopolymers as they are produced from microorganisms. They are thermoplastic polymers and are biodegradable. Physical characteristics of PHB are as follows:

1. Insolubility in water and its resistance to hydrolytic degradation.
2. It produces transparent film at a melting point of 175 °C.
3. It is biodegradable without residue.
4. Good oxygen permeability.
5. Good ultraviolet resistance.
6. Poor resistance to acid and bases.

Applications of PHB are summarized in Table 3.4:

PA 11 is a biopolymer extracted from polyamide natural oil. Rilsan is the trade name of polyamide bioplastic. PA 11 derived from castor beans is not biodegradable (Davis and Song 2006). Application of PA 11 is as follows:

- Automotive fuel lines,
- Pneumatic airbrake tubing,
- Electrical anti-termite cable sheathing,
- Oil and gas flexible pipes,
- Control fluid umbilical,
- Sports shoes,
- Electronic device components and catheters.

Polyethylene (PE) also known as fossil-based polymer is obtained from bioethanol (by dehydration). It is produced in large scale by fermentation of agricultural

feedstock such as sugarcane or corn. Bio-polyethylene is identical to traditional polyethylene and it is not biodegradable but can be recycled (Alexander 1993).

3.2.2 Production Techniques and Constraints

Market products are produced from a variety of natural feedstocks including corn, potatoes, rice, tapioca, palm fiber, wood cellulose, wheat fiber, and bagasse. These products can be used for a broad range of applications such as bottles, cups, cutlery, plates, bags, bedding, carpets, film, textiles packaging materials, and furnishings (Fischer et al. 2008; Jinghua et al. 2009).

In addition, the use of renewable resources for green polymer production should not compete with food production, promote intensified farming or deforestation, use transgenic plants or genetically modified bacteria (Tanaka et al. 2008).

Biomass can be directly converted into renewable coal and oil by using energy-efficient processes. Agricultural and forestry wastes already have utilized to manufacture renewable monomers. Processes have been designed to transform carbon dioxide into carbon monoxide, methanol, formic acid, and formaldehyde (Xiaoyun and Shuwen 2013). Vegetable oils are utilized to make biodiesel and glycerol as a byproduct, which can be further used to produce different monomers such as propane diol, acrylic acid, and even epichlorohydrin for the manufacture of epoxy resins. Similar cycle process to produce biodegradable polymers is shown in Fig. 3.7.

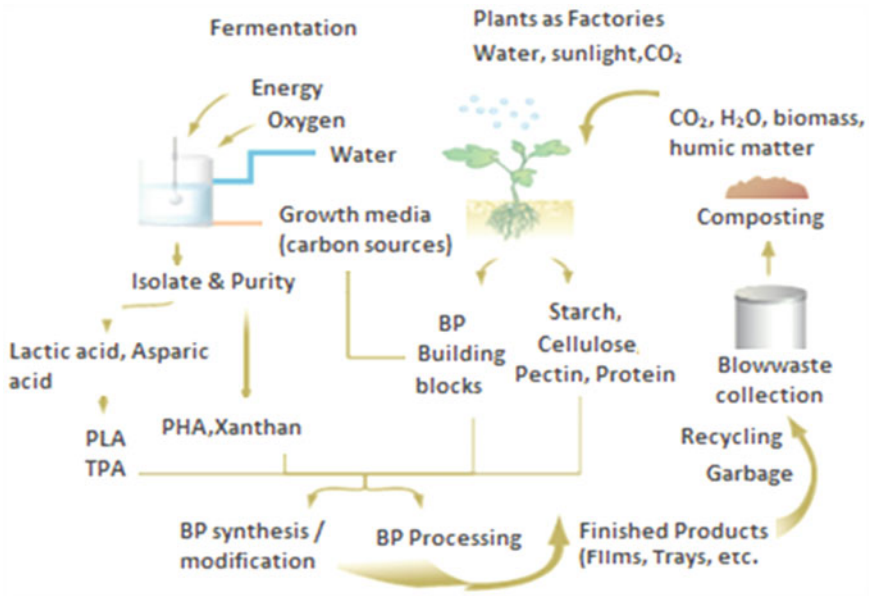

Fig. 3.7 Cyclic process of yielding of biodegradable polymers (Tanaka et al. 2008)

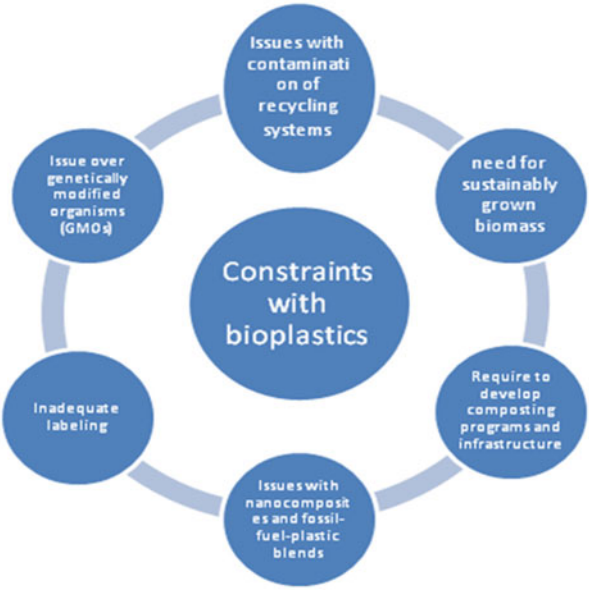

Fig. 3.8 Constraints with bioplastic (Axel 2009)

The inspiring factor for the innovative bioplastics technology was to handle our habit to dispose everything. The addiction of disposing everything eased out our lives, but on the other side it created huge problem on our environment which encouraged to the development of biodegradable disposables to reduce the problems produced by us to the environment. In 2010, disposable bioplastics contributed to more than 65% of the total bioplastic production capacity. However, 6 years down the lane, the durable bioplastics contribute to huge 77% of the overall bioplastic production capacity which is likely to grow above 80% by 2020. Bioplastics can be generally broken down into two types: durable and biodegradable. Biodegradable bioplastics break down naturally into the environment. This is unique, as the vast majority of plastics today will never break down. On the contrary, the Plant Bottle is a more durable bioplastic substitute to traditional PET bottles made by Coca-Cola. Produced with up to 30 percent ethanol sourced from plant material, the Plant Bottle will not degrade, but it can be recycled with traditional PET containers and bottles. Constraints with development and widespread acceptance of bioplastics are exhibited in Fig. 3.8 (Axel 2009).

3.2.3 Factors Affecting Degradation Behavior

The polymeric materials may break down by microbial action, chemical degradation, and photodegradation. Most of the biopolymers are considered to be discarded in landfills, composts, or soil. The materials will be broken down, if the required microorganisms are present. Microbially reduced plastics need soil bacteria and

water (Goodship 2007). Naturally grown materials based polymers (such as starch or flax fiber) are prone to degradation by microorganisms. The microorganism degrades the starch, leaving behind a porous, sponge like structure with a high interfacial area, and low structural strength.

Biopolymers can be microbially degraded by another way which involves growing microorganisms on the surface of digesting polymer materials. This is a more rigorous process which costs more, and avoids the use of renewable resources as biopolymer feedstock. The microbes under consideration are developed to target and break down petroleum-based plastics (Cheng 2010). Although this technique decreases the volume of waste, it does not help in the preservation of non-renewable resources.

Photodegradable polymers are thermoplastic synthetic polymers which are incorporated with light-sensitive chemical additives or copolymers for purpose of weakening the bonds of the polymer in the presence of ultraviolet radiation. Photo sensitizers include diketones, ferrocene derivatives (aminoalkyferrocene), and carbonyl-containing species. The effectiveness is dependent on exposure intensity and will vary with factors such as the season, geography, dirt or water cover, and shading. Photodegradable plastics may be useful in applications where littering is an issue and in those that pose a threat to animal and marine life.

Some biodegradable polymer materials experience a rapid dissolution when exposed to particular (chemically based) aqueous solutions due to the following properties:

- Chemical structure and chemical composition and molecular weight.
- Presence of low molecular weight compounds (monomers, oligomers, solvents, plasticizers, etc.) and distribution of repeating unit in multimers.
- Presence of ionic groups, chain defects, and unexpected units.
- Configurational structure and morphology (crystallinity, presence of microstructure, orientation, and residual structure.)
- Processing methods and condition.
- Method of sterilization, annealing, and storage history.
- Absorbed compounds, physicochemical compounds.
- Mechanism of hydrolysis (enzymes vs. water).

Three basic strategies to produce eco-friendly plastics are with biomass and/or carbon dioxide to produce “bio oil” and green monomers for highly resourceful and energy-effective polymer manufacturing processes, through living cells, which are transformed into solar-powered chemical reactors, using genetic engineering and biotechnology way to produce biopolymers and bio-based polymers and by activation and polymerization of carbon dioxide. In the production of biodegradable polymers, following green principles must be included (Fig. 3.9).

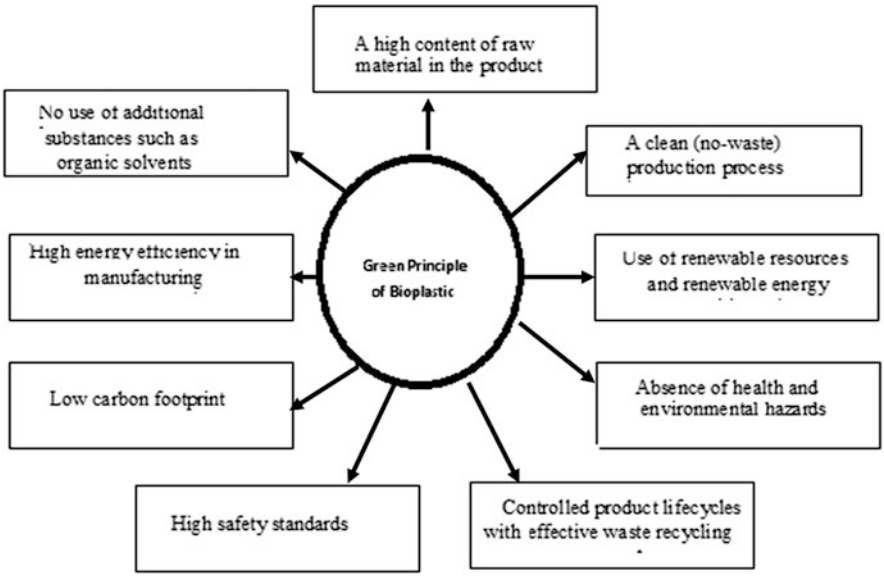

Fig. 3.9 Green principles for bioplastics

3.3 Application of Green Polymer

While 100% bio-based and biodegradable plastics are mainly used to substitute the plastics that might end up as litter (usually shopping bags, food packaging, disposables), partially bio-based plastics such as polythene manufactured from sugarcane, can provide a near-perfect substitute for oil-based equivalents in products where durability and robustness are vital. To have a quick look at the various types, feedstock, raw material, and applications of major durable bioplastics available in the current market, the following points are to consider:

- Bio-polyamide uses castor oil as feedstock and sebacic acid as raw material with processing of the dicarboxylic acid (sebacic acid), part of polyamide is produced from renewable resource (castor oil) that are used electronics, automotive, sports.
- Bio-based polyurethane (PUR) uses corn, sugarcane, tapioca as feedstock and bio-succinic acid as raw material with processing of the adipic acid used to produce conventional PUR which is replaced with succinic acid from renewable resources that are used as specialty foams, coatings, adhesives, TPU.
- Bio-based Polyethylene Terephthalate (PET)) uses sugarcane as feedstock and sugar as raw materials with processing of fermented and distilled to ethanol monoethylene glycol (MEG) from bio-ethanol, MEG is combined with Purified Terephthalic Acids (PTA) which are used in bottles, containers.

- Bio-based polyethylene (PE) uses sugarcane as feedstock and sugar with processing fermented and distilled to ethanol dehydrated to ethylene polymerization that are used in carry bags, films, and bottles.
- Bio-polycarbonate (PC) uses corn as feedstock and isosorbide (a monomer that replaces bisphenol A in conventional polycarbonate) as raw materials with processing of hydrogenation of glucose to produce sorbitol. Isosorbide is obtained from double dehydration of sorbitol that are used in a close substitute for high-performance glass components, electronic equipment, automotive housings, interior and exterior décor.

3.4 Application of Bioplastics

- Film including overwrap, shopping bags, waste and bin liner bags, composting bags, mulch film, silage wrap, body bags/coffin liners, landfill covers, packaging—O2 and H2O barriers, bait bags, nappy backing sheet, and cling wrap.
- Flushable sanitary products.
- Sheet and non-woven packaging.
- Bottles.
- Liquid paper board.
- Planter boxes and fishing nets.
- Food service cups, cutlery, trays, and straws.
- Loose fill foam.

Durable bioplastics are the main contributors of the growth of bioplastics industry, with polyurethane (PUR) producing around 43% and PET over 22% of the global bioplastics market. More than 75% of the bioplastics production capacity worldwide in 2015 was bio-based, durable plastics. In 2010, the share of durable bioplastic was only around 45% of the total share of 0.8 m metric tons of bioplastics in the global production capacity as shown in Table 3.5.

With mycelium (mushroom roots, funnily enough, the same stuff that Quorn is made from), packaging has literally grown. The Growth Trays are made out of PET plastic, which is reusable and recyclable. They are created by thermoforming over a solid form (precisely milled by a CNC router) to create the molded shape. The Growth Trays are then filled with a mix of substrate (hemp), nutrition (flour), and

Table 3.5 Markets growth trends in durable plastic (Cheng 2010)

Year	Total capacity (million tons)	Durable (%)
2010	0.7	42.3
2013	1.58	62.4
2014	1.697	60.9
2015	3.952	75
2016	4.16	76.8
2020 (Estimated)	5.95	79

mycelium which are sealed to grow for 6 days total. After 4 days, the mold pop out and further they are allowed to grow for another 2 days to get a velvety layer of overgrowth. The final stage is to dry the parts to prevent future growth.

Etiketten-Becker's stone paper consists of 80% limestone and 20% recycled polyethylene. This combination results in a 100% ecological product that can be used for several purposes, from posters, flyers to bags and is mostly used as (hang) labels and pot covers by growers. "It is a substitute for polypropylene because it is water-UV-, and tear-(more than paper) resistant. On top of that, it is also writable, even when it is wet." The paper can be printed with a thickness between 100 and 400 micron. PHA patents cover a broad range of PHAs products such as coating and packaging, cosmetic containers, bottles, golf tees, and pens. PHAs have also been converted into fibers, for a non-woven fabrics material. Emerging application areas for bioplastics include bait bags, fishing line and net, silage wrap, body bags and coffin liners, nappy backing sheet, coated paper, agricultural mulch film, shopping bags, food waste films and bags, landfill cover films, and various sanitary products.

3.5 Sustainability of Green Polymer

Engineers are trying to incorporate environmental considerations directly into material selection techniques, in order to protect the environment. The use of renewable and eco-friendly resources in the manufacturing of polymer materials is obtained in two ways. First of all, the feedstock being used can be replaced, either through intentional intervention by humans or natural cycles. The second environmental benefit of employing renewable feedstocks for biopolymer production is the biodegradable characteristics of the end products, thereby reducing potential pollution from the disposal of the equivalent volume of traditional plastics. At the end of their beneficial period, biopolymer materials are usually sent to landfills or composted. Recycling of plastic materials is encouraged, well promised, and advertised, but efforts of enhancing this attempt have been less than helpful. In the USA, recently less than 15% of plastic products must be known as a disposal technique, not a final aim for material development. A satisfied approach regarding recycling processes overlook the fact that advanced infrastructure is essential to systematic house recycling (Cao et al. 2007). This appears to be positive at the beginning, but the open systems by which the plastics are recycled allow the release of toxic gases at crucial levels.

Bioplastics are primarily important because petroleum oil price is rising enormously and its stock will finish in the near future. It is important for the global community to have a substitute for the product resulting from petroleum oil like plastics. PHAs will supposed to be an effective solution for most of the industries and societies, which mostly depend on resources made from plastic. No new inventions can prevent from the limitations and drawbacks and bioplastics too have some drawbacks. The most important drawback for PHA yield is its production cost, but the good news is that the price of PHA production is reducing, whereas,

petroleum oil price is rising constantly. As a result, the gap between the petroleum oil and PHA are becoming very less. Following are the advantages of bioplastics:

- **Reduce carbon footprint**: It should be mentioned that the carbon footprint of a bioplastic is significantly dependent on whether the plastic permanently stores the carbon produced from the air by the growing plant. A plastic produced from a biological source sequesters the CO2 captured by the plant during the process of photosynthesis. If the resulting bioplastic decomposes back into CO2 and water, this sequestration process is reversed. But a permanent bioplastic similar to polyethylene or other traditional plastics stores the CO2 forever. Even if the plastic is recycled many times, the CO2 primarily taken from the atmosphere remains sequestered.
- **Less costs of energy during manufacturing process**: On the contrary, plastics are produced from approximately 4% of the oil that the world uses every year. With oil scarcity, the production of plastics becomes exposed to fluctuating prices.
- **Do not dependent on crude oil**: On the other hand, each kilogram of plastic usually requires 20 kilowatt hours of energy to produce more than the amount required to create the same weight of steel. Almost all of this originated from fossil sources.
- **Decrease in litter and improvement in compostability**: Biodegradable bioplastics help in the reduction of permanent litter. Single use plastics are the most obvious example of how plastics can pollute the environment with huge and unsightly persistence. A large fraction of the litter in our oceans is of disposable plastic bags. Cities and countries around the world are taking actions against the litter, sometimes by banning non-degradable plastic bags completely.

The past of bioplastics is not a very old. Bioplastics have emerged as a potential alternative to ubiquitous material (fossil fuel-based polymers) which is earth-friendly. Various reasons associated with the emergence of bioplastics are that they reduce dependency on limited fossil fuel resources, reduction in GHG. They are considered because of their contribution to increased resource efficiency through closed resource cycle. The increased use of bioplastics is an important step towards the minimizing the plastic pollution and saving the earth.

The key rule to measure the impact on environment is supported by the Life Cycle Assessment. Biodegradable polymers widen the range of waste management treatment option over traditional plastics.

The most preferred disposal options for plastic materials are composting instead of landfill as shown in the Fig. 3.10, which is the least preferred disposal option. Therefore recovery of material, from biodegradable polymers can make important contributions in reducing the landfill and consumption of renewable resources (Davis and Song 2006). Recycling is the process in which the discarded waste is recovered or reclaimed, reprocessed or refined, to yield a complete new product. Recycling of plastic mainly depends on the resin code of plastic waste. A top priority of waste management has always been recycling; it not only helps us in protecting

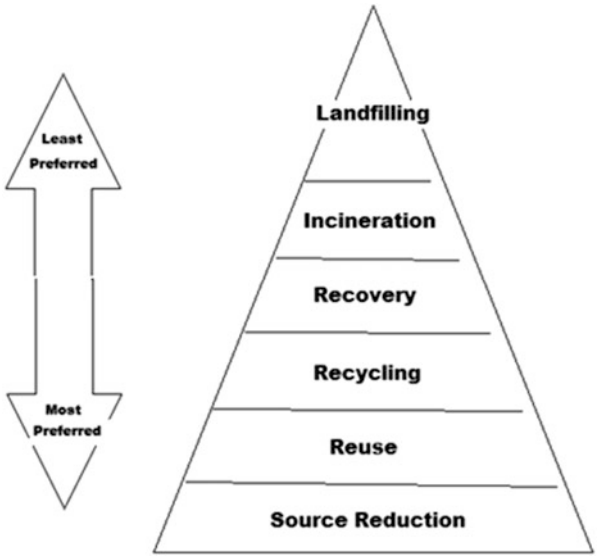

Fig. 3.10 Plastic waste management

the health of the environment, but it also contributes to reuse the waste productively thereby plummeting the space of landfill.

Incineration is the practice of burning waste products in the presence of oxygen in excess amount for thermal degradation of the waste. It is a chemical reaction in which hydrogen, carbon, and other elements mixed with waste generate lots of heat. CO2, CO, oxides of nitrogen, and water vapor are some of the gases produced in the process.

3.6 Conclusion

Crude oil prices will rise considerably in the next century, imposing the world to consider substitutes for petrochemical plastics. The renewable, eco-friendly nature and biodegradability of bioplastic consider them suitable resource to substitute synthetic plastics in various applications. In current scenario, their production is costly, but these plastics are only in their first stage of marketable development. Further research on genetically modified microbial strains, mixed cultures, proficient fermentations, recovery /purification, and the use of economical substrates can significantly reduce the production cost. Therefore, the future of bioplastics depends on the efforts towards satisfying price as well as performance necessity. Microbial synthesis of bioplastic seems to be an unlimited game; we can either make homopolymers with different monomers, or copolymers or block copolymers of different combinations. Because of their particular characteristics and wide biotechnological applications, bioplastics have a tremendously promising future. With respect to the bioplastics, future modification for improving biodegradability for

certain environments can be accomplished by metallization and better barrier properties obtained by addition of SiO_2. Thermal conductivity is increased by addition of carbon fiber, or other metals.

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Microbial Procession During Decomposition of Organic Wastes

4

Mohd Arshad Siddiqui and R. Hiranmai Yadav

Abstract

Almost every single habitat generated a huge amount of biodegradable solid organic waste which is discarded by dumping at different sites, in landfills, being burnt or is dumped in different water bodies. Because of this about all living organisms are negatively affected and the whole environment including soil, water and air are getting polluted. So it is necessary to manage the generated waste properly. There are many methods of municipal organic waste management. But the decomposition of organic waste through microorganisms (Bacteria, Fungi, Actinomycetes) is very efficient, safe and environment friendly method. For the decomposition of waste, these microbes secrete different kind of enzymes (Extracellular and Intracellular) which are responsible for the decomposition of waste. The present chapter includes generation of municipal solid waste and their management, microbes responsible for decomposition of such waste, enzymes secreted by the microorganisms, molecular characterisation of efficient bacteria, application of the identified microbes in waste management and also suggests to isolate and characterise efficient microbes which can be applied at industrial level for the proper management of municipal organic waste.

4.1 Introduction

The generation and accumulation colossal amount of municipal solid waste (MSW) everyday by human beings develops adverse effects on environment which is reflected on the biodiversity and thereby causing changes in the ecosystems. Considering these effects and changes in the environment due to accumulation of wastes

M. A. Siddiqui · R. Hiranmai Yadav (✉)
School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, Gujarat, India

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management strategies for safe waste disposal is need of the hour. Several methods of waste management have been developed and practiced based on the types of wastes and facility available in various places. Among these methods, composting could be considered as best, safe and environment friendly method that is practiced for many years and a method of recycling wastes in nature. Composting is a natural process involving a number of microorganisms (Bacteria, Fungi, Actinomycetes) that facilitates the process of decomposition. These microbes secrete enzymes (extracellular and intracellular) that hasten the breakdown process.

This chapter is a discussion about the generation and management of MSW through the process of composting and the microbial procession during the degradation phases.

4.2 Generation of Municipal Solid Waste (MSW)

Anthropogenic activities involving residential, industrial and commercial activities result in production of large quantities of solid waste materials that are of degradable and non-degradable nature. These are dumped onto soil surface that ultimately leads to soil, water and air pollution.

Characterisation and segregation of wastes could be an initial step for management of wastes that falls into categories of degradable and non-degradable materials. The adverse impact of this waste on environment and human health can be reduced by proper management strategies. The segregated waste generated from the residential, institutional and industrial sources could be converted into manures that help in economic development through its utility in farming and soil fertility improvement and enhance the quality of life in the area of waste generation.

The wastes from residential, institutional and industrial sources include newspaper, clothing materials, cafeteria waste, wood plates, office and classroom papers, disposable tableware, boxes and food scraps. The components and the rate of production of such waste are highly variable that makes it difficult to reach an exact generation rate (Singh 2011).

At present the MSW generation rate of India is about 0.34 kg/capita/day (or 109,598 tonnes/day) and it is predicted that it will be rise up to the 0.7 kg/capita/day (or 376,639 tonnes/day) by 2025 (Hoornweg and Bhada-Tada 2012). According to the survey of Central Institute of Plastics Engineering and Technology (CIPET) at the instance of CPCB in 59 cities of India the generation of municipal solid waste in 2010–2011 is about 50,592 tonnes per day. During 2013–2014, 34 states and union territories of India generated 1,43,449 tonnes per day as per CPCB. On the basis of above data, the average rate of waste generation of India is 0.11 kg/capita/day. Approximately 1,17,644 tonnes per day (82%) of municipal solid waste was collected and 32,871 tonnes per day (22.9%) was processed or treated out of the total waste generated in India (Srivastava et al. 2014).

4.3 Municipal Solid Waste Management in India

To the proper management of municipal solid waste that is being generated at a large scale and getting dumped at different sites, India has lack of the technical expertise and resources. There are many methods like incineration, pyrolysis and gasification technologies, landfilling, bioreactor landfill, refuse derived fuel (RFD) plants, composting and vermicomposting of MSW management, which are used in country (Siddiqqui 2018). Among these methods composting could be considered one of the safe and environment friendly methods of MSW management.

4.4 Composting for Waste Management

Composting is traditionally practiced in villages of India for management of solid waste. Compared to earlier days, presence of huge amount of non-organic materials in waste is making it difficult to compost the generated waste material. Decomposition of mixed waste results in poor quality of end product. Until the plastic items are not get recycled or have a secondary market, their presence in solid waste poses difficulty in degradation process.

To handle 500tonnes per day of municipal solid waste, the first large scale aerobic composting plant was start at Mumbai in 1992 by Excel Industries Ltd.

In the country, due to certain problems there is only 300 tonnes/day capacity is being utilised recently, but the plant is working very successfully and the produced compost is being sold at the rate of Rs. 2/kg. With the 150 tonnes/day capability, another plant has been working in the city of Vijayawada. In the main cities of country such as Lucknow, Bhopal, Hyderabad, Ahmedabad, Bangalore, Delhi and Gwalior, a number of other plants have been implemented over the year. To adapt composting facilities, with these cities many other cities have either signed agreement or in the process of doing so very soon. There is only 10–12% composting used in India, due to composting needs segregation of waste and shorting is not widely practiced (Sharholy et al. 2006; Reddy and Galab 1998).

4.5 Role of Microorganisms in Decomposition

There are many categories of microorganisms which are the main reason of the biodecomposition of MSW like rotifers, protozoa, algae, fungi, bacteria and many other advance animals which inhibit the aerobic biological treatment system.

In a given waste system the proper growth of any or all types of microbes which are responsible for the biodegradation of MSW depends on many factors like chemical composition of the waste, biochemical features of the microbes and environmental conditions. The microbes which are specific to grow in a specific waste have both positive and negative impact. To the whole stabilisation of waste, it is necessary to identify the role (Fig. 4.1) of each single microbe, when the waste treatment system is to be properly designed and operated for maximum efficiency.

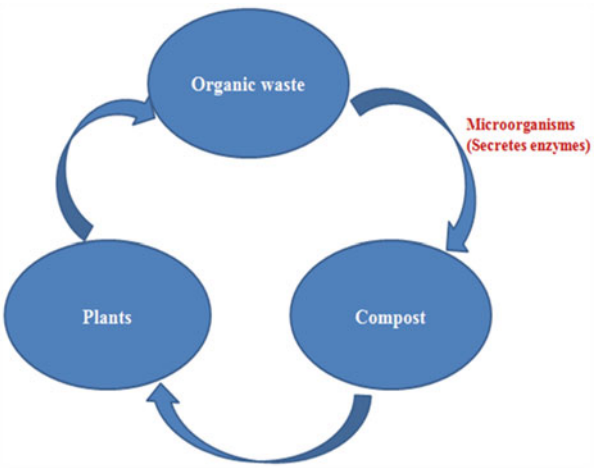

Fig. 4.1 Role of microorganisms in decomposition of organic waste

4.5.1 Bacteria in Decomposition

Bacteria, fungi, actinomycetes, algae and protozoa are major groups of the microbial population of the soil and bacteria are the most rich group among all these groups of microbes with significant role in the degradation of MSW. These bacteria secrete compounds that are useful for soil fertility, plant growth and maintaining balance of natural ecosystem. Microbes utilise the available materials for their metabolism that in turn reduces the accumulation of wastes in the soil. Composting is the manageable bio decomposition or transformation of organic compounds, mainly in aerobic conditions where it is transformed into stable and soil like end product called compost. In addition to bacteria there are many other microorganisms responsible for the degradation of solid substances. Secretion of the biological substances called enzymes by microbes hastens the process of degradation (Saha 2012).

Many bacterial species and Actinomycetes that helps in decomposition are isolated and identified by many researchers and are shown in the following Table 4.1.

4.5.1.1 Bacterial Enzymes

Conditions where the nutrients are specifically in the form of macromolecules are the best suitable for microorganisms. These macromolecules need to be cut into smaller molecules so that they can take it through absorption and utilised as nutrients. The enzymes secreted by the microbes cleave these macromolecular nutrients into absorbable molecules (Karigar and Rao 2011). Enzymes are biocatalyst responsible for many chemical reactions and are commercially exploited in the diagnostics, pharmaceutical, food, detergent and fine chemical industries. Till date more than three thousands enzymes are reported and most of them are isolated from the mesophilic microbes. A narrow range of ionic strength, temperature and pH is required for the proper functioning of these enzymes. It is observed that

Table 4.1 Bacteria identified for decomposition of waste

S. No.	Source	Bacteria	References
1	Municipal solid waste	*Bacillus* sp., *Pseudomonas* sp., *Arthrobacter* sp., and *Alicaligenes*	Stofella and Kahn (2001)
		Staphylococci	Hassen et al. (2001)
		Bacillus sp.	Abdullah et al. (2014)
		Halomonas sp., *Luteimonas marina*, *Bacillus megaterium* and *Bordetella petrii*	Siddiqqui (2017)
		Zoogloea ramigera	Adibayo and Obiekezie (2018)
2	Kitchen waste	*Xanthomonas* sp. and *pseudomonas* sp.	Zaved et al. (2008)
		Actinomycetes	
3	Municipal solid waste	*Micromonospora* sp., *Streptomyces* sp., and *Actinomyces* sp.	Stofella and Kahn (2001)

extremophiles are the most important source of the enzymes, whose specified properties are assumed to result in novel process application (Kumar and Takagi 1999). The use of enzymes is not a new phenomenon, their existence was linked with the ancient Greece where they were using enzymes in cheese making, brewing, baking and alcohol production. With the availability of engineered enzymes the application of enzymes are increased and new possibilities for industrial process have emerged with better knowledge and purification of enzymes (Beg et al. 2003). It is estimated that the worldwide sales value of industrial enzymes is about $1 billion.

Bacterial Proteases This enzyme works on the degradation of proteinaceous part of the biological materials. In municipal solid waste there is a huge amount of proteinaceous part of solid waste which are decomposed by such enzyme (Karigar and Rao 2011). Bacterial protease is one of the largest three groups of industrial enzymes with 60% of total worldwide enzyme sales. It is expected that in the industrial market the supremacy of protease is increased year by year (Rao et al. 1998). The protease enzymes which are active in neutral to alkaline pH conditions are called alkaline protease enzymes (EC.3.4.21–24,99). These are either have serine protease (serine centre) or metalloprotease (metallo-type) and the most important groups of enzymes so for exploited are alkaline serine protease (Gupta et al. 2002). Due to the high catalytic activity, very specific to the substrate, economic viability these enzymes have more advantages over the conventional chemical catalysts. Because of two-third part of detergent industry, microbial alkaline protease enzyme dominates the worldwide enzyme market. However, enzyme production is inherent property of all microbes yet only those microbes are commercially exploited which produces a good amount of extracellular protease. The alkaline proteases isolated from Bacillus sp. has a good industrial potential because of its biochemical diversity

and its wide application in detergent industry, food and tannery industries, resolution of amino acid mixtures, silver recovery, medical formulation and processes like waste treatment (Agrawal et al. 2004).

Bacterial Lignin Peroxidases Monomethoxilated, dimethoxilated and non-methoxilated phenyl propanoid subunits of lignin complex are observed (Martinez et al. 2005). This enzyme is present in secondary cell wall of plants, where it fills the space between pectin components, cellulose and hemicelluloses, due to this cell wall becomes more rigid and hydrophobic. Lignin is very helpful in finding comprehensive protection and strength from pathogens to the plants (Rubin 2008; Kumar et al. 2008). At present a huge amount of lignin and lignin related compounds are generated as waste effluent from paper and pulping industries (De los Santos Ramos et al. 2009). In the near future this amount is expected to be increased due to the aim of recent development of replacing fossil feed stocks with lignocellulosic biomass for the production of chemicals and fuels. Usually, lignin components remain as a low-value waste stream because the biorefinery process is only applicable on the hemi-cellulosic part (Stewart 2008).

The lignocellulosic biomass while incinerated generate heat and power (Zaldivar et al. 2001; Ragauskas et al. 2006; Himmel et al. 2007). For the valuable chemicals like substituted aromatics, to expand on the range of products which can be obtained from lignocellulosic biomass, instead of being incinerated for energy and heat the lignin components can be used as raw material. For lignin depolymerization, enzymes could give a most effective and specific alternative.

Besides these enzymes there are many other bacterial enzymes like bacterial oxygenases, monooxygenases, bacterial deoxygenase, bacterial laccases, bacterial peroxidases, bacterial hydrolytic enzymes, bacterial lipases, bacterial cellulases which are reason for the biodegradation of MSW (Karigar and Rao 2011).

4.5.2 Fungi in Decomposition

Fungus can metabolise about all type of organic compound present in the waste and play an important role in its stabilisation. Except that the fungi do not except under unusual environmental conditions, they have predominance over the bacteria. Since the fungi do not form a tight compact flock and settle easily, the filamentous nature of many of the fungi present in the organic waste makes them not desirable. Due to this reason many efforts are expanded to make the environmental conditions more favourable for bacteria predomination than for the predomination of filamentous fungi. The filamentous fungi are dominating over the bacteria at low pH, at low oxygen tension and at low concentration of nitrogen.

The condition of low oxygen tension is due to the low oxygen supply or from a high organic load resulting in the demand to enhance the supply. In this condition, metabolism does not proceed to water and carbon dioxide but stops with the formation of organic alcohols, aldehydes and acids under reduced oxygen levels.

Table 4.2 Bacteria identified for decomposition of waste

S. No.	Source	Fungi	References
1	Municipal solid waste	*Aspergillus* sp.	Miller (1996)
		Mucor sp.	Epstein (1997a, b)
		Humicola sp.	Wassenaar (2003)
		Pichia sp., *Dipodascaceae* sp., *Thermomyces* sp., and *Rhizomucor* sp.	Hultman et al. (2010)
		Thermomyces lanuginosus	Abdullah et al. (2014)
2	Agricultural waste	*Pleurotus* sp.	Theradimani and Sankaralingam (2012)
3	Empty fruit bunches and palm oil mill effluent	*Aspergillus niger* and *Trichoderma virens*	Empty fruit bunches and palm oil mill effluent
4	Polyester polyurethane	*Fusarium solani* and *Candida ethanolica*	Zafar and Robson (2013)
5	Organic waste	*Aspergillus* sp., *Alternaria* sp., *Mucor* sp., *Cladosporium* sp., and *Penicillium* sp.	Shouche et al. (2014)
		Saccharomyces sp.	Galitskaya et al. (2017)
6	Food manufacturing wastes	*Botrytis* sp.	Gordillo et al. (2017)

The organic acid depresses the pH to the more favourable range for fungi if the system lacks sufficient buffer. Thus, it can be observed that pH and low oxygen tension can be associated. The optimum pH of many fungi is about 4–5 but there are many bacteria that grow well at such pH and compete with fungi. Per unit mass of protoplasm fungi need less nitrogen as compared to bacteria. The fungi are capable of making more biomass from the wastes as compare to bacteria in nitrogen deficient wastes and thus fungi predominate over the bacteria. Fungi range from 5% to 6% while bacteria average range of nitrogen approximately 10% to 12%. Fungi will be found and will add in the stabilisation of the organic matter under normal environmental conditions. But the fungi are of secondary importance and will not predominate (Adibayo and Obiekezie 2018).

Many fungal species that helps in decomposition are isolated and identified by many researchers and are shown in the following Table 4.2.

Most of the fungi survive in the temperature range of 5-350C (mesophilic) in nature but some can grow at temperature of up to 600C and thermophilic in nature (Dix and Webster 1995). Thermophilic fungi are present in compost along with the thermophilic bacteria and perform an important role in composting (Gray et al. 1971). A pure bacterial community can be used in place of mixed fungal–bacterial community when the temperature exceeds 65 °C (Epstein 1997a, b). Fungi re-grow in the compost after cooling but it remains unknown that whether these stem from

vegetative cells or spores that survived the composting process or comes from the environment. Fungi are the most important community of microbes as the mixed culture of bacteria and fungi increase the rate of decomposition in both process that is early and late decomposition (Gray et al. 1971). In processes where conditions are very extensively, fungi are suitable degraders because they existing properly in dry conditions and some are also active in oxygen-limited conditions because they are facultative anaerobic (Dix and Webster 1995). Besides this fungi are also called degraders of lignin and cellulose (Tuomela et al. 2000).

4.5.2.1 Fungal Enzyme Production During Composting

Fungal exoenzymes or extracellular enzymes are synthesised within the cell and then released outside the cell, where they perform their work to decompose complex macromolecules into smaller parts to make easy to be taken up by the cell for growth and assimilation (Sinsabaugh 1994). These enzymes degrade complex organic compounds such as cellulose and hemicellulose into simple sugars that enzyme-producing organisms use like a source of energy, carbon and nutrients (Burns et al. 2013). The different enzymes are grouped as hydrolases, lyases, oxidoreductases and transferases (Sinsabaugh 1994). These exoenzymes control soil enzyme activity through efficient degradation of biopolymer.

Upon senescence, plant residues, microorganisms and animals become a source of nutrient and energy for other organisms after interring the dead organic matter pool (Cebrian 1999). Exoenzymes mainly target macromolecules like lignin (oxidases), carbohydrates (cellulases), amino sugar polymers (chitinases), organic phosphates (phosphatases) and protein (proteases) (Allison 2007) and cleaved them in to soluble sugars that are subsequently transported into cells to support heterotrophic metabolism (Sinsabaugh 1994).

Biopolymers require the combined action of diverse microbial community because they are structurally complex in nature and secreted extracellular enzymes (Fig. 4.2) to decompose the polysaccharides into easily assimilable monomers. These microbial communities are found in both terrestrial and aquatic ecosystem because by the heterotrophic soil microorganisms, the cycling of elements from dead organic matter is essential for nutrient turnover and energy transfer in terrestrial ecosystems (Gessner et al. 2010). Extracellular enzymes also aid digestion in the guts of termites (Warnecke 2007), ruminants, herbivores and humans. Microorganisms release energy by hydrolysing plant cell wall polymers that has the potential to be used by humans as biofuel (Ragauskas 2006). Besides this other human use includes bioethanol production (Wackett 2008), waste water treatment (Shackle et al. 2006) and composting (Crecchio et al. 2004).

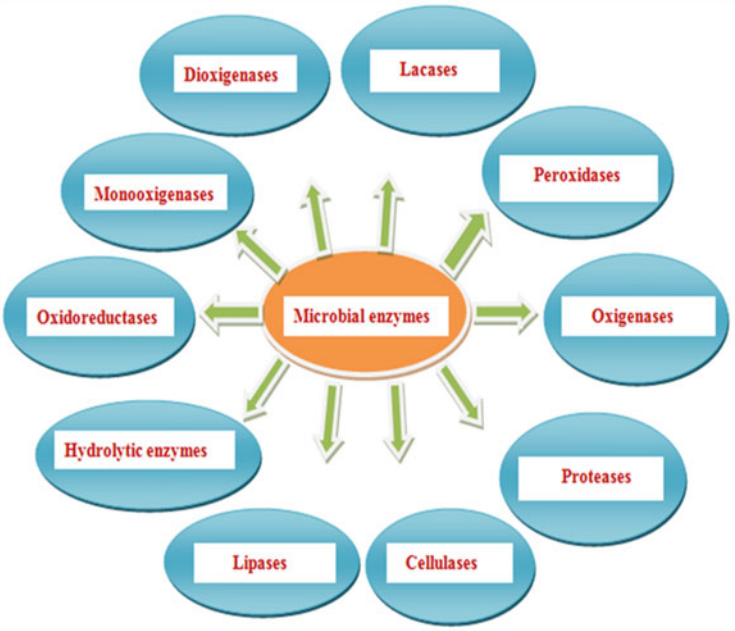

Fig. 4.2 Microbial enzymes responsible for decomposition of organic waste

4.6 Molecular Characterisation of Efficient Composting Bacteria

To determine microbial presence and diversity, a very fast and reliable molecular techniques based essentially on rDNA amplified sequences analyses have provided tools (Cardinale et al. 2004; Ranjard et al. 2000). In microbial ecology, the use of molecular techniques has made possibility the discovery of new microbes (Whitman et al. 1998; Macrae 2000). The identification of bacteria present in the environment have been depended on the determination of 16S rRNA sequence of amplified and cloned genes isolated from the soil microbiota's DNA (Kuske et al. 1997; Lee 1996; Gelsomino et al. 1999; Borneman et al. 1996). Currently the sequences of the gene have been used with success in studies of microbial ecology and phylogeny (Mota et al. 2005). This bacterial characterisation of gene 16S rRNA helps in the identification of many bacterial populations in the soil.

4.7 Application of Bacteria and Fungi in MSW Management

Microorganisms (Bacteria and Fungus) play significant role in maintenance of several natural and artificial phenomenon in the environment. Utilisation of microorganisms and their enzymes is developed for waste management strategy. It

is a great challenge to properly manage the voluminous waste which is generated by the human being through their daily activities, for this government and different environmental agencies are doing their best in whole of the world. In the whole environment microbes have many applications such as energy generation, soil and sewage treatment, oil spillage and radioactive contamination (Adibayo and Obiekezie 2018). Microorganisms are versatile in nature where they perform a variety of essential functions. Many microorganisms are uniquely adapted to specific environmental niches, such as those that inhabit the Dead Sea (Halobacterium) and Chlamydomonas nivalis that causes pink snow (Gallo and Ventresca 2016). Microbes have immense applicability in maintaining the quality of the environment in nature which can be adopted to waste treatment in large scale. They are applied in environmental protection, genetic engineering and municipal and industrial waste treatment. They perform many feasible and cost-effective roles compared to physical or chemical engineering methods (Satyanarayana et al. 2012).

4.8 Future Prospects

The environmental-friendly and cost-effective benefits of microorganisms in waste management, and considering the biodegradable part of the voluminous waste generated, the following are viable recommendation from this discussion,

1. Relevant government parastatals should consolidate on their programs and projects like of the Integrated Solid Waste Management System (ISWM) project that educate residents on proper ways to manage waste, and management of waste collection.
2. Waste separation at the source should be done to allow for more effective and efficient waste collection and management.
3. Microbiological methods of waste management should be developed and utilised, not only for environmental clean-up but also for the value-added products.
4. The waste collection systems should be enhanced for sustainable and more hygienic environmental conditions especially around populated areas of the municipalities.

4.9 Conclusion

The generation and accumulation of MSW create many problems to the human beings and to the whole environment. It is necessity of the day to manage this waste properly. The microorganisms (mainly bacteria and fungi) play a vital role in the proper management of municipal solid waste by secreting various enzymes of different categories and produce a material called compost which is rich in nutrient content and hence enhance the soil fertility and decrease the use of chemical fertilisers and pesticides. The microbial enzymes are very helpful during biodecomposition of municipal solid waste through composting method and it is

safe, efficient and environment friendly technique of municipal solid waste management.

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Electronic Waste Management: Challenges and Opportunities

5

Deepak Pant, Tenzin Dolker, Somvir Bajar, and Anita Singh

Abstract

Managing waste generated from electrical and electronic equipment is recognized as one of the major environmental challenges for the twenty-first century around the world. Every year e-waste is growing at a rate of 3–5% and is estimated to reach 52.2 million tons by the year 2021. Most of the research technologies developed for the recycling of e-waste in pilot or laboratory scale require validation in terms of design, operation, and cost. Proper management can not only protect the environment from pollution but also provide alternate secondary sources for these metals.

5.1 Introduction

Management of the fastest-growing e-waste is a severe problem and has attracted worldwide attention. The electrical and electronic devices have become a part of everyone's day to day life. Moreover, with technological advancement in the electrical and electronic sector unprecedented growth in the product has been

D. Pant (✉)
Department of Chemical Sciences, Central University of Haryana, Mahendergarh, Haryana, India

T. Dolker
Department of Environmental Sciences, Central University of Himachal Pradesh, Dharamshala, Himachal Pradesh, India

S. Bajar
Department of Environmental Sciences, J.C. Bose University of Science and Technology, YMCA, Faridabad, Haryana, India

A. Singh
Department of Environmental Sciences, Central University of Jammu, Jammu, Jammu and Kashmir, India

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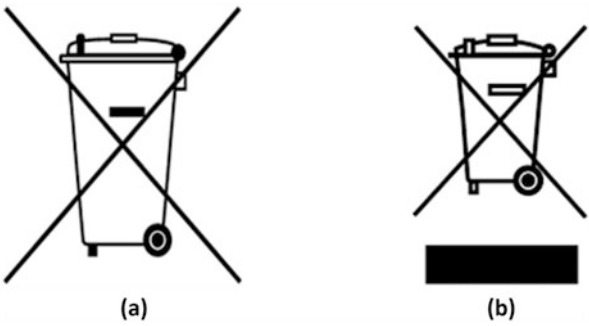

Fig. 5.1 WEEE Symbols for the products introduced in the market, (**a**) between 2002 and 2005 and (**b**) after 2005, when the Directive came into existence

reported from the last three decades. The significant boosting production and expansion to the availability of these items to the consumers in developed and developing countries, the product obsolescence rate is rapidly increasing, which further resulted in the generation of e-waste. An efficient, effective, and eco-friendly e-waste management practices are required to balance the surplus production of electronic gadgets which ultimately leads to waste generation. All the electronic devices when discarded or reached their end-of-life are considered e-waste or waste from electrical and electronic equipment (WEEE). The symbol used for WEEE is shown in Fig. 5.1a, b, which strictly forbids the dumping of e-waste. The WEEE mainly includes computers, mobile phones, televisions, LED, air conditioners, refrigerators, washing machines, and other electrical and electronic items.

These electronic and electrical equipments are a complex mixture of plastics, hazardous metals such as lead, mercury, cobalt, and valuable metals such as gold, silver, copper (Nayaka 2018; Kaya 2016; Monika 2010) (Table 5.1). Considering their hazardous nature, disposal of e-waste in a landfill may cause a detrimental impact on the environment and living beings and further causes loss of valuable metals (Cui and Forssberg 2003). However, precious metals can be recovered and reused for other purposes, turning waste into a useful secondary source from the recycling of the waste and mitigating the environmental impact of WEEE (Widmer et al. 2005). Recycling also prevents the depletion of natural/primary sources. Therefore, the recycling of WEEE is highly significant from both environmental and economic perspectives as many countries have issued regulations for formal recycling of e-waste (Widmer et al. 2005).

The management of e-waste follows rules and regulations formulated by the European Union (EU) in January 2003 and later on the WEEE (Waste Electrical and Electronic Equipment) and the RoHS (Restriction of the Use of Certain Hazardous Substances) Directives have also issued directions for safer use and disposal of e-waste (Nnorom and Osibanjo 2008; Kumar et al. 2005; Realff et al. 2004). According to WEEE Directive, industries are accountable for collecting, recycling, and disposing of waste from electrical and electronic equipment (Widmer et al. 2005). In July 2006, RoHS Directive came into existence, which prohibits the use of cadmium (Cd), mercury (Hg), lead (Pb), hexavalent chromium (Cr (VI)),

Table 5.1 Types and composition of valuable materials in WEEE

S. No.	Type of e-waste	Valuable materials	Reference
1.	Computers	Aluminum, copper, cadmium, gold, lead, mercury, silver	Bazargan et al. (2012)
2.	Mobile Phones	Gold (Au), silver (Ag), and palladium (Pd)	Osibanjo and Nnorom (2008)
3.	Washing machines	Silver particles	Reijnders (2006)
4.	LCD	Indium, tin oxide	Zhang et al. (2015)
5.	Printed circuit board	Tin, silver, non-ferrous and ferrous metals, silicates, ceramics, and polymers; plastics	Işıldar et al. (2018), Yamane et al. (2011), Veit et al. (2005)
6.	Cathode ray tubes (CRT)	Lead oxide and cadmium	Singh et al. (2016), Li et al. (2009)
7.	Capacitors	Palladium, polychlorinated biphenyls (PCBs)	Işıldar et al. (2018)
8.	Photovoltaic (PV) panels	Silicon, cadmium, tellurium, indium, gallium	Cucchiella et al. (2015)

polybrominated biphenyls (PBB), and polybrominated diphenyl ethers (PBDE) in the manufacture of all electronic equipment.

5.1.1 E-waste Scenario in Developing and Developed Countries

Rapid development in technology and boom in the electronic industry has resulted in the generation of an enormous amount of e-waste in developing and developed countries. The waste from this specialized section is increasing with the rate of 3–4% every year. In the nineteenth century, around 14 Mt of e-waste was produced, which reached to 41.8 Mt in the year 2014 (Kumar et al. 2017; Kaya 2016). According to the UN report (2019), waste generated from electrical and electronic equipment has reached 48.5 Mt in 2018 and further, it is expected that it will grow by 2–8 times by the year 2020 (Schluep et al. 2009). Besides, this enormous production of e-waste, only 20% of global e-waste is recycled.

The current generation of e-waste is relatively higher in developed countries rather than in developing countries (Pathak and Srivastava 2019). However, with the rapidly increasing pace in demand for electrical and electronic equipment (EEE) among developing countries, the waste generation will go beyond the developed countries in few years (Zhang et al. 2015). According to UNU and UNEP reports, China is the leading country with its generating potential of 64% of the global e-waste, followed by India (13%) and Brazil (11%). It is expected that e-waste from personal computers (PCs) in developing countries will increase to twice in comparison to developed countries by the year 2030 (Sthiannopkao and Wong 2013; Yu et al. 2010). The technology in the telecommunication sector is rapidly evolving and with the availability of EEE devices and changing technology (Ala-Kurikka 2015),

while the life expectancy of electronic products is continuously decreasing (Zhang et al. 2012). These factors have resulted in the availability of e-waste for disposal, especially at dumpsites and landfill sites. The rapid economic growth is also responsible for increasing amounts of WEEE generation in Asian countries. Besides, Asian countries have emerged as an open market for the disposal of e-waste generated globally. The absence of proper obligation on unauthorized vendors and importer has stimulated the transboundary flow of e-waste from the different parts of the world. The movement of hazardous components from e-waste in developing countries has also encouraged the world to initiate the enactment and implementation of the Basal Convention in 1989 to control international trading of WEE and other hazardous material.

5.2 Types of WEEE or E-waste

The e-waste or waste electrical and electronic equipment (WEEE) is one of the complex wastes streams that needs a better understanding before the finalizing recycling, reuse, and disposal opportunities. The WEEE Directive 2002/96/EC has described EEE as devices/tools or equipment whose working either based on electric current or electromagnetic field or involved in production, transfer, or measurement of such currents and electromagnetic fields. The waste produced by electrical and electronic gadgets is quite different from traditional municipal solid waste. Considering their heterogeneous and complex nature, WEEE comprises a wide range of products to meet a diversity of associated functions. Moreover, the electrical and electronic equipment have great variety in terms of composition, covering several valuable as well as hazardous substances. The conventional mechanism and policies available in different countries are more suitable to handle traditional municipal waste and cannot be followed for disposal and management of e-waste. The wide variety of hazardous components in the WEEE stream may be harmful to human health and the environment. Moreover, the valuable components can be recovered from the waste and can be reutilized as raw material. The base metals in the e-waste can be recovered up to 90%, whereas precious metals can be recycled up to 97–98% from the electronic waste stream (Huisman 2003). Moreover, it has been revealed that the environmental impacts of recycling of e-waste are negligible in comparison to the primary production of various raw material utilized for the production of electrical and electronic items (Robinson 2009; Hischier et al. 2005). Such observation indicates the significance of recycling as environmentally perfect option management of WEEE and diminishing the negative impact on human health and the environment. In this context, the categorization of different items of e-waste is vital to improve the e-waste statistics for its safe, secure, and sustainable management.

However, no universal acceptance of the definition and applied scope have been identified across the globe. Mostly, e-waste is misinterpreted as waste comprises computers and related IT equipment only. Many prevalent definitions have been reported to describe the WEEE and e-waste (Widmer et al. 2005). As per OECD,

e-waste is defined as "any appliances using an electric power supply that has reached its end-of-life." "Any appliances using an electric-powered appliance that no longer satisfies the current owner for its original purpose" is called as e-waste (Sinha-Khetriwal et al. 2005). As described earlier, WEEE comprises the wide variety of components covering both "white" goods (e.g., refrigerator, washing machine, microwaves, etc.) and "brown" goods (e.g., computer, radio, television, etc.) that remain no longer in use for their current owner and reached to their end-of-life.

Within the principle of harmonized scope in the WEEE (Waste Electrical and Electronic Equipment) Directive and RoHS (Restrictions on Hazardous Substances) Directive, variations in scope have been observed due to differences at national implementation level and defining the product list of used by Producer Responsibility Organizations. In February 2003, the European Community Directive 2012/19/EU and WEEE Directive in association with RoHS Directive 2011/65/EU on WEEE, became European Law. The WEEE directive was formulated to frame guidelines for enactment and achievement of targets reading collection, recycling, and recovery of all types of electrical and electronic goods. Simultaneously, the RoHS Directive imposed restrictions on the European manufacturers about the material content used for the manufacturing of electrical and electronic gadgets.

The European Council has implemented a symbol of "crossed-out wheelie bin with or without a single black line underneath the symbol" to represent electrical and electronic equipment. The black line underneath the symbol highlights the EEE goods, which have been introduced in market after 2005 when the Directive came into existence. Such EEE items are counted as non-historic WEEE and the responsibility to provide provision for safer management of e-waste, covering collection, recycling and disposal, is governed by producers/distributors. Whereas, EEE goods, which are manufactured and available in the market between 2002 and 2005, are counted as "historic WEEE" and represented through the WEE symbol without the black line. Such WEEE fall outside reimbursement via producer compliance scheme and the responsibility to make provision for their recycling is maintained by the owner itself.

The categories of EEE (electrical and electronic equipment) specified by the EU WEEE Directive (EU Directive 2002/96/EC) are the most widely accepted classification of e-waste. The Directive covers both households as well as professional EEE, however, the e-waste generated from the products used at the industrial and commercial level is covered by WEEE Regulations. The Directive has defined a list of EEE under ten different categories in Schedule-I of the WEEE Regulations, following the end-of-life (EoL) management.

The list of different e-waste categories as per WEEE Directive 2002/96/EC is mentioned in Fig. 5.2.

The details of EEE covered in individual WEEE Directive categories are enlisted in Fig. 5.3 (Directive, EC 2012; Directive 2012/19/EU). The WEEE Regulations also concealed professional versions of electrical and electronic items, which are covered in categories 1, 2, and 4 of WEEE Directive. The compliance of WEEE Regulations is not required for the products fall other than these categories.

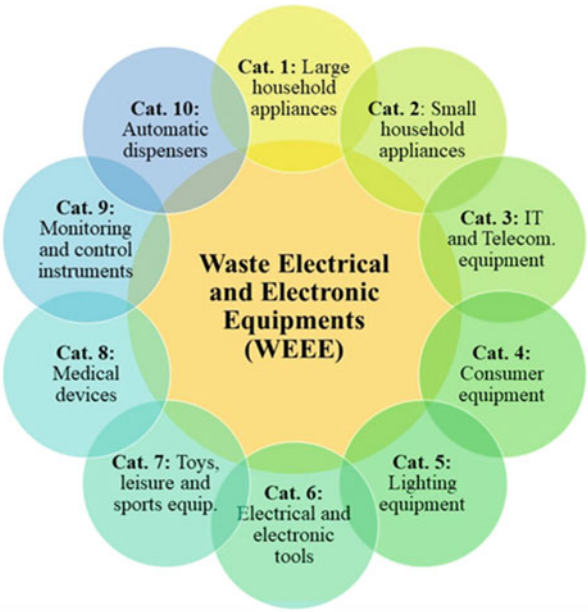

Fig. 5.2 Different categories of Waste Electrical and Electronic Equipment (WEEE)

The scope and categorization of electrical and electronic products classified as per WEEE Directive are comprehensive enough to adopt for the rest of the world. The Directive imposed a flat target for the collection of e-waste for each Member State of the European Union. However, no target has been specified relative to the e-waste generation or consumption pattern within the state, which faded the effectiveness of waste management. Therefore, based on applicability, the WEE Directive 2002/96/EC was foreseen towards the possibility of revision for suitable management of e-waste. Moreover, the Directive was set to attain the mandatory targets of e-waste collection, reuse, recycling, and recovery by the end of the year 2008. In subsequent consequences, the Directive was amended and recast in the form of WEEE Directive 2012/19/EU in 2014. The revised directive was more focused to strengthen the e-waste statistics, their collection, treatment, overall management and check on the illegal export of electrical and electronic equipment, disguised as "used equipment." With this new directive, the WEEE categories have been revised and recategorized from 10 to 6 categories.

These recast categories of WEEE Directive 2012/19/EU are mentioned in Fig. 5.4

There are a wide variety and variability of EEE products, which are available in the market. However, despite categories documented in EC-WEEE Directive, 2012, almost every country has its scope of e-waste categorization to tackle with challenges of EEE waste management. It leads to the introduction of too many sensible indicators to effectively harmonize the e-waste management process to minimize the threats on human health and the environment. However, the classification system should not be too aggregated that will make a complicated system to interpret the information. Considering all these facts The United Nations University

Fig. 5.3 Classification of electrical and electronic items in different categories as per WEEE Directive 2002/99/EC (in spite of electrical and electronic items, large scale industrial tools are exempted from the category 6 "Electrical and Electronic Tools" of WEEE)

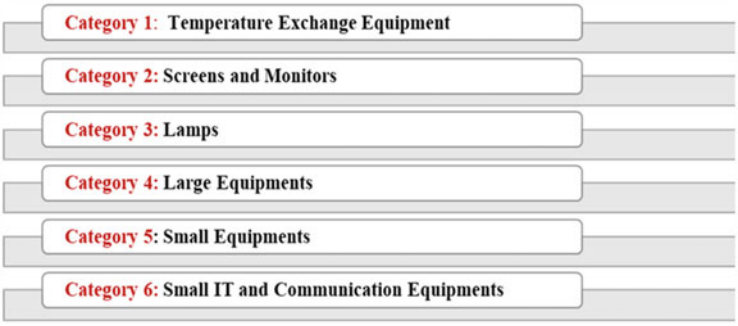

Fig. 5.4 The revised categories of electrical and electronic items waste as per WEEE Directive 2012/19/EU

(UNU) categorized the waste generated from electrical and electronic items based on similar functioning, material composition, average weight, end-of-life characteristics, and distribution of life span. The classification system which is popularly known as UNU-Keys has identified 54 categories of e-waste that are further grouped under the primary categories of old EU-WEEE Directive (Balde et al. 2015). The classification is also linked to reframed categories of the revised WEEE Directive. In general, the UNU-Keys classification acts as a connecting link between the electrical and electronic products with old EC-WEEE Directive and recast WEEE Directive. The details of UNU product classification and its correlation to other WEEE Directive classifications is shown in Fig. 5.5.

5.3 Biological/Microbiological Remediation Techniques

Biological remediation is one of the key concepts that include a gamut of processes that are engaged to remove contaminants from the environment and recover to its original form. These processes may utilize different components which are biological in origin like microorganisms or their enzymes or plants to achieve the desired trait of results, however, the key principle remains the same. Micro remediation and phytoremediation techniques are such widely acceptable bioremediation techniques that are effective to eliminate or transform the hazardous contaminants into non-hazardous form through their activities or their metabolites (Mulligan et al. 2001). In the case of micro remediation, 6 major mechanisms were identified to detoxify the contaminants, including, bioleaching, biosorption, bioaccumulation, biotransformation, biomineralization, and microbially enhanced chemisorption of metals.

5.3.1 Bioleaching

Bioleaching is an attractive technology for the recycling of e-waste due to its low-cost and environmentally friendly nature. The e-waste is complex with the presence of heavy metals, which can be recovered/ recycled with the help of microorganisms. The recovery/recycling of metals from e-waste is an important aspect from both economic as well as environmental point of view. The bioleaching process can be performed by different microorganisms like bacteria and fungi, which secrete inorganic/organic acids or cyanides that help in enhancing enzymatic reactions like oxidation–reduction, proton-promoted mechanism, ligand, and complex formation, etc. (Wu et al. 2019). The bioleaching process involves three mechanisms—acidolysis, redoxolysis, and complexolysis. Table 5.2 shows different microorganism groups and their mechanism of action for the removal of toxic substances and Table 5.3 shows the bioleaching of metals from the e-waste part with the help of microorganisms. Kumar et al. (2018a, b) carried out a bioleaching study for gold and silver using bacterial strain Pseudomonas balearica SAE1 from waste printed circuit boards. The strain was isolated from the e-waste recycling

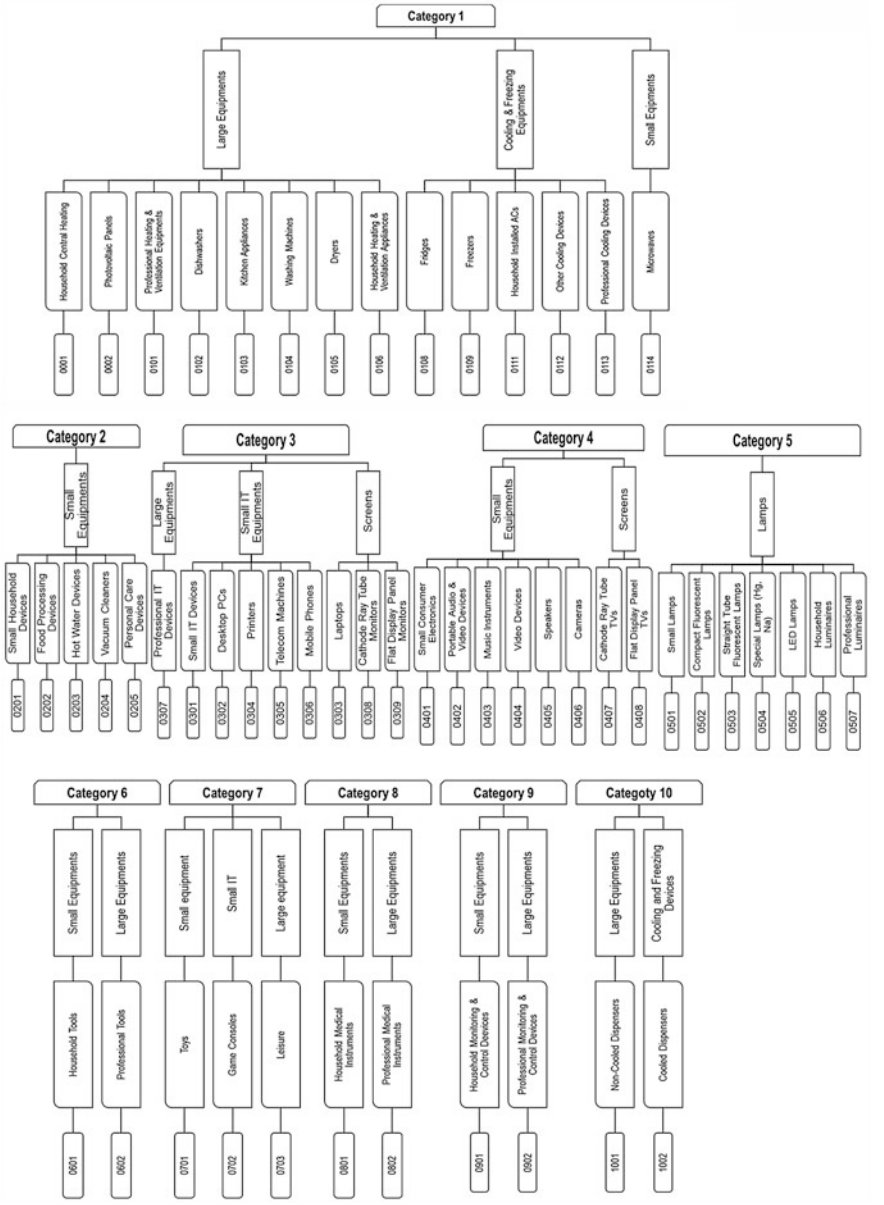

Fig. 5.5 UNU classification of WEEE and their linkage with the WEEE Directives (2002/96/EC and 2012/19/EU) categorization

facility and studied for its metal toxicity tolerance level. Rozas et al. (2017) isolated bacteria from Hymeniacidon heliophila sponge cells and found that microbial strain Hyhel-1 (*Bacillus* sp.) showed the highest bioleaching activity and can be used in

Table 5.2 Microorganism Group and Mechanism involved in bioleaching process

Microorganism Group	Mechanism	Microorganism	Reference
Chemolithoautotrophic Bacteria	Acidolysis and redoxolysis	*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*	Johnson (2018)
Organic acid producing fungi	Acidolysis and complexolysis	*Aspergillus niger*, *Penicillium simplicissimum*	Baniasadi et al. (2019)
Cyanogenic bacteria	Complexolysis	*Chromobacterium violaceum*, *Bacillus megaterium*	Baniasadi et al. (2019)

Table 5.3 Microorganisms involved in bioleaching of toxic metals

Microorganism	E-waste component	Metal	Reference
Acidithiobacillus ferrooxidans	Spent coin cells	Li, Co, and Mn	Naseri et al. (2019)
Bacillus mucilaginosus (BM) and *Bacillus circulans* (BC)	Electrolytic manganese residue	Mn, Co, and Ni	Lv et al. (2019)
Aspergillus niger	Spent lithium-ion phone mobile batteries	Cu, Li, Mn, Al, Co, and Ni	Horeh et al. (2016), Bahaloo-Horeh and Mousavi (2017), Bahaloo-Horeh et al. (2018)
Chromobacterium violaceum (*C. violaceum*)	Waste mobile phone printed circuit boards (PCBs)	Au and Co	Chi et al. (2011)
Acidithiobacillus ferrooxidans	PCB	Cu and Ni	Arshadi and Mousavi (2014a, b), Arshadi and Mousavi (2015)
Aspergillus niger	Waste printed circuit boards	Zn, Ni, and Cu	Faraji et al. (2018)
Acidiplasma sp.	PCB	Au	Rizki et al. (2019)
Consortium of three *Aspergillus* strains (*Aspergillus niger*, *Aspergillus foetidus*, *and Aspergillus carbonarius*)	Spent fluid	Al	Das et al. (2019)

copper recovery from e-waste. The biotic and abiotic factors play an important role in the bioleaching process, so the optimization of factors affecting microbial growth like pH, temperature, dose of nutrients, etc., is equally important (Priya and Hait 2017). Different optimization tools like one factor at a time (OFAT) and statistical models like response surface methodology (RSM) were also used for the optimization of parameters for enhancing metal recovery. Arab et al. (2020) optimized factors like pH, temperature, glycine concentration affecting copper bioleaching for cyanide production by cyanogenic bacteria using OFAT or batch mode experiments and revealed 1.5–5 times more metal recovery under optimal conditions. Kumar et al. (2018a, b) used a central composite design of RSM for optimization of parameters

like pH, temperature, glycine concentration, and pulp density for enhanced recovery of gold and silver from the computer printed circuit boards.

5.3.2 Biosorption

Biosorption is a physicochemical process that includes a sorbate and sorbent where the sorbent is of biological origin. In this process, the sorbate is concentrated with the help of living or dead biomass-based sorbent (Chojnacka 2010; Lovley and Coates 1997). Many organisms like plants, microalgal biomass, bacteria, fungi, etc., as well as biogenic components (like chitosan, alginate), can act as a biological agent for the biosorption process. The main factors like pH, temperature, the concentration of other competing ion/interfering ions (both cations and anions) are physicochemical limiting parameters for the biosorption process (Naja et al. 2009). The biosorption process involves absorption, adsorption, ion exchange, surface complexation, and precipitation mechanisms by which the metal/mixture of metals is recovered once biosorbed onto biomass (Bindschedler et al. 2017). Table 5.4 shows different microorganisms used as sorbent for heavy metal biosorption. Savitha et al. (2010) used Helminthosporium solani for the biosorption of manganese from e-waste and also studied the effect of various environmental factors like metal concentration, pH, and dry biomass concentration on biosorption process. Gurung et al. (2013) used biological origin biosorbent prepared from persimmon tannin for recovery of gold and silver from incinerated samples of PCB's of mobile waste. Sheel and Pant (2018) used a hybrid technique including a chemical agent (ammonium thiosulfate) and biological agent (*Lactobacillus acidophilus*) for biosorption of gold from electronic waste.

5.3.3 Bioaccumulation

Bioaccumulation is a type of biosorption process which includes active as well as passive uptake of metals. The metals are accumulated intracellular to protect against metal toxicity (Bindschedler et al. 2017). The bioaccumulation process depends on

Table 5.4 Microorganisms involved in biosorption of toxic metals

Microorganism	Metal	Reference
Lactobacillus acidophilus	Gold	Sheel and Pant et al. (2018)
Bacillus amyloliquefaciens	U(VI)	Liu et al. (2019)
Bacillus badius AK	Cd(II)	Vishan et al. (2019)
Sargassum sp.	Ni and Cu ions	Barquilha et al. (2019)
Saccharomyces cerevisiae	Cu (II)	do Nascimento et al. (2019)
Scenedesmus sp.	Cr(VI)	Pradhan et al. (2019)
Bacillus cereus	Ag ions	Li et al. (2011)
Bacillus sp. dwc-2	U	Li et al. (2014)

Table 5.5 Microorganisms involved in bioaccumulation of toxic metals

Microorganism	Metal	Reference
Monopterus albus	Cu, Zn, Cd and nickel	Sow et al. (2013)
Geobacillus toebii subsp. *decanicus* and *Geobacillus thermoleovorans* subsp. *stromboliensis*	Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Mn^{2+}	Özdemir et al. (2012)
Geobacillus thermantarcticus and *Anoxybacillus amylolyticus*	Cd^{2+}, Cu^{2+}, Co^{2+}, and Mn^{2+}	Özdemir et al. (2013)
Vanadium-resistant bacteria V-RA-4 and S-RA-6	Vanadium	Ueki (2016)
Saccharomyces cerevisiae	Cu^{2+}, Co^{2+}, and Cd^{2+}	Brady and Duncan (1994)
Aspergillus foetidus	Al, Co, Cr, Cu, Fe, Mg, Mn, Ni, and Zn	Ge et al. (2011)
Candida tropicalis	Copper	Gönen and Aksu (2007)
Corynebacterium glutamicum MTCC 2745	As (III) and As (V)	Podder and Majumder (2018)
Aeromonas hydrophila	Pu and Am	Giesy and Paine (1977)

organisms behavior towards metal and physicochemical factors. This approach includes the absorption and concentration of contaminants within the organism. The processes involved in bioaccumulation include—intracellular accumulation and oxidation/reduction reactions (Yilmazer and Saracoglu 2009). The metals accumulation in fruiting bodies of fungi is a well-known example of the bioaccumulation process, but this process is species-specific, e.g., Ag accumulation in fruiting bodies of *Agaricus bisporus* (Byrne et al. 1991). Table 5.5 shows few studies on bioaccumulation of heavy metals by microorganisms. Shang et al. (2013) carried out bioaccumulation studied of polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) from e-waste dismantling area in China using earthworms. The authors also concluded that earthworm showed different bioaccumulation characteristics towards different studied contaminants. Wu et al. (2019) used apple snail as bioindicator for the bioaccumulation study of polychlorinated biphenyls (PCBs) from an abandoned e-waste site in China and found that apple snail was having 11-fold higher mean PCB concentration as compared to reference site. Liu et al. (2020) studied bioaccumulation and biomagnifications of hexabromocyclododecane occurrence in insects, in terrestrial birds, frogs, toads, lizards, etc., collected from a former e-waste contaminated pond and its surrounding area.

Table 5.6 Microorganisms involved in biotransformation of toxic metals

Microorganism	Metal	Reference
Alcaligenes strain	Mercury (Hg), lead (Pb), cadmium (Cd), nickel (Ni), arsenic (As), tin (Sn), selenium (Se), zinc (Zn), chromium (Cr), and copper (Cu)	Gupta and Nirwan (2015)
Aspergillus flavus	As (III)	Mohd et al. (2019)
Azospirillum brasilense	Selenite	Vogel et al. (2018)
Clostridium sphenoides	Bidentate Fe(III)–citrate complex	Francis (1998)
Desulfovibrio desulfuricans	U(VI)	Ganesh et al. (1997)
Shewanella alga	U(VI)	Ganesh et al. (1997)
Clostridium sphenoides	U(VI)	Francis (1998)

5.3.4 Biotransformation

This approach includes the change of a substance from one chemical form to another chemical form by chemical/microbial reactions by which the oxidation state of the toxic metal is changed. Robrock et al. (2009) investigated the biotransformation ability of polybrominated diphenyl ethers degrading bacteria Rhodococcus jostii RHA1 and Burpkolderia xenovorans LB400. Chen et al. (2014) carried out a field study near e-waste site in china on enantioselective metabolism of chiral PCBs in plants and found that atropisomeric PCBs occur in plant leaves. Table 5.6 shows few studies on biotransformation of metals with the help of microorganisms.

5.3.5 Phytoremediation

Phytoremediation also known as agro-remediation, botano-remediation, or green remediation (Ashraf et al. 2019) refers to the utilization of plants and associated microorganism for removal of various organic and inorganic pollutants from the environment (Jacob et al. 2018). In 1983, this concept was first introduced and to date, this is one of the best approaches for removal of low to average contaminants from the environment. Phytoremediation is a green, sustainable, socially acceptable, environmentally friendly way to detoxify contaminants. This approach uses plants for toxic substances removal and many plant species can accumulate toxic substances in their vegetative as well as reproductive parts (Kotrba et al. 2009). Different plants like Populus, Salix, Picea, Pinus, etc., were successfully studied for pollutant removal (Ashraf et al. 2019). There are different mechanisms by which plants remove toxic substances like phytoextraction, phytofiltration, phytovolatilization, phytostabilization, etc.

5.3.5.1 Phytoextraction

The phytoextraction process is also known as phytoaccumulation or phytoabsorption, where contaminants removal from soil and wastewater by plant roots and transported to the aerial parts of the plant (Lam et al. 2016). Some plants are having a natural property for the accumulation of a higher dose of metals known as hyperaccumulators. These hyperaccumulators can accumulate 100–1000 times higher concentrations of metals as compared to normal plants and transport metals to their aerial organs (Khan et al. 2019). Natural and chemically induced phytoextraction are two approaches for the extraction of metals from water and soil. In a natural or continuous process, metals are extracted by the root system of plants and transported in aerial parts of the plant. The harvested plant can be used for the biogas process, which can be used as an energy source. The harvested plant can be combusted and metals can be recovered from combusted material, a process called phytomining (Salam et al. 2019). This approach reduces the concentration of heavy metal concentrations and contaminants in soil by extracting those using plants without affecting the soil properties. Fast-growing energy crops (e.g., Salix) can be used for phytoextraction purposes due to their extensive root system, high biomass yield, and rapid growth phytoextraction purpose (Salam et al. 2016). Yang et al. (2019) studied Cd removal with the help of the tobacco plant.

5.3.5.2 Phytofiltration

In this approach, the contaminants are absorbed by the plant roots from an aqueous medium like surface and groundwater and wastewater. In phytofiltration, when roots are used for absorption, the process is called rhizofiltration; when seedlings are used, the process is named as blastofiltration, while caulofiltration when plant shoots are used (Ashraf et al. 2019).

5.3.5.3 Phytovolatilization

The process phytovolatilization, comprises of conversion of contaminants/metals into less toxic volatile form before their release into the environment (Chatterjee et al. 2013). This technique is best suited for organic metals and a few inorganic metals (Patel and Kasture 2014). In this approach harvesting of plants is not required as the transformed less toxic volatile form is directly released into the environment through the stem and leaves of the plant. This method is promising for the removal of mercury (Hg) and selenium (Se) (Jacob et al. 2018; Shahid et al. 2018).

5.3.5.4 Phytostabilization

This process is also known as phytoimmobilization or phytorestoration process. In this approach, the toxic metal is immobilized using plant and due to reduced mobility and bioavailability, the leaching and entry of heavy metal in groundwater and food chain are prevented (Barik et al. 2017; Khalid et al. 2017). In this approach, the plants have an extensive root system for absorption and accumulation of heavy metals. This process is achieved by absorption and accumulation by roots, adsorption onto roots, and precipitation within the root zone or reduction in metal valence in the rhizosphere (Wuana and Okieimen 2011). The main objective of this process

is the reduction in water percolation, decrease migration of contaminants, and reduce soil erosion (Akhtar et al. 2013).

5.3.5.5 Biomining

Biomining is an interesting technique of processing and extraction of specific metals from their ores using biological agents like bacteria (e.g., *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus*, etc.), fungi (e.g., *Aspergillus niger*, *Penicillium simplicissimum*). This process of mining is used for the extraction of metals like Cu, U, Au, Mn, Cd, etc., (Kathi and Padmavathy 2019). The microorganisms involved in biomining process gain energy by breaking down of minerals into their constituent elements. Extraction of metals from oxidized ores and selective removal of metals from waste streams of e-waste using acidophiles is a new technique of metal recovery (Johnson 2018). Schippers and sand (1999) studied that two indirect mechanisms either thiosulfate or polysulfides are involved in bacterial leaching of metal sulfides. Das et al. (2011) extensively reviewed manganese biomining including manganese mineralogy, manganese geomicrobiology, and manganese-oxidizing and reducing microorganisms. Liao et al. (2019) used a mixed culture of *Leptospirillum ferriphilum* and *Acidithiobacillus thiooxidans* for the biomining of low-grade base-metal sulfide tailings and found that mixed culture gave better metal recovery as compared to pure culture. Maass et al. (2019) synthesized iron-containing nanoparticles from coal tailing by *Rhodococcus erythropolis* ATCC 4277 free cells for reuse of sulfur minerals from coal mining.

5.4 Lab-Scale and Pilot Studies

E-waste is a complex mixture of different organic and inorganic materials and some of them are hazardous, rest are precious or rare earth metals (Sahin et al. 2015), which make the recycling of e-waste complicated process. Most of the research technologies developed for the recycling of e-waste is still in pilot or laboratory scale. To scale up processes, batch laboratory experiments were performed to optimize the design, operation, and cost of the process. In lab-scale studies, all experiments environmental conditions and laboratory resources play a significant role, while in the pilot-scale process, the relative environmental impacts were greatly reduced (Pérez-López et al. 2014).

The physical method (pyrometallurgy and hydrometallurgy) and the biological methods (bacteria and fungi) were used for the recycling of metals and non-metals in e-waste. Both physical treatment process pyrometallurgical (mostly) and hydrometallurgical (to a smaller extent) processes were applied for recovery metal from e-waste (Cui and Zhang 2008; Xu et al. 2008). Though, the majority of these processes have not reached the pilot and industrial scale. In this section, some of the processes that were used at the pilot scale and commercial level were discussed. Li et al. (2009) studied a case of Suzhou pilot-scale project developed for the treatment of computer waste. The pilot project comprises the semi-automatic

disassembling system; the thermal separator for CRT leaded and panel glasses with an electrical thermal belt; the combined system for recycling PCBs, LCD screen, and Li-ion battery, respectively. Physical treatment process was also used for recycling e-waste. Though, the loss of metal and the high energy consumption is a huge hindrance to its scale-up (Chancerel et al. 2009).

The development level of the available technologies for e-waste recycling is emphasized below:

- Smelting and pyrolysis techniques of pyrometallurgical processes require the heating of WEEE at very high temperatures (up to 1500 °C) to separate materials and are used at the commercial level. Lemieux et al performed a pilot-scale rotary kiln incinerator experiment to investigate the emissions and operational behavior during the incineration of e-waste.
- Hydrometallurgical processes are still in the stage of preliminary investigation and laboratory research scale.

In recent years, bioleaching technology has been applied for the recovery of metals from e-waste. Bioleaching technology is based on similar principles where the metabolites and biooxidants such as HCN or organic acid are biologically produced to facilitate metal dissolution (Wu et al. 2019; Willner et al. 2015). Bioleaching technology for the extraction of metals from mines has achieved industrial application; however, in the case of metals recovery from e-waste is still at a laboratory scale (Bai et al. 2019).

5.5 Conclusion

E-waste production is massive and is increasing each year resulting in environmental pollution. The complex toxic material present in e-waste needs to be efficiently remediated from the disposal sites to minimize their impacts on human health and the environment. The biological techniques including microbiological hybrid processes have the future to reduce the toxicity associated with e-waste. Simultaneously, thier efficiency could be enhanced on using these biological techniques in combination with different methods such as nanoparticles or with some non-polluting biodegraded ligands. Moreover, there is an urgent need to bridge the knowledge gap through further continuous efforts on reducing, reuse, and recycling strategies for effective and sustainable e-waste management.

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Heavy Metal Pollution: An Insight Towards Its Infiltration, Impact and Remediation

6

M. K. Ramkumar, K. Preeti, K. Varuna, Maulin P. Shah, and S. Senthil Kumar

Abstract

With the environment being constantly prone to threats, the sources of pollution have increased drastically over the past few years. Heavy metals are one of those pollutants that are naturally present in the environment and their accumulation has increased due to human interventions. These heavy metals find their way in to the environment through industrialization. These processes had affected the biological community in such a way that heavy metals are part of the living system. Although certain metals fall under the category of essential components of biological mechanisms, most of the heavy metals are toxic to living organisms. These heavy metals cause oxidative stress in living organisms and also interfere in major molecular mechanisms and change the functional properties, thereby affecting the cell organelles. Due to the adverse effects of these heavy metals,

M. K. Ramkumar
Geobiotechnology Laboratory, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India

National Institute for Plant Biotechnology, New Delhi, India

K. Preeti
Department of Energy and Environmentl, TERI SAS, TERI University, New Delhi, India

K. Varuna
PG and Research Department of Biotechnology and Microbiology, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India

M. P. Shah
Industrial Waste Water Research Lab, Division of Applied & Environmental Microbiology Lab Enviro Technology Limited, Ankleshwar, Gujarat, India

S. S. Kumar (✉)
Geobiotechnology Laboratory, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India

PG and Research Department of Botany, National College (Autonomous), Tiruchirappalli, Tamil Nadu, India
e-mail: senthil@nct.ac.in

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developed countries have formed several organizations that emphasize on the permissible limits of these heavy metals emission into the environment. Yet, the levels of heavy metals are not contained. Bioremediation is a promising approach to deal with this issue since this method is highly sustainable. Microbial bioremediation is a highly adopted method to tackle the heavy metal polluted area following different methods. This book chapter discusses the different ways by which these heavy metals are leached into the environment, their mode of affecting the biological systems and the different remedial process that could be incorporated to tackle heavy metals pollution.

6.1 Introduction

Physical conditions and external components encompassing living organisms and influencing their survival are collectively termed as environment (Asati et al. 2016). Pollutants are substances that affect the equilibrium of the environment, hindering survival resulting in increased mortality. These pollutants find their way into the environment through all possible routes. There are different types of pollutants and they are not always man made. They can be naturally occurring as well. Nonetheless, they could be harmful depending on their site of occurrence and concentration. Around 75% of the elements in the periodic table are classified as metals, whose properties include 'metallic luster, malleability, sonority, ductility, electrical conductivity and the ability to lose electrons forming basic oxides'. In terms of biology, they can be further divided into metalloids (semi-metals), light metals, heavy metals, essential metals, toxic metals, abundant metals, trace metals and micronutrients (Duffus 2002). Heavy metals include a group of 19 elements with similar physical and chemical properties and are remarkably distinct from the remaining 97 metals (Rajeswari and Sailaja 2014). Though we have been using the term 'heavy metal' for almost thousand years, there exists no definite description that would make us understand the term undoubtedly. Metals with physical properties such as specific gravity greater than 5 g/cm^3, density fivefold higher than water (RoyChowdhury et al. 2018), and chemical properties such as high molecular weight and ability to produce highly reactive chemical entities like free radicles, are called as heavy metals. However, the chemical properties are prioritized during classification.

Although certain metals such as zinc and iron, termed as micronutrients, are essential for biological processes, heavy metals are known to induce toxicity even at low concentrations (RoyChowdhury et al. 2018). Even these essential heavy metals could become toxic beyond permissible levels (Pandey and Madhuri 2014). Heavy metals are natural constituents of the earth's crust. And natural phenomena like weathering and volcanic activities, anthropogenic activities including mining, smelting, industrial and agricultural use have increased the abundance of heavy metals in the environment either by releasing or concentrating them (RoyChowdhury et al. 2018). Heavy metals have infiltrated the environment and modified the living organisms in such a way that life forms are now a combination of

both organic and inorganic substances. Heavy metals, which were found in 'trace' before, have now become a serious threat to the environment due to human interventions. Since the middle of nineteenth century, production of heavy metals increased steeply for around 100 years owing to their regular emission into environment (Järup 2003). Heavy metal seeps into the environment through various channels. Atmospheric emission tops all of them due to easy dissemination which affects almost all of living organisms and is of human health concern.

In order to control heavy metal pollution, numerous committees have been formed to create rules and regulations on the emission of heavy metals. The Aarhus Protocol on Heavy Metals was formed in 1998 to control the emission of heavy metals with the focus on three major heavy metals, viz. cadmium (Cd), lead (Pb) and mercury (Hg). The Protocol set guidelines to restrict emissions from various industrial sources, selected combustion and waste incinerators. Furthermore, it laid down stringent limit values for emissions from stationary sources and suggested best available techniques (BAT) for these sources. At the end of twentieth century, however, it decreased and fell by over 50% from 1990 to 2000 but this progress was confined only to developed countries (Ilyin et al. 2004). Despite the measures, pollution by heavy metals has not been successfully contained. This book chapter is focused on expounding the toxic effects of heavy metals with special emphasis on the molecular mechanism behind their toxicity and the different remediation processes that can be utilized to eliminate the prevailing heavy metals in the environment.

6.2 Heavy Metal Ingression into the Soil Ecosystem

Since soil cannot be confined, control measures for soil contamination, instigated by the excess concentration of heavy metals, have never been strict unlike water and air contamination. However, in recent decades, the developed countries have taken soil contamination into consideration and thus soil contamination by heavy metals has now become a prime focus worldwide (Su 2014). The list of heavy metals has increased in the recent years, apart from already existing ones (Tchounwou et al. 2012; Zojaji 2014). Generation rate of heavy metals via man made cycles is more rapid than natural cycles. The concentrations of the metals in discarded products are relatively higher compared to receiving environment. Furthermore, the chemical forms in which they are present in the soil can be changed leading to depreciation in severity (Wuana and Okieimen 2011). The sources by which these heavy metals find their way into the soil ecosystem are as follows:

6.2.1 Natural Sources

Weathering of Rocks During pedogenesis, heavy metals like Cr, Sn, Hg, Pb, etc. are naturally added to the top soil through weathering and breaking of the parent rock. However, these are considered as trace and are rarely toxic.

Volcanoes Volcanoes are known to release high levels of Zn, Pb, Cu and Mn along with harmful gasses. Winds blowing against volcanoes carry dust rich in heavy metals and add particulate compounds in the surrounding areas apart from eruptions.

Evaporation from Seas Sprays and mists from ocean water carry heavy metals in it. These are released into the atmosphere through bubble bursting and evaporation which in turn results in rainfall enriched in heavy metals. (Nagajyoti et al. 2010).

6.2.2 Agricultural Sources

Fertilizers In order to supply the nutrients which are deficit in the soil, fertilizers are added to ensure robust growth of plants. Commonly these fertilizers are organic compounds along with macronutrients like Cu and Mn. However, certain fertilizers are conjugates of inorganic compounds which contain heavy metals as impurities. With the frequent use of these artificially synthesized compounds, heavy metals such as Cd and Pb get accumulated in the soil, eventually leading to toxicity (Zhang and Zhang 2007; Arao et al. 2010). Phosphoric (P) fertilizers are widely used, since their deficiencies directly reflect on the global productivity (AlKhader 2015). Generally, P fertilizers contain higher concentrations of heavy metals than other fertilizers. These fertilizers are predominantly loaded with cadmium (Cd), lead (Pb) and arsenic metalloid (As) (Galadima and Garba 2012). When these types of fertilizers are used for agricultural practices, Cd easily infiltrates into the soil and could be transported to the crops. The proportion of this event has increased rapidly over years (Boyd 2010). A study revealed that P fertilizers such as diammonium phosphate (DAP), monoammonium phosphate (MAP) and single super phosphate (SSP) encompasses heavy metals such as Cd (0.5–7.9 ppm), Pb (1.8–2.2 ppm) and As (2.8–43.0 ppm) (AlKhader and Abu Rayyan 2014).

Pesticides and Manures Use of pesticides to control the insect attack is yet another widely applied modern day agricultural practice. These pesticides are chemical compounds containing heavy metals like Cu, Hg, Mn, Pb and Zn. For example, lead arsenate and Bordeaux mixture (copper sulphate) have been employed for a long time without check, which has created hot spots for these heavy metals in and around agricultural lands (Wuana and Okieimen 2011). Livestock manures and composts that are applied in agricultural lands obtained from poultry wastes and cattle wastes are reported to contain heavy metals like Cu and Zn, as these metals are an important component in their diet. Prolonged application of these animal wastes as manure in agricultural fields, though considered beneficial, also has side effects like accumulation of heavy metals in the soil (Sumner 2000). A study conducted in the northern region of china revealed that application of animal manures led to the accumulation of Cd, Pb, Zn, As, Cu and Cr in the soil (Zhang et al. 2012a, b). In Austria, usage of poultry and pig manures reflected in elevated levels of Cd in the soil (Sager 2007). Cd content was recorded in extremely high quantity in animal manures in the farmlands of Beijing and Fuxin (Li et al. 2010).

6.2.3 Industrial Sources

Human intervention in nature is the biggest cause of elevated heavy metal levels in soil. During industrial mining and tailing, unearthing of rocks and metal ores results in contamination of surrounding areas with heavy metals present in the ores, which are processed and removed as waste. Due to improper management of this waste, it gets leached into the top soil. There are lot many ways by which industrialization could lead to heavy metal build-up in the soil. Some of the important means by which heavy metals enter the soil are discussed below.

- Atmospheric effluents: Atmospheric heavy metal concentration has increased drastically due to the emission of gases from the industries. These heavy metals get dispersed in the atmosphere, which then deposited on the soil surface through natural sedimentation processes (Zhang et al. 2012a, b). In Sweden, effluents in the form of gas from copper and sulphuric acid factories lead to Pb contamination of the surrounding environment and affected the locality owing to the condensation and precipitation over the time (Lin et al. 1998). A Russian chemical factory dealing with sulphuric acid production was found to emit arsenic along with sulphur and vanadium. Urbanization leading to extensive usage of automobiles had led to the contamination of atmosphere and soil with Pb, Zn, Cd, Cr, Cu, etc. Around 50 μg of Pb per litre of the fuel was exhausted from cars and other heavy vehicles (Su 2014).
- Sewage and solid wastes: The use of sewage water in agricultural field has been known to enhance the soil properties for effective crop yield (Behbahaninia et al. 2009). Wastewater management is an important aspect to be considered in cities. Heavy metals present in wastewater sludge are most likely to be transferred to the soil and subsequently in to the ground water table. Improper maintenance can lead to the deposition of metals and metal containing compounds (Tijani 2009). Though sewage irrigation aids in crop irrigation issues, it depends on the type and processing of sewage. Generally, sewage is categorized as sanitary sewage, chemical wastewater, industrial mining wastewater and urban mining mixed sewage (Su 2014). Heavy metals such as Hg, Cd, Pb and Cr are predominantly present in most of the sewage disposals. Solid wastes that are generated from mining pose a major threat to the environment as they are heavily stocked with heavy metals and have easy access to both soil and aquatic environments. These solid wastes could also be household and electronic wastes which to some extent could be removed by incineration. Even so, huge tons of solid wastes remain untreated (Asgele and Gebremedhin 2015).

6.3 Metabolic Destabilization Due to Heavy Metals in the Living System

Metals are indispensable part of metabolic pathways as they bind to S–H groups of enzymes and are called as 'co-factors'. Nevertheless, research evidences suggest that these metals in quantities more than required would lead to adverse effects in living organisms (Strydom et al. 2006). Metal toxicity leading to impaired metabolism is generally classified into three broad mechanisms as follows:

A. Production of ROS (Reactive Oxygen Species) by auto-oxidation and Fenton's reaction
B. Blocking of essential functional groups in bio-molecules
C. Displacement of essential metal ions from bio-molecules (Schutzendubel and Polle 2002).

Owing to their chemical properties and foreign nature, they escape counter mechanisms such as homeostasis, pass through compartments and bind to specific cellular constituents. This in turn has an effect on cellular organelles like mitochondria, lysosomes, endoplasmic reticulum, nuclei and even cell membranes. Metal ions tend to interact with original metals or displace them from their native binding sites in DNA, nucleic acids and other proteins causing DNA damage and conformational changes resulting in cell cycle modifications, carcinogenesis and cell apoptosis (Tchounwou et al. 2012). Moreover, these heavy metals have huge impact on the microbial community.

Diversity and abundance of soil microbes is an indicator for good soil quality. Their stability could be easily disturbed by infiltration of heavy metals that could cause shifts in density, size, pH, respiration rate and enzyme activity of soil and microbes present in it. Heavy metals indirectly interfere with enzymatic actions, toxicity of which results in inhibition of certain key processes, decreasing the population and activity of microbes. Heavy metals display varied effect on the metabolism owing to the differences in their chemical properties and affinities towards the enzymes. For instance, Cu inhibits β-glucosidase activity, Pb decreases activities of urease, catalase, invertase and acid phosphatase, As affects phosphatase and sulphatase activity. Cd negatively impacts protease, urease, alkaline phosphatase, and arylsulphatase activity. Permissible levels and mechanism of toxicity of significant heavy metals are listed in Table 6.1.

6.3.1 Cadmium

Cadmium affects oxidative metabolism in the following ways:

- Decrease in the levels of glycogen due to higher rates of glycogenolysis resulting from the elevated levels of phosphorylase.

Table 6.1 Details on permissible levels and mechanism of toxicity of heavy metals (Source: Sharma et al. 2014; Asati et al. 2016)

S. No.	Metals	Permissible levels		Toxicity in animals	Toxicity in plants	Toxicity in microorganisms
1	Arsenic	50 µg/day	40 mg/kg	1. Lipoate, an intermediate in Krebs cycle, is depleted by As^{3+} leading to inhibition of oxidative phosphorylation and resulting in ATP depletion 2. As^{5+} hydrolyses stable phosphate ester bond in ATP with arsenate ester bond, depleting ATP reserves 3. Inhibits cellular respiration and uncoupling oxidative phosphorylation leads to cellular energy reduction and eventual cell death 4. Binds to thiol or sulphydryl groups and inactivates over 200 enzymes 5. It modifies gene expression and participates in DNA repair, duplication etc.	1. It is an analogue of P and thus competes with it for uptake. It gets metabolized as organo-phospholipids and arseno-sugars and bio-accumulates in the cells 2. In tomato, it reduces fruit yield and leaf's fresh weight 3. In canola, it stunts growth leads to chlorosis and wilting 4. Arsenic reduces seed germination, decrease in seedling height, leaf area and dry matter production in rice	
2	Cadmium	0.4 µ/kg/day	100 mg/kg	1. Decreases levels of glycogen due to increased activity of phosphorylase which participates in glycogenolysis 2. Increases protease activity leading to excessive proteolysis 3. Enzymes participating in Krebs cycle like succinate dehydrogenase, malate dehydrogenase are decreased and thus impairs	1. Decreases nitrogen fixation and ammonia assimilation in soya beans 2. In wheat, seed germination, reduced root and shoot length 3. Inhibits the activity of Fe(III) reductase thereby reducing photosynthesis 4. Depress activity of nitrate reductase in shoots and so decreases the mobility of the nitrate from roots to shoots	1. Denatures proteins 2. Destroys nucleic acids 3. Hinders cell division and transcription 4. Impacts enzymes like protease, urease, alkaline phosphatase, and arylsulphatase

(continued)

Table 6.1 (continued)

S. No.	Metals	Permissible levels		Toxicity in animals	Toxicity in plants	Toxicity in microorganisms
				mitochondria's oxidative metabolism	5. Negatively impacts the uptake, transport and use of elements like Ca, Mg, P and K resulting in chlorosis, growth inhibition, browning of root tips and finally death	
3	Lead	–	200 mg/kg	1. It demonstrates ability to displace bivalent cations (Ca, Mg, Fe) resulting in changes in cell adhesion, protein folding, release of neurotransmitters by interfering with calcium dependent signalling 2. Lead binds to biomolecules like enzymes having sulphydryl and amide groups, altering their configuration 3. It competes with essential metal ions inhibiting action of enzymes like catalase, and δ-amino levulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase, which are responsible for GSH synthesis	1. Lead bind to sulphydryl groups of enzymes inhibiting their activity 2. Lead acetate inhibits protease and amylase in rice endosperm, inhibiting seedling growth 3. Cell growth is inhibited due to induced stimulation of IAA oxidation 4. Carboxylating enzymes are inhibited thus reducing photosynthetic activity and growth and biomass such as in oats 5. Inhibits root and shoot elongation in Allium sp. and barley 6. It usually gets accumulated in the roots 7. It also decreases protein content, alters water balance and membrane permeability and thus mineral nutrition 8. It is responsible for Inducing oxidative stress	1. Destroys nucleic acids and proteins 2. Inhibits enzyme action and transcription

4	Mercury	1.3 µg/kg	0.3 mg/kg	1. Binds to sulphydryl group of enzyme, affecting heme synthesis in animals 2. It dissociates salts, thereby precipitating proteins and destroying mucosal membranes 3. Induces ROS activity through increased electron mobilization in mitochondria 4. Oxidative stress negatively impacts Ca homeostasis which is essential for activation of several enzymes such as proteases, endonucleases and phospholipases	1. Binds to water channel proteins, obstructing water flow and thus inducing stomatal closure 2. Induces oxidative stress interfering with mitochondrial activities 3. Destroys bio-membranes lipids and cellular metabolism in plants 4. In rice, it decreases plant height, reduces tiller and panicle formation, and reduction in yield 5. In tomatoes it reduces plant height, flowering fruit weight germination rate 6. Chlorosis is noticed on the entire plant on exposure to mercury	1. Denatures proteins 2. Inhibits enzyme action 3. Disrupts cell membrane
5	Chromium	5 µg/m^3	100 µ/kg	1. Cr(IV) is highly mobile and penetrates cell membranes though SO_4^{2-} and HPO_4^{2-} channels 2. Induces production of ROS which denatures proteins and DNA	1. Depresses activity of amylase affecting transport of sugars to the seed, but increases activity of protease, resulting in overall reduction in germination of Cr treated seeds 2. Induces oxidative stress which degrades photosynthetic pigments (chlorophyll and anthocyanins) present in mainly PSI 3. Inhibits electron transport on the enzymes of Calvin cycle	1. Inhibits growth by prolonging lag phase 2. Inhibits oxygen uptake by the micro organism

- Drop in lactate dehydrogenase activity leading to increased lactate levels indicating reduced mobilization of pyruvate to citric acid cycle. Decreased levels of Krebs cycle enzymes such as succinate dehydrogenase and malate dehydrogenase suggesting impairment of mitochondrial oxidative metabolism.
- Enhanced glucose oxidation owing to elevated activity of glucose-6-phosphate dehydrogenase.
- Decreased protein and amino acid levels with increased ammonia, urea and glutamine levels.
- Increased protease activity suggesting increased proteolysis.
- Elevated levels of arginase and glutamine synthetase resulting in production of urea and glutamine through detoxification of ammonia.

6.3.2 Copper

- Inhibitory effect on 8 different phosphofructokinases (PFKs) with a broad phylogenetic range.
- Studies on long-term exposure to copper and in vivo production of CO_2, suggested that activities of 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase could be used as a biomarker for copper toxicity in toad oocytes

6.3.3 Fluoride

Fluoride is known to inhibit a large number of biological processes such as photosynthesis, respiration, protein synthesis and enzyme activities of higher plants, green algae, cyanobacteria and bacteria.

- Fluoroaluminate could bind with ADP, GDP and cations, which causes translocation of ATPases and inhibition of metabolic energy synthesis and release, thereby producing negative effect on physiological processes that require energy.
- It could also cause translocation of ions essential for algal growth and multiplication. The reduction in the number of ATPs at low pH could possibly be attributed to the inhibition of the electron transport chain as it is the major energy producing system in cyanobacteria. The reduction in ATP content also brings about the inhibition of 14C assimilation at low pH. This could lead to the decrease in the availability of carbon skeletons. It is postulated that this is responsible for the reduction in nitrogenase and nitrate reductase activities. This reduction in energy may also lead to an inhibition of other enzymes such as glutamine synthetase and urease (Rai et al. 1996).

6.3.4 Zinc

Despite being an essential element for normal enzymatic functioning, Zn in excess quantities is attributed towards reversible anaemia and damage to pancreas and kidneys.

- The two glycolytic enzymes that might be inhibited by increased Zn concentrations are glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphofructokinase which could result in the build-up of glycolytic metabolites upstream of GAPDH and depleted intermediate products downstream and resulting in lower ATP production.
- Zinc is also known for its inhibition of glycolysis, tricarboxylic acid cycle, electron transport chain and glutamate release through its involvement with α-ketoglutarate dehydrogenase complex (KGDHC), lipoamide dehydrogenase (LADH) subunit of the tricarboxylic acid cycle and photosystems (Cytochrome C1) (Dineley et al. 2003).

6.3.5 Lead

Lead is a highly toxic metal that is abundantly found in various products of day-to-day life. The ill-effects of Pb at higher quantities are listed below.

- The ability of lead metal ions to replace other bivalent cations like Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations like Na^{+} ultimately disturbs the biological metabolism of the cell. The ionic mechanism of lead toxicity causes significant changes in various biological processes such as cell adhesion, intra- and inter-cellular signalling, protein folding, maturation, apoptosis, ionic transportation, enzyme regulation, and release of neurotransmitters. Pb could replace calcium even in picomolar concentration affecting protein kinase C, which regulates neural excitation and memory storage (Flora et al. 2012).
- Pb is involved in the inhibition of glycolysis, heme and globin synthesis thereby influencing the metabolism of erythrocytes.
- Formation of covalent attachments is one of the properties of Pb. These attachments are formed between Pb moiety and the sulphydryl groups present in antioxidant enzymes, which are the most susceptible targets for Pb and which eventually get inactivated. Pb also inactivates glutathione by binding to sulphydryl groups present in it. This results in synthesis of GSH from cysteine via the γ-glutamyl cycle, which is usually not effective in replenishing the supply of GSH (Jan et al. 2015). Similarly, Pb inactivates enzymes like δ-aminolevulinic acid dehydratase (ALAD), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione-S-transferase, which further depresses the glutathione levels (Ahamed and Siddiqui 2007). A few other notable antioxidant enzymes that are rendered inactive by Pb includes superoxide dismutase (SOD) and catalase (CAT). Decrease in SOD concentration reduces the disposal of

superoxide radical, whereas reduction in CAT impairs scavenging of superoxide radical. Apart from targeting sulphydryl groups, Pb can also replace Zn ions which serve as the vital co-factor for these antioxidant enzymes and thereby inactivating them (Flora et al. 2006).

- Lipid peroxidation is another biomarker of oxidative stress and is one of the most investigated consequences of ROS on lipid membranes. The generated free radicals capture electrons from the lipids present inside the cell membranes and damage the cell. Apart from lipid peroxidation, Pb also causes haemoglobin oxidation, which directly causes RBC hemolysis. This occurs due to inhibition of ALAD, which results in an increased concentration of substrate, ALA, in both blood and urine. These elevated ALA levels generate hydrogen peroxide, superoxide radical and also interact with oxyhaemoglobin, resulting in the generation of hydroxyl radicals. Progression of all the above mentioned mechanisms makes the cell extremely vulnerable to oxidative stress and may lead to cell death.

6.3.6 Arsenic

- As generates severe effects by binding with the sulphydryl groups of vital enzymes and depleting lipoate by trivalent arsenite. Lipoate is involved in the synthesis of key intermediates in the Krebs cycle. Hence, the depletion of lipoate results in inhibition of the Krebs cycle and oxidative phosphorylation leading to ATP depletion.
- Pentavalent arsenate, on the other hand, can replace the stable phosphate ester bond in ATP with arsenate ester bond, thereby rapidly hydrolyzing ATP (arsenolysis) and uncoupling oxidative phosphorylation resulting in the depletion of ATP stores. The combined action of inhibiting cellular respiration and uncoupling oxidative phosphorylation results in cellular energy depletion, causing cell death in tissues with high energy requirement.

6.3.7 Mercury

- Mercury binds to sulphydryl groups (–SH groups) of proteins and inhibits heme biosynthesis. Heme is the essential structural component of haemoglobin, myoglobin, and cytochromes.
- Hg is involved in the destruction of mucosal membranes, necrosis of proximal tubular epithelium and precipitation of proteins through dissociation of salts.

6.4 Microbial Remediation of Heavy Metal Poisoning

Remediation is employed to control and minimize heavy metal toxicity in contaminated water, soil and to sustain the living organisms dependent or residing in it. It is essential to ensure their healthy growth and to maintain biodiversity of that region. Figure 6.1 represents the broad classification of remediation based on the mode of operation.

6.4.1 Physical Methods

Techniques used under physical remediation are as follows:

Soil Replacement Contaminated soil is excavated, so that the site could be filled with uncontaminated soil. This is employed only for extreme cases of contamination as it is expensive and requires working in large volumes.

Soil Isolation Using sub-surface barriers, contaminated soil is physically separated from uncontaminated area. It restricts further contamination and used for highly contaminated soils.

Vitrification Bioavailability of heavy metals could be reduced by forming vitreous material from either decomposition or volatilization of organic matter, when heating the soil at temperatures around 14,000C–20,000C. It is easily applicable to lot of contaminants though it is expensive due to high energy requirement.

Electrokinetic Remediation This is an in-situ process where the soil is heated using electrodes, which pass direct current (DC) through damp soil for heavy metals' removal. It is easy, cost effective and maintains the nature of soil (Khalid et al. 2017; RoyChowdhury et al. 2018).

As discussed above, most of the physical remediation methods are generally tedious and expensive.

6.4.2 Chemical methods

It involves the usage of various chemical compounds to remove heavy metals from the area of contamination. Some of the methods are as follows:

Immobilization Heavy metals such as clay, cement, organic compost and zeolites are used to immobilize the soluble forms of metals that are bio-available, by forming stable complexes.

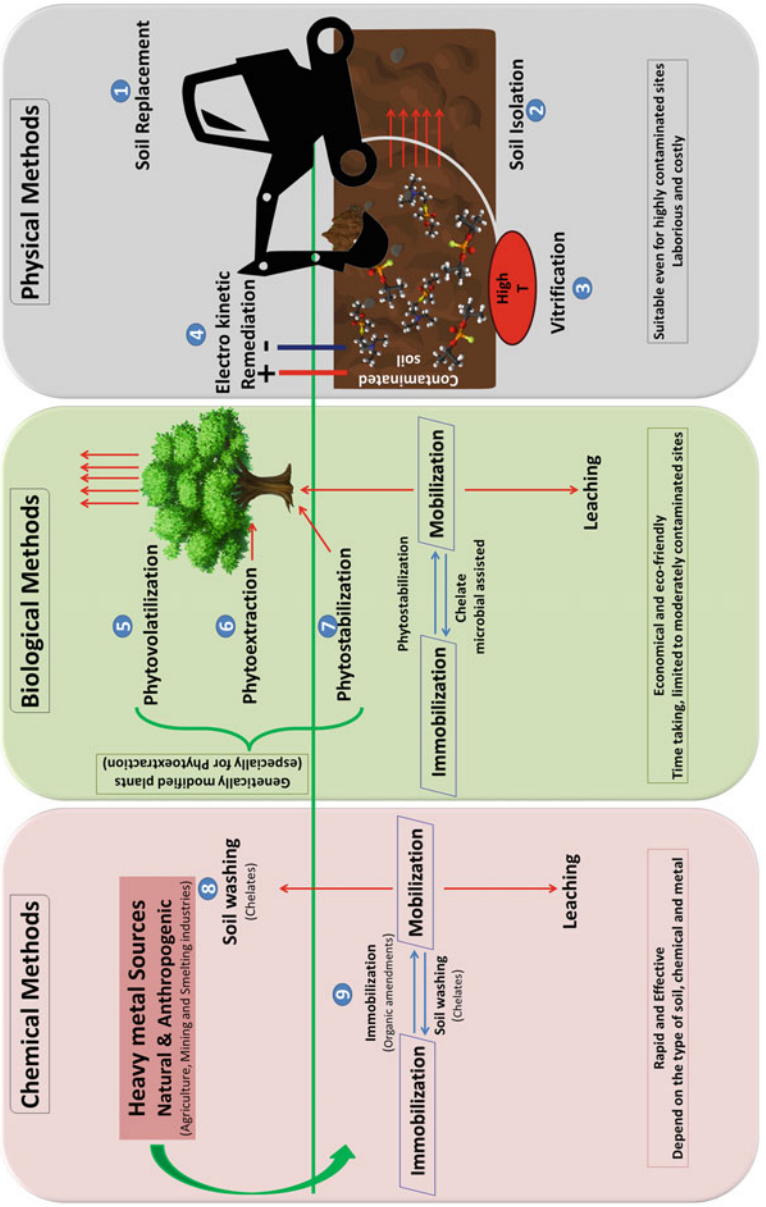

Fig. 6.1 Methods of heavy metal remediation (source: Khalid et al. 2017)

Extraction It is also called soil washing because chemical agents (organic and inorganic) which can chelate or elute the heavy metals are added to bring the metals into aqueous phase by forming stable complexes, and thus extracting them from the system (Khalid et al. 2017; RoyChowdhury et al. 2018).

6.4.3 Biological Remediation

Biological remediation is the application of living organisms for the treatment of polluted areas (RoyChowdhury et al. 2018). It could be microorganisms or plants. Through bioremediation, only oxidation state of heavy metals is changed, therefore it is not degraded. However, due to this modification, alterations in their vaporization and solubilization properties occur, which make the process of leaching out (more water soluble) or precipitation (less water soluble) of heavy metals easier. Though it is a natural method, it takes long time and happens gradually over the span of years.

Phytoremediation This method is employed for wide-spread contamination which is present till the root zone of the plant. The plants utilized for this method have hyper-accumulating properties towards certain heavy metals and thus remove it from the soil.

Phytoextraction Hyper accumulator plants (10–500 times more than ordinary plants) (Chibuike and Obiora 2014) are used which have high tolerance to toxic effects of heavy metals, rapid growth rate, high biomass and extensive roots. They absorb, translocate and concentrate heavy metals in their tissues. These plants are then harvested and incinerated and thus purifying the soil from heavy metals. Pteris vittata for As, Brassica juncea for Pb and Arabidopsis halleri for Cd are a few examples for hyper-accumulators.

Phytostabilization Specific plants are known to immobilize ions by absorption and accumulation. These ions are absorbed into the roots or precipitated in rhizosphere. Through this, bioavailability of metals to living organisms is reduced via sequestration in plant roots. Solanum nigrum is shown to phytostabilize Zn.

Phytovolatilization Plants take up heavy metals from the soil and release it into the atmosphere in the form of vapour. This method is mostly used for remediation of soil from Hg contamination. Toxic Hg ions are converted into elemental Hg and transpired into the atmosphere. *Nicotiana tabacum*, *Arabidopsis thaliana*, *Liriodendron tulipifera* are used for Hg and *Brassica juncea* and *Brassica napus* for Se remediation through phytovolatilization (Chibuike and Obiora 2014; Dixit et al. 2015; Khalid et al. 2017).

6.4.3.1 Microbial Remediation

Microbial remediation involves the usage of microbes to reduce heavy metals through absorption, precipitation, oxidation or reduction of these metals. Algae,

fungi and bacteria could perform microbial remediation. Microbes are known to display biosorption of heavy metals. Biosorption is defined as sequestering metals, intra or extracellularly, and thus removing it from the surrounding areas. The mechanism is of two types, viz. Active and Passive.

Active Mechanism (Direct Changes in Metals)

Precipitation Soluble metal ions are converted into insoluble products thereby reducing the transportation and bioavailability of these heavy metals. Anaerobic bacteria like Desulfovibrio and Desulfotomaculum produce hydrogen sulphide which reacts with these metal ions (cadmium, copper, lead and mercury) to produce insoluble metal sulphides.

Intracellular Accumulation This process takes place in two steps: firstly, the heavy metals bind to the surface in a passive manner and then enter the cell slowly with the expenditure of energy, that being the second step. Complexation of these ions occurs in the cell's cytoplasm through various compounds. *Rhizobium leguminosarum* and *Pseudomonas putida* have demonstrated resistance to Cd and its ability of intracellular sequestration.

Reduction Toxic oxidation states of heavy metals are converted into non-toxic forms thus eliminating its harmful effects. For example, Bacillus subtilis, Pseudomonas putida and Enterobacter cloacae have been found to reduce Cr(VI) to Cr(III). Similarly, Desulfovibrio vulgaris precipitates soluble U6+ to U4+. Reductase enzymes produced by these bacteria are responsible for this mechanism.

Passive Mechanism (Alteration of Heavy Metal Surroundings)

Extracellular Complexation This occurs from interactions between metal ions and chelating agents released by the microbes present in the soil. These chelating agents could be extracellular polymers like polysaccharides, proteins and nucleic acids or could be low molecular ligands called siderophores (Fe complexing molecules). These agents form complexes with the heavy metals, and negatively impact its bioavailability. *Bacillus thuringiensis* and *Bacillus subtilis* are known to secrete siderophores that increase extraction of Cd and Zn by increasing its mobility and thus their ability to be extracted.

Surface Adsorption It is the accumulation of heavy metals on the complexes and components present on the periplasm of the cell. It occurs in three ways, namely ion exchange with peptidoglycan and teichoic acid present on the cell wall, precipitation through nucleation reaction and complexation with nitrogen and oxygen ligands.

Dead Microbes Dead microbes also tend to accumulate heavy metals on their surface (Gavrilescu 2004; Igiri et al. 2018).

6.4.3.2 Microbes Involved in Bioremediation

Bacteria for Bioremediation Bacteria are the most versatile and abundant microorganisms for the remediation of heavy metal based on certain characters such as number of surface binding sites, binding strength for different ions and its binding mechanisms. They are highly preferred because of their small size, ubiquitous nature and the capacity to grow in a wide range of controlled and environmental conditions due to which they have developed many mechanisms to detoxify and develop resistance against heavy metals.

Algae for Bioremediation Algae are cheap and efficient microbes owing to their autotrophic nature and minimal requirement for nutrients. Algae can absorb from 15.3% to 84.6% of heavy metals due to their high biosorptive capacity. Out of all the algae, brown algae sequester most of the heavy metals. This is due to the presence of several chemical moieties on its surface such as hydroxyl, carboxyl, phosphate and amide groups which act as metal binding sites.

Fungi for Bioremediation Fungi are useful in both dead and alive condition due to their remarkable capacity for heavy metal uptake. They are easy to grow in fermentors and are easily available from industries. Fungus produces large quantities of extracellular enzymes and chemicals and also provides larger surface, thus increasing the availability of cell wall materials. Filamentous fungi are advantageous over yeast because the former is easily filtered and is less sensitive to variations in nutrients, aeration, pH and temperature (Abbas et al. 2014; Mustapha and Halimoon 2015).

6.4.3.3 Types of Microbial Bioremediation

In a broad spectrum, microbial bioremediation is categorized into two types, viz. in situ and ex situ bioremediation depending on the mode of the utilization.

In Situ Bioremediation His method involves application of bioremediation in the sub-surface which provides thorough end result. It is less expensive and more effective. It is further divided into two categories.

1. *Intrinsic in situ bioremediation*: It utilizes the naturally occurring microbes and does not incorporate any engineering techniques and employs only the capabilities of already present microbes. These microbes are fed nutrients and it is regularly aerated to increase their metabolic activity.
2. *Engineered in situ bioremediation*: Certain microbes of known beneficial properties are artificially introduced in to the site. Detoxification rate is increased by controlling physiochemical conditions for maximum growth of microorganism.

Ex Situ Bioremediation In this method, soil is excavated and lined above the ground in a treatment area and is subjected to aeration ad several processes so as to

enhance microbial degradation. Ex situ bioremediation has several sub-categories such as

1. *Slurry-phase treatment*: Also known as bioreactors; it changes the soil into slurry by addition of water and placing in a bioreactor after removal of stones, rubble and debris. Followed by this, the soil is removed and contaminated water is sent for further treatment. Meanwhile the soil is dried up using pressure filter, vacuum filter or centrifuges.
2. *Solid-phase treatment*: This method directly uses the contaminated soil without processing it. With the help of networked pipe system, the entire pile of soil is aerated for encouraging the action of microbes already present in the excavated soil. This method involves more time and space as compared to slurry-phase treatment.

6.4.3.4 Implications of Bioremediation

Advantages

1. Bioremediation is a natural process and is widely accepted.
2. Less energy is required as compared to other processes.
3. It is less expensive than other technologies.
4. It is a complete process where the toxic substances are completely transformed into harmless products.
5. Instead of transferring contaminants from one place to another, the pollution is contained and degraded at the site itself.

Disadvantages

1. Biological processes are highly specific and require optimum conditions for its success.
2. Requires longer duration than other treatment methods.
3. It is a dynamic process and it is difficult to predict the outcome.
4. Only biodegradable compounds can be degraded through this method (Kulshreshtha et al. 2014).

6.4.3.5 Genetic Engineering in Bioremediation

Genetically Engineered Microorganisms (GEMs) include those organisms that have undergone genetic modifications, either to alter the expression of certain characters or to incorporate certain traits into an organism that was originally lacking it. Through genetic engineering, biosensors have also been developed which could detect and measure the degree of contamination in a polluted site rapidly and accurately, for heavy metals such as Hg, As, Cd, Ni, Cu, etc. GEMs are engineered with characteristics such as the ability to tolerate high levels of heavy metal stress and the ability to produce metal chelating (Bae et al. 2000). Some of the commonly used GEMs are Escherichia coli (ELP153AR) to target As, Saccharomyces cerevisiae (CP HP3) to target Cd^{2+}, Zn^{2+} and also for their remediation. Chlamydomonas reinhardtii was engineered to increase resistance to Cd toxicity and accumulation (Dixit et al. 2015; Igiri et al. 2018).

6.5 Future Endeavours Towards Controlling Heavy Metal Pollution

Human intrusion has led to the contamination of environment with high levels of heavy metals. International protocols such as Convention on Long-Range Transboundary Air Pollutants (LRTAP), Rotterdam Convention, The Basel Convention, and International Convention on the Control of Harmful Anti-Fouling Systems on Ships, etc., aim to reduce and stabilize the existing levels of heavy metals. Apart from these protocols and norms, alternatives to heavy metals can also be used so as to control the emission of heavy metals. Some of the alternatives corresponding to the heavy metals are listed below.

Lead Lithium-ion polymer batteries can be used instead of lead acid batteries. Steel, soft iron, wolfram, bismuth and tin maybe used as alternatives to lead in ammunition. Iron, copper and plastic pipes could also be used in industries instead of lead pipes. In paint industries, instead of lead based siccatives, calcium based siccative can be used.

Mercury Amalgams and cast fillings in dentistry can use ceramics, gold or silver as alternative to mercury. Electrical thermometers can be used instead of the traditional glass thermometers. Pesticides using mercury could be avoided. It is better not to use pesticides at all.

Cadmium Lithium-ion polymer batteries can be used instead of cadmium–zinc batteries. Voltage stabilizers made of PVC containing calcium instead of cadmium can be used. Photovoltaic cells made of traditional crystalline cells could be an alternative to cadmium film based cells.

Chromium Chromium based leather tanning could be replaced with synthetic organic compounds based tanning. Cu, Cr, As based wood preservatives could be replaced with Cu, B—organic compounds based preservatives.

6.6 Conclusion

Heavy metal contaminated soils pose a serious threat to the global food security considering the fact that heavy metals are considered as one of the major abiotic stress. And also, the agricultural ecosystem is being deeply affected leading to serious health effects in humans. In order to contain the toxicity levels in the soil and to recover fertility of the heavy metal affected area, developed countries have taken necessary steps to reduce and to regulate their emission. Despite these precautions, the reduction in the heavy metal levels is nominal. Moreover, it is rarely being considered as a threat among the developing countries. To achieve a global success in reducing metal toxicity, awareness has to be created among people and government has to frame strict rules and regulations for factories emitting such toxic

compounds. Traditional agricultural practices have to be followed in order to avoid the usage of chemical fertilizers and pesticides which is a two-way benefit. With the tremendous development in technology, instruments for the accurate measurement levels of heavy metals could be developed. As far as the containment of the prevailing contamination is concerned, genetically engineered microbes with multiple metal targets and new molecular biological tools could be developed and utilized. Phytoremediation is yet another option as it not only detoxifies the soil but also purifies the atmosphere. Therefore, containment and maintenance of heavy metal concentration in the environment could be brought by the combined efforts of the research community, industrial sector and government. Success comes with commitment after all!

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Biotransformation of Chitinous Waste into Value-Added Products

7

Manish Kumar, Vivekanand Vivekanand, and Nidhi Pareek

Abstract

Chitinous wastes are the key source of coastal area pollution and it can be managed by converting into value-added products like chitosan, chitooligosaccharides, and glucosamine. The conversion of chitinous wastes into the more beneficial products can be carried out through microbial enzymes which not only provide products with improved properties but also give advantage over the chemical conversion in terms of environment protection. The microbial-derived biotransformations of chitin into their derivatives have numerous applications in food, pharmaceutical, cosmetics, medical, wastewater treatment, and agriculture industries. Microbial enzymes like chitinase, chitosanase, chitin deacetylase, and N-acetyl glucosaminidases have specific affinity towards the polymer chitin and they can be proved vital for the industrial-scale production of chitin derivatives. Other non-specific enzymes like cellulases, lysozymes, lipases, etc. can play critical role in enhancing the production level of the chitin derived bioactive compounds. The present chapter covers the different aspects like structure, mode of action, properties and application of the various chitinolytic specific enzymes as well as their bioactive products.

M. Kumar · N. Pareek (✉)
Department of Microbiology, Central University of Rajasthan, Ajmer, Rajasthan, India
e-mail: nidhipareek@curaj.ac.in

V. Vivekanand
Centre for Energy and Environment, Malaviya National Institute of Technology, Jaipur, Rajasthan, India

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7.1 Introduction

The rapid increasing population of world is exploring different natural resources for their energy requirement in the form of food. Being almost two-third part of the world marine resources are becoming one of the most potent natural resources for feeding the huge population. This resulted in the establishment of numerous sea food processing industries along the vast marine coast leading the generation of gigantic amount of chitinous waste. The chitin-containing waste has become one of the major concerns of coastal area pollution. On some extent these waste are utilized for chitin extraction but still the annual worldwide production of chitin is approximately 1010–1012 ton (Gortari and Hours 2013). Chitin stands second in the term of natural abundance on earth just after cellulose, but its applicability is limited due to high crystallinity and low solubility. Chitin can be transformed into derivatives with improved properties, i.e. chitosan (CHS), chitooligosaccharides (COS), and N-acetylglucosamine (GlcNAc), which has variety of utilizations in the field of agriculture, medicine, cosmetics, wastewater treatment, and drug delivery (Kumar et al. 2018b). These transformations can be carried out with the help of microbial resources.

The microbial biotransformation is the process of conversion of organic waste into its structurally related products by the mean of microorganisms or their enzymes. Substrate specificity, stereospecificity, mixed reaction condition, environment-friendly, ease of alteration, and scale up with less cost makes microbial biotransformation preferable one (Smitha et al. 2017). The very popular and effective approach for chitin biotransformation is the application specific of enzymes such as chitinases, chitosanases, chitin deacetylase (CDA), and N-acetyl glucosaminidases (NAG). Moreover, it can also be achieved through the use of non-specific enzymes viz. cellulases, lipases, pectinases, lysozymes, and hemicellulases. The present chapter is focused on the exploration of various enzymes used in chitin biotransformation and the application of the derived products along with their structure and bioactive properties.

7.2 Chitin: Structure and Properties

Chitin is an amino polysaccharide comprised of β-(1-4) linked N-acetyl-D-glucosamine residues. It is one of the amplest natural structural polysaccharides. Chitin is found as an ordered crystalline microfibrils which is the structural components of exoskeletons of arthropods, crustaceans, and mollusks as well as in the cell wall of fungi and algae (Dutta et al. 2004). On the basis of source, chitin is present in three forms i.e. α, β, and γ. The predominantly occurring form, i.e. α-chitin is formed by the antiparallel organization of microfibrils which is found in fungal cell wall, tendons, shells of lobster and crabs, shrimps shells, and insect cuticle. In case of β-chitin, sheets are arranged in parallel fashion and are mainly present in squid pens, vestimentiferan worms, and Aphrodite chaetae. The γ-chitin contains both the

parallel as well as the antiparallel sheets and is found in the insect cocoons. But, some researchers considered γ-chitin as the variant of α-chitin (Kumar 2000).

Structurally, chitin is much akin of cellulose expect the two-hydroxyl group of each glucose unit in cellulose is replaced by an acetylated amino group (-NHCOCH3). Chitin is white, hard, inelastic, highly basic, nitrogenous polysaccharide that is insoluble in water and most of organic solvents (Rinaudo 2006). However, chitin was reported to be soluble in hexafluoroisopropanol, hexafluoroacetone, and chloralcohols when it combined with aqueous solutions of mineral acids and dimethylacetamide containing 5% lithium chloride (Pillai et al. 2009). Chitin has several exceptional properties like non-toxicity, film and fiber forming capacity, biocompatibility, biodegradability, adsorption of metal ions, and coagulation of suspensions or solutes (Knorr 1982). These distinctive biological activities make chitin as one of the most demanding biofunctional polymers. The chemical structure of chitin contains the acetyl amino group that open the door to chemical modifications of the polymer in order to construct more useful derivatives (Rinaudo 2006).

7.3 Biotransformation: Importance and Demand

Bioransformation is the method of transformation or synthesis of an organic compounds into the structurally related another one by the biological means like microorganisms or enzymes. This structural modification is towards either the reduction of persistence and toxicity of a compound or its conversion to enhance its applicability. Natural biotransformation is slow, and nonspecific and less productive. So, the artificial or induced biotransformation by the help of microorganisms and enzymes are gaining more attention (Smitha et al. 2017). Microorganisms have high biodegrading potential of natural organic compounds in order to use them as energy source or cell building blocks and so, they can play a key role in global recycling and maintaining ecological balance (Dave et al. 2014). In recent years, microbes induced biotechnological approaches are employed for the biotransformation of various industrial, agricultural, and sewage wastes into the valuable products with improved utility and less toxicity (Perkins et al. 2016). Biocatalysis and biotransformation have resulted into the development of several pharmaceutical, agricultural, and cosmetic products along with providing novel tool in drug delivery and development (Zaks and Dodds 1997; Kebamo et al. 2015). One of the most extensively explored wastes is sea food processing industries wastes. These chitinous wastes are generated in huge amount throughout the world and are emerging as a serious threat to the coastal area pollution. However, the microbial-derived products of chitin have proved its importance in various sectors. This biotransformation of chitin to the value-added products are carried out through various specific microbial enzymes that function as biocatalyst.

7.4 Biocatalysts for Chitin Transformation: Microbial Production and Mode of Application

The vast application of chitin transformed products and the environmental concern lead to the intensive research in order to find novel biocatalysts. In this regard, enzymes like chitinase, chitosanase, CDA, and NAG are of utmost interest due to their high potentiality of transforming chitin into valuable (Patil et al. 2000). These enzymes are commonly produced by insects, plants, and microorganisms. However, for industrial-scale production of chitin derived products bacteria, fungi, and yeast are most explored due to ease of extraction process and highly specific ability of biotransformation (Table 7.1). In the coming section of this chapter, the major

Table 7.1 Microbial enzyme derived biotransformation of chitinous waste and polymers

Substrate/raw material	Microorganism	Enzymes	Biotransformed products	Reference
Swollen chitin	*Myceliophthora thermophila* C1	NAG	GlcNAc	Krolicka et al. (2018a)
Chitin and colloidal chitin	*Humicola grisea*	Chitinase	$(GlcNAc)_3$ $(GlcNAc)_2$ GlcNAc	Kumar et al. (2018c)
Chitin flakes, swollen chitin, colloidal prawn shell, ground prawn shell	*Aspergillus terreus*	Chitinase	GlcNAc	Das et al. (2018)
Shrimp waste	*Paenibacillus* sp.	Chitinase	$(GlcNAc)_4$ $(GlcNAc)_3$ $(GlcNAc)_2$ GlcNAc	Kumar et al. (2018a)
Crab shell powder	*Bacillus subtilis*	Chitinase	GlcNAc	Wang et al. (2018)
Chitin and $(GlcNAc)_{2\text{-}12}$	*Myceliophthora thermophila* C1	Chitinase Chi1	$(GlcNAc)_2$	Krolicka et al. (2018b)
α-chitin	*Aeromonas caviae*	Chitinase	GlcNAc	Cardozo et al. 2017
Chitosan	*Penicillium decumbens* CFRNT 15	Exo-β-D-glucosaminidase	D-glucosamine	Nidheesh et al. (2015a)
Crayfish shell wastes	*Serratia proteamaculans*	chitinase	GlcNAc	Wei et al. (2017)
Crab shell chitin	*Streptomyces coelicolor* A3(2)	Chitinase C and *N*-acetylhexosaminidase	GlcNAc	Nguyen-Thi and Doucet (2016)

enzymes have been studied in detail regarding their microbial source and mode of action with the special attention on the latest work done in this area.

7.4.1 Chitinases

Chitinases (EC 3.2.1.14) are glycosyl hydrolases with systematic name (1→4)-2-acetamido-2-deoxy-β-D-glucan glycanhydrolase, that can randomly cleave the glycosidic linkages of chitin and chitodextrin in a non-progressive manner, resulting into the generation of COS and free ends upon which exochitinases and endochitodextrinase can act (Lombard et al. 2014). Chitinases are reported in bacteria, fungi, yeasts, plants, actinomycetes, arthropods, and humans (Hamid et al. 2013; Kumar et al. 2018e). The molecular weight of chitinases is found in the range of 20 kDa to about 90 kDa. Chitinases are well classified and mainly belongs to the family 18 and family 19 of the glycosyl hydrolases (http://www.cazy.org/Glycoside-Hydrolases). Based on mode of action chitinases can be either endochitinases or exochitinases. The endochitinases randomly split chitin chain at internal sites forming diacetylchitobiose and soluble low molecular mass oligomers viz. chitotriose and chitotetrose. On the other hand, exochitinases shows a progressive catalytic action originating from the non-reducing end of the chain releasing of chitobiose or glucosamine units. Further, the exochitinases can be divided into chitobiosidases (EC 3.2.1.29) and 1,4-β-glucosaminidases (EC 3.2.1.30). The former catalyzes the progressive release of diacetyl chitobiose originating at the non-reducing end of the chitin, whereas the later cleave the oligomeric products resulting from action of endochitinases and chitobiosidases with release of glucosamine.

Chitinases have tremendously studied in the recent years due to their remarkable role in controlling the fungal pathogens and harmful insects. The specific action of chitinases on chitin helps in protection against the chitin-containing pathogens (Kumar et al. 2018d). One of the most exploited sources of chitinases is microorganisms which can produce extracellular chitinases that can be extracted for valuable applications. A wide range of bacteria viz. *Escherichia* (Kim et al. 2018), *Paenibacillus* (Kumar et al. 2018a), *Streptomyces* (Karthik et al. 2015), *Aeromonas* (Halder et al. 2016), *Bacillus* (Laribi-Habchi et al. 2015), and *Serratia* (Tuveng et al. 2017) have been reported as chitinases producers. In case of fungi, *Aspergillus* (Alves et al. 2018), *Humicola* (Kumar et al. 2017), *Trichoderma* (Sandhya et al. 2004), *Thermomyces* (Khan et al. 2015), and *Monacrosporium* (Soares et al. 2015) are the potential chitinases producers. Apart from the role in conversion of chitinous waste materials into value-added COS (Kumar et al. 2018a) microbial chitinases have also shown applicability as antimicrobial (Farag et al. 2016), insecticidal (Binod et al. 2007), and biopesticidal (Wu et al. 2010) agents.

7.4.2 Chitosanase

Chitosanase (EC 3.2.1.132) is glycoside hydrolase having molecular mass in the range of 20–75 kDa, that catalyzes the endohydrolysis of β-(1→4) linkages between D-glucosamine residues of partly acetylated CHS and thus also known as CHS N-acetylglucosaminohydrolase (Lombard et al. 2014) (http://www.cazy.org/GH46). On the basis of amino acid sequences chitosanases are present in families 5, 8, 46, 75, and 80 of glycoside hydrolase. Further chitosanases are categorized into three subclasses viz. subclass I (split both GlcN–GlcN and GlcNAc–GlcN linkages), subclass II (split only GlcN–GlcN linkages), and subclass III (cleave both GlcN–GlcN and GlcN–GlcNAc linkages) (Thadathil and Velappan 2014). Apart from the above chitosanases, another enzyme with exo-β-D-glucosaminidase activity has been reported from various microorganisms (Fukamizo et al. 2006). The exochitosanase acts on CHS and/or COS from the non-reducing termini in order to remove successive GlcN residues. It can degrade GlcN–GlcNAc linkages but is unable to act on GlcNAc–GlcNAc linkages.

The major sources for chitosanases are bacteria, fungi, cyanobacteria, and plants (Somashekar and Joseph 1996). The most extensive studied bacterial chitosanases are from *Bacillus* spp. (Choi et al. 2004; Sinha et al. 2016; Liaqat et al. 2018), *Serratia* spp. (Wang et al. 2008), *Paenibacillus* spp. (Zitouni et al. 2013; Pagnoncelli et al. 2010), *Acinetobacter* spp. (Wang et al. 2011; Shimosaka et al. 1995), *Janthinobacterium* spp. (Johnsen et al. 2010), *Streptomyces* spp. (Boucher et al. 1992; Sinha et al. 2012). In case of fungal chitosanase production there are comparatively less study reported. Chitosanase production has been reported in fungi viz. *Penicillium* spp. (Rodríguez-Martín et al. 2010; Nguyen et al. 2014), *Fusarium* spp. (Shimosaka et al. 1993; Liu and Bao 2009), *Aspergillus* spp. (Chen et al. 2005; Zhang et al. 2012a; Singh and Vidyasagar 2017), *Gongronella* spp. (Zhou et al. 2008), *Trichoderma* spp. (da Silva et al. 2012). Recently, chitosanase from cyanobacteria viz. *Anabaena* spp. (Gupta et al. 2012; Prasanna et al. 2010) has also been reported. Chitosanase has application in the generation of bioactive COS (Nidheesh et al. 2015b; Liang et al. 2016; Qin et al. 2018). Moreover, it has also potential of enhancing resistance against phytopathogenic fungi (Punja and Zhang 1993).

7.4.3 Chitin Deacetylase

CDA (EC 3.5.1.41) belongs to family 4 of the carbohydrate esterase which can hydrolyze the N-acetamido groups of N-acetyl-D-glucosamine residues in chitin (Lombard et al. 2014). CDA catalyzes the elimination of acetyl groups from the native chitin chain through a set of multiple-attack mechanism which lead to the generation of both Glc and GlcNac monomers in the polymer (Ghormade et al. 2017). The primary structure of this family members shares a conserved region assigned for the "NodB homology domain" or "Polysaccharides deacetylase domain." Apart from the CDA, the other members of the family are acetyl xylan

esterase (EC 3.1.1.72), chitooligosaccharide deacetylase (EC 3.5.1.-), peptidoglycan GlcNAc deacetylase (EC 3.5.1.-), and peptidoglycan N-acetylmuramic acid deacetylase (EC 3.5.1.-) according to the CAZy database (http://www.cazy.org/CE4). Generally, the molecular mass of CDA is found to be in the range of 25–80 kDa (Zhao et al. 2010).

The main sources of CDA are fungi, yeast, bacteria, and insects. Among fungi, CDA production from *Mucor* spp. (Kafetzopoulos et al. 1993; Chatterjee et al. 2005); *Aspergillus* spp. (Alfonso et al. 1995; Narayanan et al. 2016; Karthik et al. 2018); *Rhizopus* spp. (Jeraj et al. 2006; Chatterjee et al. 2008; Zhang et al. 2014); *Metarhizium* spp. (Nahar et al. 2004); *Penicillium* spp. (Pareek et al. 2011, 2014); and *Colletotrichum* spp. (Suresh et al. 2011) has been extensively studied. Saccharomyces cerevisiae (Martinou et al. 2002) and Schizosaccharomyces pombe (Matsuo et al. 2005) are the yeast species reported for CDA production. In case of bacteria mostly marine bacteria, *Vibrionaceae* spp. (Kadokura et al. 2007; Li et al. 2007; Pascual and Planas 2018) and *Bacillus* spp. (Bhushan 2000; Zhou et al. 2010) are known for CDA production. Moreover CDA has also been detected in insects viz. *Helicoverpa armigera* (Han et al. 2015), *Tribolium castaneum* (Noh et al. 2018), *Mamestra configurata* (Toprak et al. 2008), *Drosophila melanogaster* (Wang et al. 2006). Due to the different catalytic mechanisms CDA has shown potentiality in variety of biological applications. The major utilization of CDA is its generation ability of CHS from chitin (Pareek et al. 2013; Narayanan et al. 2016). Moreover, CDA has also applications in biological control of fungal pathogens and insects (Alfonso et al. 1995; Tsigos et al. 2000; Cord-Landwehr et al. 2016; Pusztahelyi 2018).

7.4.4 N-Acetyl Glucosaminidase

Endo-β-N-acetylglucosaminidase (EC 3.2.1.96) (NAG) catalyzes the endohydrolysis of N,N″-diacetylchitobiosyl unit containing high mannose glycopeptides and glycoproteins (Murakami et al. 2013). In this reaction one GlcNAc residue remains linked to the protein and the remaining oligosaccharides are released in an intact manner (Lombard et al. 2014). Corresponding to the CAZy database, NAG belongs to family 3, 20, 73, 84, and 85 of glycoside hydrolase based on their amino acid sequence (http://www.cazy.org). In human body it is found in many tissues and helps in breaking chemical bonds of glycosides and amino sugars which forms the structural components of various tissues. It is also vital for the degradation and disposal of several parts of cell (Wen and Kellum 2012). Moreover, NAG has also been detected in animals, plants, insects as well in fungi and bacteria such as Endo-T from *T. reesei* (Stals et al. 2010) and Endo-D from *Flammulina velutipes* (Hamaguchi et al. 2009). The optimum pH of NAG is in the range of pH 5.0–8.0, whereas the optimum temperature lies in between 37 °C and 60 °C (Zhang et al. 2018). Matano et al. (2016) reported the overproduction of active NAG by fusing it with TAT (Twin-arginine Translocation) or Sec (General secretion route) signal peptides. In a study, NAG gene from *Sphingobacterium* sp. HWLB1 was

cloned and expressed in *E. coli* BL21 and the purified enzyme was showing significant NaCl tolerance (Zhou et al. 2016). The study also stated that the enzyme retained 73.6% of its activity upon addition of 30% (w/v) NaCl in the reaction mixture (Zhou et al. 2016). Currently, researchers are also focusing on the easy and much sensitive methods for NAG detection. Recently, a fluorescence-quenching-based assay system was developed to determine the hydrolytic activity of NAG (Ishii et al. 2018). In this method the fluorescence signal emitted was directly proportional to the amount of the tetrasaccharide derivative which allowed easy evaluation and quantification of NAG. Apart from the development made in the NAG detection enhancement in its production level has also been investigated. The NAG production has been reported to be enhanced up to 9.3 mg from 20 silkworm larvae by using recombinant baculovirus expressing Endo-H without the exogenous peptide (Masuda et al. 2015).

7.4.5 Chitin Derived Products: Structure and Physicochemical Properties

Chitin is mainly obtained from the sea food processing industries wastes that include mainly crustacean wastes from processing of shrimp, crab, lobster, and krill. Chitin itself is a highly crystalline and insoluble polymer that limits its applications but the various derivatives of chitin and its deacetylated products CHS has shown tremendous applicability in diverse fields such as agriculture, medicine, cosmetics, and environment (Hamed et al. 2016). These wide ranges of applicability studies are possible by exploring the different structural and physicochemical aspects of the chitin derived products. In the following section, the most extensively studied derivatives of chitin, i.e. CHS, COS, and GlcNAc have been reviewed in regard of their different structural and bioactive properties.

7.4.6 Chitosan

CHS is a semicrystalline deacetylated form of chitin having degree of deacetylation more than 50% and soluble in acidic aqueous medium (Kumar 2000). CHS is a linear polymer of β(1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose which is derived from the deacetylation of chitin. Its molecular weight varies from 300 to >1000 kDa with a deacetylation ranging from 30 to 95% (Rinaudo 2006). These large extents of variations are due to differences in the source of origin material and the process of preparations. CHS also contains 5–8% nitrogen which are present in the form of primary aliphatic amine groups. CHS is more active as compared to chitin due to the primary and secondary hydroxyl groups present in its structure on the each repeat unit and the amine groups on the deacetylated units (Franca et al. 2008). The solubility of CHS in a solution depends upon the protonation of the $–NH_2$ function on the C-2 position of the D-glucosamine repeat units, the ionic concentration, pH, nature of the acid used for protonation and the distribution of acetyl groups along the

chain (Dutta et al. 2004). CHS is derived from the extracted chitin of the sea food industries crustacean wastes. There are four steps required for the conversion of CHS from crustacean shells i.e. (a) deproteinization, (b) demineralization, (c) decolouration, and (d) deacetylation (Younes and Rinaudo 2015). CHS has chemical properties like presence of linear polyamine, reactive amino groups, reactive hydroxyl groups, and metal chelating affinity (Dutta et al. 2004). Moreover, CHS also exhibited various biological properties viz. biocompatible, antimicrobial, hemostatic, anticoagulant, mucoadhesive, antitumor, anticholesteremic, central nervous system depressant, immunoadjuvant, and speed up bone formation (Tavaria et al. 2008; Dash et al. 2011). CHS being non-toxic in nature craft it cytocompatible that helps in improved cell adhesion and proliferation. Additionally, the excellent processability of CHS enables it to fabricate in various medicinally important forms viz. microspheres, films, sponges, hydrogels, nanofibers, etc. (Islam et al. 2017). Owing numerous biological properties CHS extracted from fish scales, crab and shrimp shells were characterized and their physicochemical properties were determined (Kumari et al. 2017). In this study, Kumari et al. (2017) extracted CHS from the starting materials, i.e. fish scales, shrimps and crab shells through the deacetylation of chitin using 40% KOH at 90 °C for 6 h and the prepared CHS degree of deacetylation were found to be 75%, 78%, and 70%, respectively. Kumari et al. (2017) also studied the various physicochemical properties of the prepared CHS and found that the solubility of CHS from crab, fish, and shrimp was found to be 60%, 78%, and 70%, respectively. Similarly, the fat binding capacity (246%, 226%, and 104%); water binding capacity (492%, 138%, and 358%); ash content (1%, 2.5%, and 0.03%); and elemental analysis (7.62%, 7.79%, and 6.20%) were determined of the CHS obtained from fish scale, shrimp scales, and crab scales, respectively (Kumari et al. 2017). Recently, Muley et al. (2018) extracted 173 kDa CHS with 78.40% degree of deacetylation from prawn shells waste. The extracted CHS was reported to be soluble in 1% acetic acid along with water and fat binding capacities of 923% and 598.05%, respectively.

7.4.7 Chitooligosaccharides

COS are the derivatives of chitin or CHS. COS are composed of homo- or heterooligomers of GlcNAc and D-glucosamine units in varying proportions (Aam et al. 2010). The average molecular weight of COS are found to be less than 3900 Da with degree of polymerization less than 20 (Mourya et al. 2011). COS can be prepared by physical, chemical as well as biological methods. Physical methods include microwave (Wang et al. 2016), hydrothermal (Sato et al. 2003), gamma-rays (Li et al. 2001), and ultrasonication (Savitri et al. 2014). Among the chemical methods of COS preparation, the use of hydrochloric acid (Cabrera and Van Cutsem 2005; Kazami et al. 2015) and hydrogen peroxide (Chang et al. 2001; Lin and Zhang 2009) are well documented. The functional properties of COS are dependent upon the degree of polymerization and the sequence of acetylated and deacetylated units. The biological properties of COS viz. antimicrobial, anticancer, antioxidant,

immune stimulating activity are directly proportional to the degree of polymerization (Das et al. 2015). However, the biological activities are also dependent on solubility of COS which decreases with the degree of polymerization (Kim and Rajapakse 2005). Moreover, the enzymatic preparation of COS is becoming more popular due to safety and ease of controlled generation of desired COS. For enzymatic preparation of COS enzymes like chitinases (Kidibule et al. 2018; Kumar et al. 2018c), and chitosanases (Pechsrichuang et al. 2013; Liang et al. 2016) alone or in synergy of both (Frankowski et al. 2001; Kim et al. 2018) or with other chitinolytic enzymes viz. lysozyme, pepsin, cellulases has been reported (Lin et al. 2009; Laokuldilok et al. 2017). El-Sayed et al. (2017) prepared COS by employing immobilized pepper chitosanases from CHS. The prepared COS were grouped into four groups on the basis of their molecular weights. The molecular weight of group I, group II, group III, and group IV COS were more than 100 kDa, 100–10 kDa, 10–1 kDa and less than 1 kDa, respectively. The prepared different groups of COS were showing antimicrobial activity against different microorganisms. Group (I, II, III) COS were active against B. cereus, whereas group (I, II, IV) were showing antimicrobial activity against *B. subtilis*. Moreover, group (II, IV) were active against *Staphylococcus aureus*, group (II) against *Pseudomonas aeruginosa*, group (I, II, IV) against *Candida albicans,* and group (III) was active against *S. chevalieri* (El-Sayed et al. 2017). Recently, a 13 kDa COS with degree of deacetylation of 54.83% were prepared from white-leg shrimp shells by the mean of hydrochloric acid (Rakkhumkaew and Pengsuk 2018). The synthesized COS were showing water solubility of 97 g/100 ml, molecular weight of 13 kDa, and 54.83% of degree of deacetylation. The prepared COS were reported to enhance the shelf life of bread due to strong inhibitory effects against *B. cereus* and *Rhizopus* sp. (Rakkhumkaew and Pengsuk 2018).

7.4.8 Glucosamine

Glucosamine (GlcN) is an amino sugar predominantly present as the part of the structure of the polysaccharides, CHS, and chitin. It is one of the most abundant monosaccharides. GlcN is found in various forms viz. glucosamine sulfate, glucosamine hydrochloride, and GlcNAc. One of the most extensively explored forms of GlcN is GlcNAc due to its vast biomedical potentiality. GlcNAc has molecular weight of 221.209 g/mol with chemical formula C8H15NO6. In chemical terminology GlcNAc is an amide between glucosamine and acetic acid that is found in the skin, cartilage, and blood vessel of humans (Chen et al. 2010). Moreover, it can also be extracted from the shell of cutaneous. Nowadays, researchers are focusing on environment-friendly microbial enzymatic process with prominence on the metabolic and process engineering tactics for the controlled and improved conversion of chitin into GlcNAc which has wide applications in food, cosmetics, and pharmaceuticals industries (Sitanggang et al. 2012; Liu et al. 2013). Liu et al. (2014) improved the GlcNAc production from the recombinant *B. subtilis* strain through modular engineering of the GlcNAc synthesis related metabolic network.

They achieved 3.8-fold increments in GlcNAc titer by using a 3 L fed-batch bioreactor. The GlcNAc production enhancement study from *Aspergillus* sp. BCRC 31742 was also carried out by Zhang et al. (2012b) through optimizing the dissolved oxygen in the medium. The study elicited the highest GlcNAc production level during 0–12 h and 12–60 h at 30% and 50% level of dissolved oxygen, respectively. The highest GlcNAc production level was 14.37 g/l that was 1.3 times more than the control (Zhang et al. 2012b). Recently, GlcNAc production from a marine origin *Aspergillus terreus* has been documented (Das et al. 2018). In this study 50 mg/ml of substrate was hydrolyzed by using 6 U of enzyme. The hydrolysis of ground prawn shell, chitin flakes, colloidal prawn shell, and swollen chitin were resulted into the GlcNAc production level of 15, 36.5, 40, and 46 mg/ml, respectively (Das et al. 2018).

7.5 Applications

Chitin derived products are among the most explored bioactive compounds in the last few decades. The major reason behind the prime attention of researchers is due to their applicability in several sectors (Fig. 7.1). The intensive work done in the field of chitin derive products result in the development of some nutraceutical, agricultural, and biomedical products especially in wound dressing (Table 7.2). In the coming section of this study, the recent progress made in the application of CHS, COS, and GlcNAc has been presented.

7.5.1 Biomedical

Chitin derivatives have been well reported for their potential applicability in the field of biomedicine viz. antimicrobial, anticancer, anti-metastasis, wound dressing material, gene therapy agent, bone strength enhancer, etc. (Zhang et al. 2010; Xia et al. 2011). The effectiveness of the bioactive derivatives of chitin can be enhanced by grafting it with other bioactive compounds for specific need. Bioactive derivative of COS can be used against the proliferation of AGS (Adenocarcinoma gastric cell line) human gastric cancer cells. Gallic acid-grafted COS was reported to inhibit gastric cancer cell growth when it was used at a concentration of 200 and 400 μg/ml (Ryu et al. 2017). The study proved that the gallic acid-grafted COS induced apoptosis was connected to the rise in the expression of p53, p21, Bax, and caspases (−9 and −3) and decline in the activation of Bcl-2, P-lkB-α, and NF-kB (p50 and p65). Similarly, Vo et al. (2017) synthesized gallic acid-grafted COS and evaluated its antioxidant and anti-inflammatory activities in human lung epithelial A549 cells. In this study, the prepared gallic-acid grafted COS was reported to possess high DPPH radical scavenging activity as well as was showing protective effects against H2O2 induced DNA damage. Additionally, it was also able to inhibit the production of cytokines including IL-8 and TNF-α (Vo et al. 2017). The gallic-acid conjugate COS with polyvinyl alcohol hydrogel was reported to have better antioxidant and

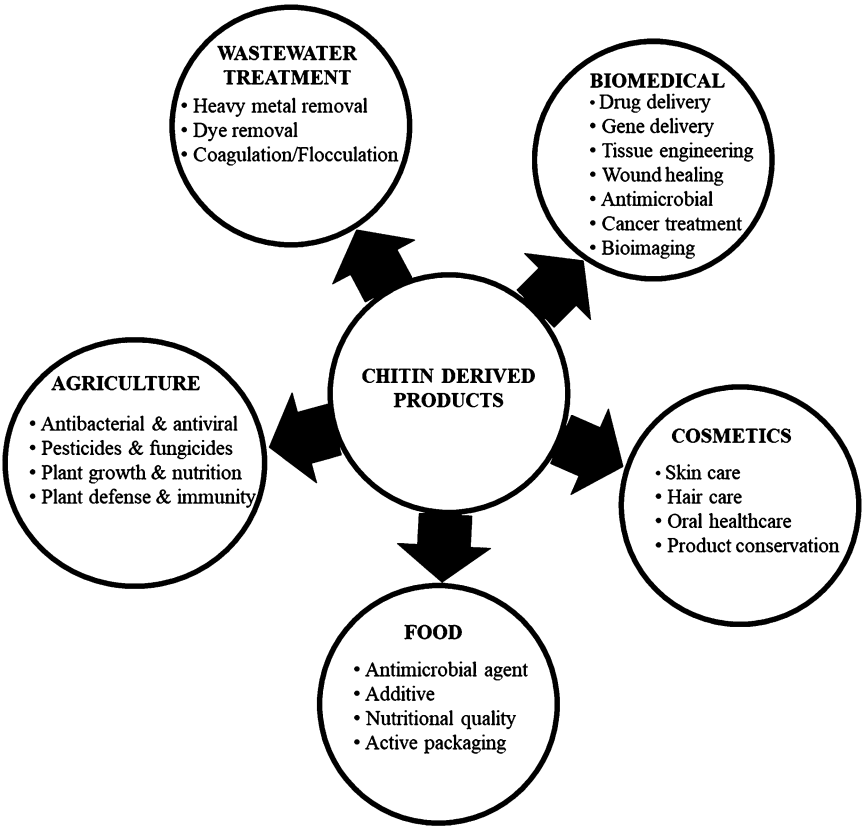

Fig. 7.1 Applications of chitin derived products in different sectors

antimicrobial activity against Propionibacterium acnes as compared to COS used alone (Park et al. 2018). CHS has been extensively used as a novel carrier for delivering other drugs molecules. Somasundaram et al. (2018) developed Hesperidin-loaded CHS nanosuspension through ion gelation method and evaluated its efficiency for the treatment of Parkinson's disease. The developed suspension average particle size was of 188 nm with zeta potential of 48.7 mV and drug content of 0.470 ± 0.25 mg/ml along with the entrapment efficiency of 78.2%. In another study, a nanoaggregate carrier of CHS was prepared by using 2-chloro-N,N-diethylethylamine hydrochloride (DEAE) and dodecyl aldehyde as grafting agents (de Oliveira Pedro et al. 2018). The prepared nanoaggregate was a pH-responsive system and a bioactive flavonoid, quercetin was encapsulated in it which was reported to show the synergistic effects on the control of the viability of MCF-7 cells (de Oliveira Pedro et al. 2018). GlcNAc has been also well studied for its biomedical applications and found to have vital potential in osteoarthritis, tissue repairing, and anti-inflammatory (Chen et al. 2010). Kubomura et al. (2017) analyzed the impact of GlcNAc administration on healthy subjects in order to find

Table 7.2 Some available products developed from chitin derivatives and their applications

Product name	Company	Product applications claimed
Chito-Max 750	Douglas Laboratories, USA	Inhibit dietary fat absorption from gastrointestinal tract
ChitoCare®	Primex ehf, Iceland	Rapid wound healing and relief from pain, sunburns, redness, and itching
Chitosan 1000 mg	Natural Balance, USA	Reduces dietary fat and cholesterol
LipoSan Ultra™	Primex ehf, Iceland	Facilitate weight loss and reduces body fat
KiOfine®-B	KitoZyme, Belgium	Removes Brettanomyces from beverages
GNC Total Lean™	GNC Live Well, Australia	Improve body composition and fuels energy and fat metabolism without stimulants
Fat Blocker	Vitabase, Georgia	Contains chitosan fibers which promotes a feeling of fullness
CELOX Gauze™	CELOX, UK	Rapid hemostatic agents
Axiostat®	AXIO, India	Wound care and dressing, having mucoadhesive property
Chito Max	AURA Biotech, India	Fungicides, plant growth and immunity booster, anti-viral, anti-bacterial

out that if the healthy subjects developed symptoms of arthritis upon GlcNAc administration. For this study they selected 68 healthy male and female and were randomly given GlcNAc (500 or 1000 mg/day) for 16 weeks. The results were evaluated by the help of biomarkers for type II collagen degradation and formation, collagen type II cleavage, procollagen type II carboxy-terminal propeptide and their ratios. The study concluded GlcNAc to be an attractive dietary supplement and its oral administration could improve the type II collagen metabolism of articular cartilage in healthy subjects without the development of arthritis symptoms (Kubomura et al. 2017). Another study evaluated the in vivo potential of the polyethylene glycol-GlcNAc-doxorubicin for the treatment of tumors in terms of improved efficacy and reduced toxicity (Pawar et al. 2017). The anticancer efficacy study was done on the mice-induced with skin melanoma by evaluating tumor volume, body weight, organ index, and percent survival rate of the animals and it was found twofold better tumor suppression with >70% of the survival rate compared to the standard (Pawar et al. 2017).

7.5.2 Food

The derivatives of chitin are well known for its application in improving the quality and shelf life of foods in the food industries. The major uses of CHS, COS, and GlcNAc in food industries are as edible film, additive, antimicrobial agent, and nutritional quality enhancer (Shahidi et al. 1999). The application of CHS and its

derivatives in beverages are one of the most attractive areas in food industries. The chitin-based bioactive derivatives can be used as natural preservatives or active packaging material in the beverages industries. Yang et al. (2017) utilized COS at 0.001–0.01% in forced aged beer to inhibit development of staling compounds and scavenge radicals. The study also detected the lower level of 5-hydroxymethylfural trans-2nonenal and phenylacetaldehyde development by 105%, 360%, and 27%, respectively, in contrast to the stale beer with no COS. CHS with 73% deacetylation at a concentration below 1% can improve the clarity of juices without affecting the nutritional value with strong scavenging activity of 90% (Ghorbel-Bellaaj et al. 2012). Similarly, CHS can be used as food preservatives to increase the shelf life in orange juice and ginger paste (Sarwar et al. 2014). The antimicrobial, antioxidant, and clarification properties of CHS and its derivatives are responsible for the development of the active packaging material (Rocha et al. 2017). Graphene oxide-CHS nanocomposites films were prepared for food packaging by cross-linking reaction at 120 °C with 6471.6 ± 1775.5 MPa tensile strength at break (Grande et al. 2017). The prepared nanocomposites films (0.6 wt %) showed the 22.83% and 54.93% of microbial inactivation against *E. coli* and *B. subtilis*, respectively. CHS coating over eggs can preserve the internal quality of eggs as well prevent it from microbial contamination when stored at tropical room condition. In a study conducted by Suresh et al. (2015), the eggs were coated with CHS solution with viscosity of 2206 mPa S and it increased the shelf life of eggs by nearly 4 week at 22 ± 1 °C and 3 week at 32 ± 1 °C as compared to the non-CHS coated eggs. The study also suggested the three-time CHS coating in order to maintain the internal quality of the stored eggs (Suresh et al. 2015). The addition of propolis extracts in CHS films enhances the tensile strength, total phenolic content, elongation at break, and antioxidant activity that result in the deeper orange color films with inhibiting activity against *S. aureus*, *Salmonella enteritidis*, *E. coli,* and *P. aeruginosa* (Siripatrawan and Vitchayakitti 2016). CHS in combination with cornstarch blend films can be monitor and function as an indicator of the food condition in real-time based on pH monitoring. The film was developed by using CHS, cornstarch, and red cabbage extracts and was able to show a strong reaction to pH change of the food samples (Silva-Pereira et al. 2015). The enzymatic browning of apple juice can be controlled by the application of oligochitosan and thus help in enhancing the shelf life of the juice with better antioxidase activities (Zhang et al. 2017). Similarly, CHS and oligochitosan have been reported to enhance the resistance induction of peach fruit against brown rot caused by *Monilinia fructicola* (Ma et al. 2013). CHS and COS have also been observed to increase the shelf life in bread (Rakkhumkaew and Pengsuk 2018), inhibit vitamin C formation in strawberries, and support synthesis of vitamin C in cherries (Kerch et al. 2011) and beneficial ingredient of fermented foods (Gurovic et al. 2015).

7.5.3 Agriculture

In recent years, the utilization of environmental-friendly natural compounds with properties like biocompatibility, biodegradability, and bioactivity for controlling crop diseases has becoming popular alternative to the chemical pesticides (Katiyar et al. 2014). In this regard CHS, COS, and GlcNAc have been shown huge potentiality due to their antifungal, antiviral, pest managing, and anti-bacterial properties (Iriti and Varoni 2015; Orzali et al. 2017). The bioactive compounds are known for their eliciting activities resulting into the several defense responses in host plants (i.e., accumulation of phytoalexins, pathogens related proteins and proteinase inhibitors, lignin synthesis, and callose formation) in response to microbial infections (El Hadrami et al. 2010). CHS can control plant disease development or trigger innate defense system in plants against pathogen by various applications mode viz. seed coating, foliar treatment, and soil amendment (El Hadrami et al. 2010). Chitin and its derivatives generate synergistic effect in soil by favoring the growth and activity of chitinolytic organisms which are the natural enemies to many vegetables plagues and diseases causing agents (Ramírez et al. 2010). COS can be proved to be a novel elicitors in plants in order to induce the innate immunity against pathogen attack (Das et al. 2015). Boonlertnirun et al. (2017) showed the ability of CHS in enhancing rice production when used in seed soaking and in soil for four times during the cropping season. Similarly, CHS foliar use on the growth, yield, and fruit quality of strawberry plants were evaluated by spraying CHS solution three times starting at 10 weeks after transplanting with 4 weeks interval in concentration of 0 (control), 1, 2, 3, and 4 cm^3/l until runoff (Abdel-Mawgoud et al. 2010). The study concluded that CHS foliar applications enhanced plant height, number of leaves, fresh and dry weight of leaves, and yield components in terms of numbers and weight (Abdel-Mawgoud et al. 2010). Recently, CHS from mushroom industry waste was applied on fresh-cut melons and it was observed that CHS application enhanced fruit firmness, inhibited off-flavors, and reduced microbial counts up to 4 log CFU/g (Poverenov et al. 2018). The study also illustrated that the CHS coated melons had higher esters contents (79.93%) than of uncoated melons (57.15%), resulted into the fruit flavor (Poverenov et al. 2018). CHS has also shown its potential application in enhancing growth and yield of strawberry (Mukta et al. 2017) and enhancing yield and curcumin content in turmeric plant (Anusuya and Sathiyabama 2016). Van Phu et al. (2017) prepared the mixture of oligochitosan and nanosilica by using 2% oligochitosan and 2% nanosilica. The study showed that upon foliar use of oligochitosan and nanosilica on soybean seed, there was rise in the yield (17%) compared to oligochitosan alone (10.5%).

7.5.4 Wastewater Treatment

Water is one of the most vital components required for the survival of all known life forms on the earth. Due to the speedy population growth there is a huge volume of wastewater generation which is contaminated with various organic and inorganic

wastes (Vakili et al. 2014). The major source of water pollution is discharge from industries like textile, dairy, tannery, chemical, and food processing. The depleting level of natural water resources and eruption of number of wastewater generated diseases lead to need for the development of efficient wastewater technology, so that it can be reused as well as control of water-borne diseases. In this concern natural bioactive compounds like CHS, COS, and GlcNAc can play vital role (Sudha et al. 2014). CHS has been much explored for its efficiency in removal of metal ion and other contamination from wastewater (Gamage and Shahidi 2007). Chi and Cheng (2006) utilized CHS as coagulant to remove high content of fat and protein from waste water. The study reported pH 7 with coagulant dose of 25 mg/l as the optimum condition for the waste water treatment with CHS. In another study, CHS aerogel was developed as an absorbent for organic pollutants and heavy metal ions (Li et al. 2016). The prepared CHS aerogel was reusable with excellent elasticity and the maximal thickness recovery was up to 96.8% of the original thickness. The study reported the adsorption capacities for crude oil (41.07 g/g), diesel oil (31.07 g/g), and copper ion (21.38 mg/g) (Li et al. 2016). Similarly, CHS was reported to be having high molybdate adsorption capacity (265 $\pm$ 1 mg/g) at 20 °C and pH 2.7 from the groundwater samples (Bertoni et al. 2018). The study also suggested that hydroxyl group were responsible for the molybdate adsorption. The combination of nanotechnology with chitin derived products has proved to be more efficient in wastewater treatment. The reticulated CHS micro/nanoparticles were used for the treatment of wastewater contaminated with hexavalent chromium, Cr (VI) and it was found that the prepared formulation was efficiently reduced the toxic Cr (VI) to the less toxic Cr (III) (Dima et al. 2015). Similarly, Alarcón-Payán et al. (2017) synthesized the CHS nanoparticles loaded with versatile perocidase with an average diameter of 120 nm with elevated enzyme loading capability. The prepared enzymatic nanoparticles were also reported to transform ten phenolic compounds including pesticides, halogenated compounds, endocrine disruptors, and anti-bacterials. Gokila et al. (2017) used CHS and alginate nanocomposites for the removal of Cr (VI) from wastewater, whereas Kahu et al. (2016) utilized ionic solid impregnated CHS for Cr (VI) removal with an adsorption capacity of 266.67 mg/g. Chitin derived products have also been well documented for its application in color absorbent from wastewater. The fermented squid pen powder consisting of COS with degree of polymerization three to nine showed better adsorption rate (96%) for the disperse dyes. Allura Red Ac and Tartrazne (Liang et al. 2015). CHS can be used with other dye absorbent like *S. cerevisiae* to enhance the dyes removal from the wastewater (Dilarri and Corso 2018). The study utilized contact immobilization as well as encapsulation techniques to immobilized *S. cerevisiae* in cross-linked CHS beads and showed the significant removal of dyes from effluents along with its toxicity reduction (Dilarri and Corso 2018). The modified CHS by (3-chloro 2-hydroxypropyl) trimethylammonium chloride was evaluated to reduce the color and turbidity of industrial wastewater and its optimum conditions for the color removal were found to be pH 3.0, concentration of dye 1000 mg/l, settling time 78.93 min, and dose of coagulant 3 g/l with the removal of 82.78% of color (Momeni et al. 2018).

7.5.5 Cosmetics

Cosmetics are the formulation or preparation meant to apply on human external body parts viz. nails, lips, epidermis, hair, and teeth in order to get one or all aspects like cleaning, purification, protection, maintenance of native texture or change in the appearance (Aranaz et al. 2018). Thus, cosmetics are for external use only and any claim related to disease treatment do not falls under this category. There are many natural resources known for their biologically active substance which can be used in cosmetics. But, in recent years a lot of attentions are focused on the marine resources especially on chitin derivatives like CHS, COS, and GlcNAc due to their various cosmeceutical properties viz. anti-aging, antioxidant, anti-wrinkling, anti-whitening, anti-tyrosinase, anti-acne, anti-inflammatory, UV photo protective, and cytoprotective (Kim 2014). In this regard, CHS and their derivatives can play crucial role due to their valuable functions like film former, gelling agent, moisturizer, antimicrobial, viscosity enhance, etc. (Jimtaisong and Saewan 2014). Sionkowska et al. (2017) prepared a thin film from blend of collagen, CHS, and hyaluronic acid for hair care cosmetics. They reported that the supplement of hyaluronic acid to the collagen/CHS blend enhanced the mechanical resistance of the prepared film. Similarly, quaternized carboxymethyl CHS and organic montmorillonite nanocomposites based cosmetic cream were reported to negligible dermal irritation with a prominent moisture-retention efficacy on stratum corneum (Chen et al. 2017). In another study, neutralized CHS citrate and acetate films were synthesized and evaluated for the hyaluronic acid release in a skin model (Libio et al. 2016). The CHS films neutralized in citrate buffer was showing greater physical integrity resulted from better thickness, decreased moisture absorbance, lower tendency to solubility in the acid medium, and better swelling capacities (Libio et al. 2016). The study also reported that the neutralized CHS citrate films promote skin exfoliation in both presence and absence of hyaluronic acid. Bissett et al. (2007) studied in vitro genome expression in SkinEthic skin equivalent culture by treating with GlcNAc and found that GlcNAc was effective in reducing melanin production along with the alteration in several pigmentation-related genes. Recently, anti-photogenic application of COS has been reported in UV-irradiated hairless mouse skin (Kong et al. 2018). They suggested that GlcNAc attenuated UV-induced skin photoaging by regulating the antioxidant and anti-inflammatory status that maintain the morphology and collagen level (Kong et al. 2018).

7.6 Future Roadmap

The efficiency of microbes in transforming chitin into value-added products have been well documented along with their various applications. There is also some product development in the area of wound dressing, cosmetics, and nutraceutical from chitin derived CHS, COS, and GlcNAc. However, still there is a long way to go in terms of successful product development for medicinal and agricultural purposes. Therefore, the requirement of novel strains which are capable of producing highly

effective enzymes to convert chitin into more valuable products with more specificity is utmost. But, the research progress in this field along with technological advancement is promising that chitin derivatives will prove a noble asset for human welfare in near future.

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Part II

Agricultural Utilization

8 Utilization and Management of Agricultural Wastes for Bioenergy Production, Weed Control, and Soil Improvement Through Microbial and Technical Processes

Nicholas E. Korres

Abstract

The utilization and management of agricultural wastes for plant growth and fertilization or crop production and protection purposes can facilitate the transition of current agricultural systems or "productivism agriculture" to a post-productivism agriculture or "production of nature" era. The availability of agricultural wastes, a theoretical estimation indicates that approximately 3.5–17.0 billion tons of agricultural wastes can be produced worldwide, in combination with the various technologies for the conversion of these wastes to bioenergy are significant factors that enhance the value of this type of feedstock. In addition, the role of agricultural wastes as mean for weed control (e.g. maize gluten meal, Brassicaceae seed meal, abrasive grit), soil amendments and fertilizer substitute (e.g. abrasive grit, biochar, Brassicaceae oilseed meal, digestate from anaerobic digestion), or plant growing medium (e.g. composts) signifies the added value of these materials toward sustainable production systems. Nevertheless, the utilization of agricultural wastes should not disturb the soil carbon and other nutrients dynamics. This can be avoided by setting appropriate limits and continued monitoring, hence, reducing uncertainty about their beneficial environmental performance.

8.1 Introduction

The need for new agricultural production approaches such as integrated food and energy systems (Bogdanski et al. 2010) or the utilization of agricultural wastes (or residues or by-products) for plant growth and fertilization (Zhang et al.

N. E. Korres (✉)
Global Change Photosynthesis Research, USDA-ARS, Urbana, IL, USA
e-mail: nkorres@illinois.edu

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2013a, b) or crop production and protection purposes (Korres et al. 2019, 2013; Waqas et al. 2019; Diaz 2007) necessitates the rapprochement of the agricultural practices that promote sustainability (Korres et al. 2019, 2013; Korres 2013). Hence, a transition from the traditional model of a productivism agricultural era i.e. production of food and fiber (Wilson 2007) should focus on environmental management that leads to a post-productivism or "production of nature" era (Marsden 1999).

The benefits of this transition are multiplicative and include, among others, potential increases of the non-farm income through the incorporation of emerging technological opportunities into the production system that satisfy the preferences of the consumers for environmental friendly and sustainable production systems (Bogdanski et al. 2010; Korres 2013). Agricultural wastes hold an important role in this transition toward the new production paradigm. It should be noted that the use of the term "waste" in this chapter does not imply materials of no value but materials that can be reused for the production of energy, substitutes of fertilizers, or livestock feed additives (Zhang et al. 2013a, b). More specifically, the term agricultural wastes normally relates to all types of wastes produced at agricultural premises (i.e. land used for row and horticulture crops, cash crops and plantations, orchards and vineyards, seed growing, animal-based production land, market gardens and nursery grounds, or commercial woodlands) as a result of an agricultural activity i.e. harvesting of the raw material (e.g. fruits, vegetables, meat, poultry, dairy products, and crops) (Anonymous 2006) or processing it into intermediate and final products (Obi et al. 2016).

Agricultural wastes, therefore can be defined as biomass that includes wastes from crops (corn stalks, sugarcane bagasse, drops and culls from fruits and vegetables, pruning), animals (manure and animal carcasses), or food processing activities (e.g. only 20% of maize is canned and 80% is wasted) (Fig. 8.1) (Obi et al. 2016).

Hazardous and toxic agricultural wastes i.e. waste or combination of wastes, that could adversely harm human and environmental health if improperly used or managed (EPA 2017) (e.g. insecticides, herbicides, fungicides, rodenticides, etc.) or wastes from the process or consumption of the treated raw material including municipal (e.g. institutional, commercial), or social (i.e. health care, household, sewage sludge) wastes are far beyond the scope of this chapter and will not be discussed.

Lignocellulosic biomass of agricultural or forest origin is the most investigated type of agricultural wastes for the production of renewables (Kim et al. 2002; Jorgensen et al. 2007) such as biogas (Korres et al. 2013), biohydrogen (Korres and Norsworthy 2017), bioethanol (Singh et al. 2010), or biodiesel (Smyth et al. 2010).

The availability of the lignocellulosic materials, according to Pitkanen et al. (2003), can support the sustainable production of liquid transportation fuels. Kim et al. (2002) have estimated that bioethanol production by the utilization of the dry waste material from the crops (approx. 74×10^5 t) is equal to 49.1×10^6 L year^{-1}, a quantity that is 16 times higher compared to the current bioethanol production

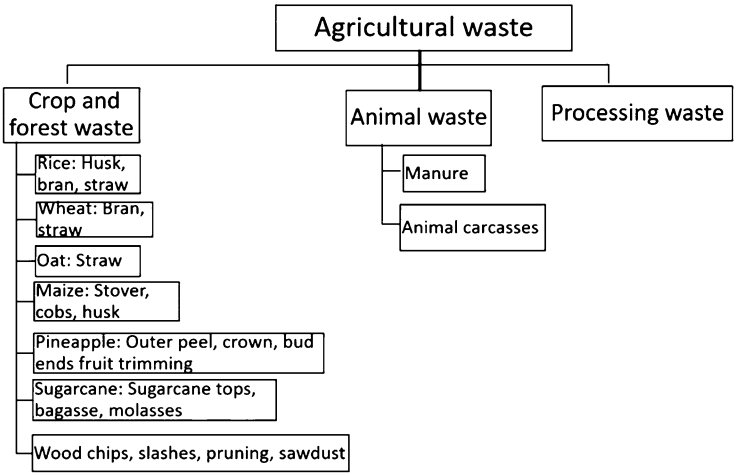

Fig. 8.1 Classification of agricultural wastes (based on Obi et al. (2016), Korres and Norsworthy (2017), Korres et al. (2013), Korres et al. (2013), Zhang et al. (2013a, b), Nagendran (2011), Holm-Nielsen and Oleskowicz-Popiel (2008), Jorgensen et al. (2007), Kim et al. (2002))

worldwide. It has been reported that a significant portion of the European renewable energy will be based on feedstock from farming and forestry (Korres et al. 2013). In addition, 25% of the future EU bioenergy production, as reported by Holm-Nielsen and Oleskowicz-Popiel (2008), will be originated from biogas produced by wet organic materials (e.g. animal manure, whole-crop silages, wet food, and feed wastes).

Agricultural wastes can also be used, directly or indirectly, as plant growth promoting material (e.g. seed priming, soil amendments, fertilizers, or for weed management) as it will be discussed in the following sections of this chapter.

Ideally, agricultural wastes should be immediately returned to the soil although this usually is not feasible and economically justifiable due to the involvement of many variables that influence their incorporation into the soil. Wastes of animal-based agricultural operations, such as manure that is biologically and chemically active, often require intermediate processing before their final utilization (Krider et al. 2009). In addition, application of manure in the field is labor-intensive and may be difficult or prohibited for use if the ground, for example, is frozen or saturated or the application is inhibited by the growth stage of the crop (Krider et al. 2009). The purpose of this chapter is to discuss the need for agricultural waste use and management through biochemical and microbiological processes.

8.2 Quantitative and Qualitative Characteristics of Agricultural Wastes

8.2.1 Quantitative Characteristics

Agricultural wastes (i.e. residues and by-products) for the production of renewables, soil amendments, or plant growth agents can be classified into three categories namely arable land or herbaceous wastes, woody wastes, and other forms of wastes.

Residues from arable land include herbaceous residues (i.e. these directly originating from agricultural crops) such as straw, stalks, etc. and the residues from the process of the agricultural raw material such as sugarcane bagasse, rice husks, etc. (Fig. 8.1) (Singh et al. 2010). Nevertheless, there is a differentiation of herbaceous residues based on crop type and region of production. Oilseed crops such as soybeans or oilseed rape, for example, tend to produce fewer residues compared with cereal grain crops (Nelson 2007) whereas sugarcane, in tropical and sub-tropical regions, exhibits the higher bagasse production (Kumar et al. 2008). Herbaceous residues are partly used on farms, remain on the fields, and incorporated into the soil or burned (Cooper et al. 1999).

Woody wastes are these originating from permanent crops e.g. orchards, vineyards, olive, and palm plantations and they are produced mostly by pruning. In this category materials such as empty fruit bunches, effluents can also be included (Anonymous 2016).

There are large quantities of agricultural biomass available for non-agricultural uses. A theoretical estimate of agricultural biomass residues indicates that approximately 3.5–17.0 billion tons can be produced worldwide (Fig. 8.2).

These, as mentioned earlier, include straw, husk, cobs, kernels, plant leaves, plant tops, etc., which are generated during crop harvesting operations (Fig. 8.3) and raw material processing.

The major crops contributing more to the production of agricultural residues (or by-products) include cereals, oil seeds, and sugar crops. The estimated theoretical energy potential (low and high) of the agricultural residues from these crops is within 0.6 EJ to approximately 100 EJ (Fig. 8.4).

Factors that contributing to high variation of agricultural residues energy potential include moisture content, residue-to-product ratio, and heating value.

8.2.2 Chemical Composition of Agricultural and Woody Residues

The composition of the residues from arable crops along with the carbohydrate composition of cereal residues are shown in Figs. 8.5 and 8.6.

The structural carbohydrate content, particularly cellulose and hemicellulose, of sugarcane bagasse despite its lower dry matter percentage, followed by oat, grain sorghum, barley, maize, rice, and wheat is noticeable in Fig. 8.5. The non-structural carbohydrate content of sugarcane baggage is higher compared with most cereal crops except barley and sorghum (Fig. 8.6).

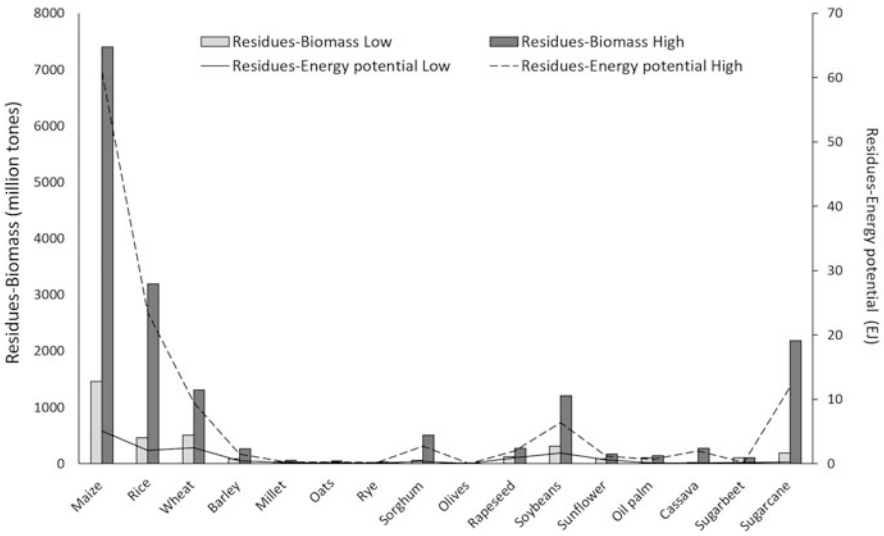

Fig. 8.2 Range (low and high) of the theoretical energy potential (EJ) and production (million tons) of agricultural waste (residues) in 2014 globally (based on Kummamuru et al. 2017); *EJ* Exajoule or 1.055×10^{18} J

Fig. 8.3 Biomass generation during harvesting operations in a (**a**) soybean straw spread across the entire field, (**b**) maize straw placed in a narrow windrow for burning as a preventive weed control method, and (**c**) winter wheat as straw bales

The percent chemical composition (i.e. structural and non-structural carbohydrates) of soft- and hardwood type residuals is shown in Fig. 8.7.

The similarities of chemical composition between hard and soft wood concerning their non-structural (e.g. cellulose, hemicellulose, and lignin) and structural carbohydrates (e.g. glucose, xylose, mannose, galactose, etc.) are shown in Fig. 8.7. Nevertheless, the content of lignin, the major factor that affects the efficient utilization of cellulose fibers from woody materials (Li et al. 2003; Chen and Dixon 2007) is higher in hardwood (angiosperms) compared to softwood (gymnosperms)

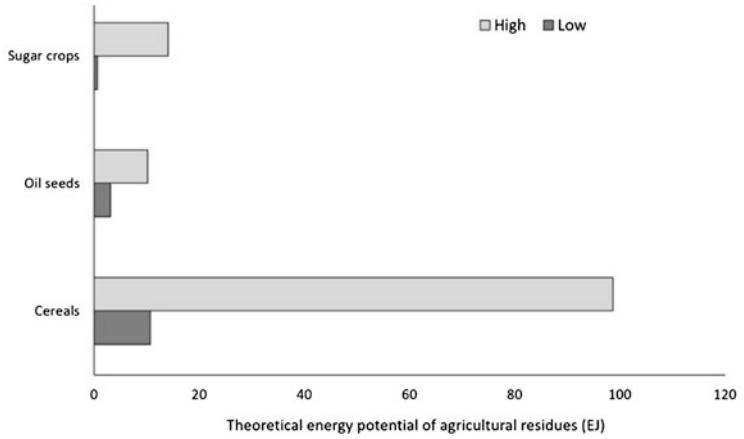

Fig. 8.4 Range (low and high) of the theoretical energy potential of major crop wastes (based on Kummamuru et al. 2017); *EJ* Exajoule or 1.055×10^{18} J

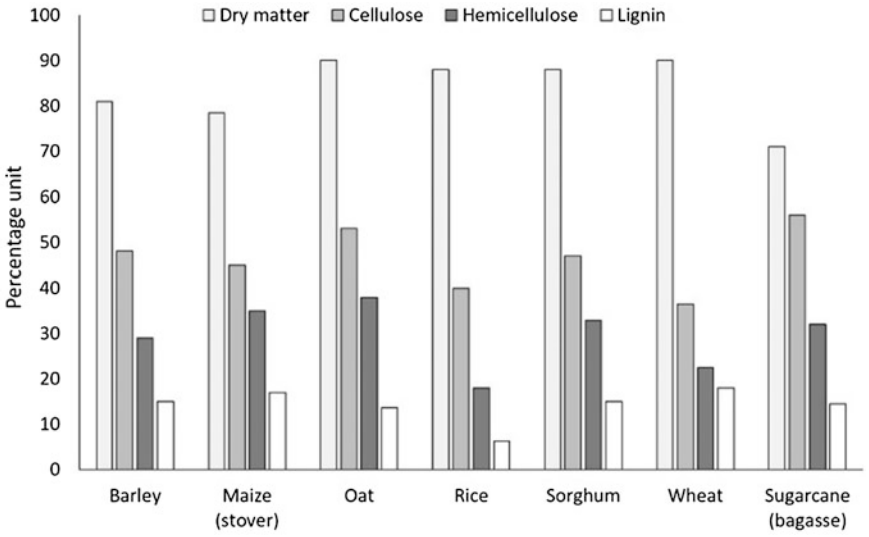

Fig. 8.5 Percentage composition of residual structural carbohydrates from arable crops (based on Demirbas 2005; Atchison 1997; Mabee and Roy 1999)

most probably due to the differences in gravitropic stimulation or mechanical stress (known as reaction wood) (Timell 1986; Andersson-Gunneras et al. 2006), a characteristic that should be taken under consideration when woody waste is treated for useful purposes.

It is typical that cereal straw is considered as the most available biomass source for various non-agricultural purposes particularly in the main cereal growing areas. Morphologically, fibers of cereal straw are more heterogeneous than wood fires

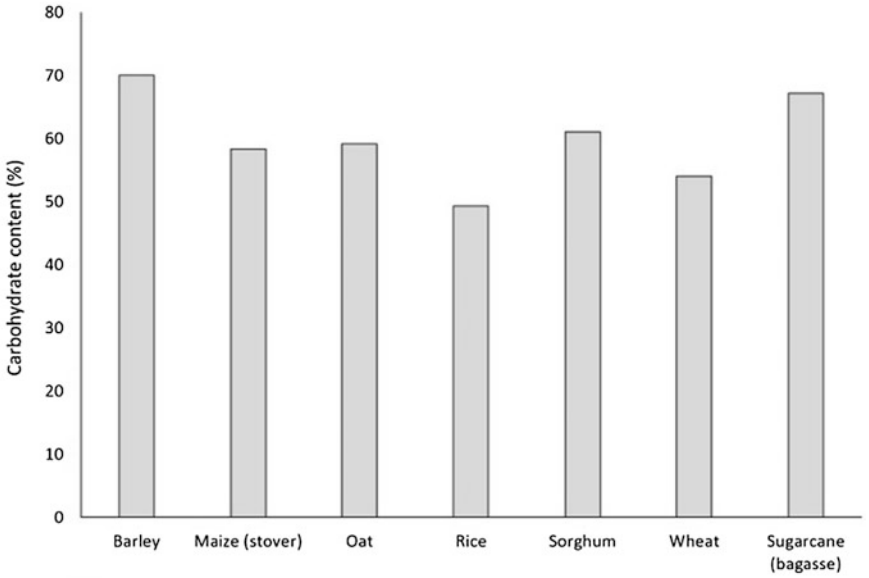

Fig. 8.6 Percentage composition of residual non-structural carbohydrates from arable crops (based on Demirbas 2005)

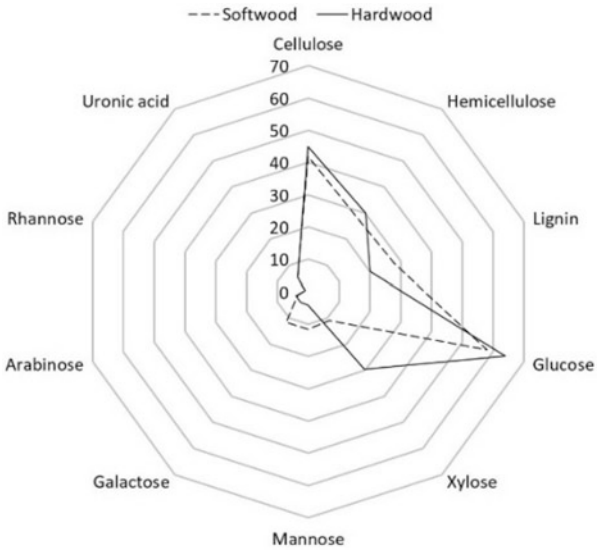

Fig. 8.7 Percent chemical composition of woody waste material (based on Demirbas 2005; Singh et al. 2010)

(Cooper et al. 1999). In comparison with wood, cereal straw contains about the same amount of holocellulose, but much less alpha cellulose. In addition, pentosan is higher whereas lignin is lower in cereal straw than wood (Atchison 1997). In particular, wheat straw contains about 35–45% alpha cellulose or simply cellulose and 30 % hemicelluloses. Cellulose has the same chemical structure (i.e. a linear polymer of D-glucopyranose units) regardless of plant source. Hemicellulose is variable between plant species. In wheat for example hemicellulose accounts for 30–40% of the plant tissue (Atchison 1997; Mabee and Roy 1999; Demirbas 2005; Singh et al. 2010) whereas the lignin content accounts for 20–30%. Lignin is a complex amorphous polymer that exhibits chemical differences between the basic building blocks in softwoods, hardwoods, and agricultural plants (Novaes et al. 2010).

8.3 Need for Agricultural Wastes Management

The production of food material and fibers is related with various sources of environmental pollution and waste generation (e.g. manure, oil, silage or horticultural plastics, effluents from silage or slurry, wastes from farms, poultry houses and slaughterhouses; veterinary medicines) (Ramirez-Garcia et al. 2019). Improper management of the agricultural wastes can result in environmental pollution through the day-to-day activities e.g. fertilizer leaching, silage effluent or slurry run-off from fields or disposal areas; pesticides that enter into water, air, or soils through leaching, volatilization; and salt and silt drained from fields (Nagendran 2011).

Agricultural wastes based on their physical state can be categorized as liquid or solid, based on their harmful potential as hazardous or non-hazardous waste (Box 8.1).

Box 8.1 Hazardous and Non-hazardous Agricultural Waste

Hazardous wastes are defined as wastes that have one or more of the following properties:
- Corrosive
- Ignitable
- Toxic
- Reactive
- Infectious

Potential sources of hazardous waste in agricultural premises and rural households include obsolete pesticides (e.g. herbicides, insecticides, rodenticides, etc.) or improperly disposable empty pesticide containers (Korres 2019)

Non-hazardous agricultural wastes include all other types of waste as it has been mentioned above

As earlier mentioned only management of non-hazardous wastes will be discussed in this section and more particularly only herbaceous wastes and wastes

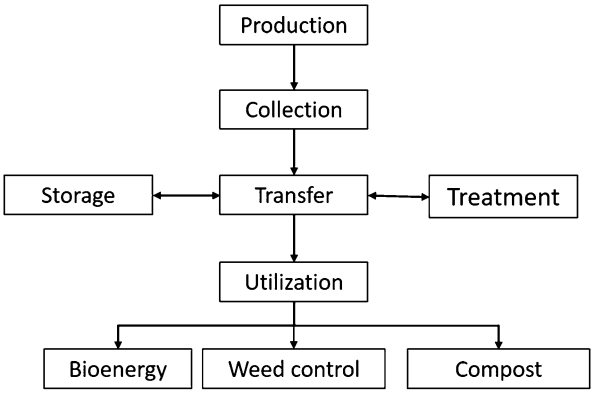

Fig. 8.8 Operational constituents of an agricultural waste management scheme (based on Krider et al. 2009; Tumuhairwe et al. 2009). Only the operational treatment that lead to the production of bioenergy, compost (fertilizers or soil amendments), plant promotion, and weed control will be discussed in this chapter

from livestock husbandry and production operations except those produced in slaughterhouses. These include solid wastes such as biomass, manure, slurry, and wastewater (Obi et al. 2016). In general, agricultural wastes if left untreated can generate greenhouse gases, imposed problems on soil fertility, and potentially cause water pollution. They also attract and contain significant amounts of microorganisms and parasite eggs that can cause serious human health problems (Hai and Tuyet 2010).

Agricultural waste management refers to methods and handling processes of waste from generation and collection through final disposal (Fig. 8.8). Waste management is needed in order to prevent direct or indirect contact of this material with humans and their environment (Tumuhairwe et al. 2009).

Proper management and processing are contributing significantly to waste transformation into useful materials. The following sections will discuss briefly the most important biochemical and microbial processes for the transformation of agricultural waste into energy, plant growth promoting medium, or crop protection agents.

8.4 Utilization of Agricultural Wastes

Increased demand for bioenergy production using feedstock of agricultural origin will be a significant factor for the development of new agricultural markets over the next years (FAO (Food and Agriculture Organization) 2008). Consequently, agricultural income and the quality of life in rural areas through new market opportunities (FAO (Food and Agriculture Organization) 2008) will be improved. Fossil fuels are expected to remain the main source of the primary energy mix but the use of biofuels with lignocellulosic origin will be on rise (Pitkanen et al. 2003). In addition, agricultural waste for the production of soil amendments, substitution of fertilizers, or usage as crop protection products have been thoroughly investigated and were proved a reliable added value source (Waqas et al. 2019; Korres et al. 2019).

8.4.1 Biogas Production Through Anaerobic Digestion

Lignocellulosic material and other organic wastes suitable for biogas production can be crops such as grass or maize silage (Korres et al. 2010a, b; McEniry et al. 2013; Neureiter 2013), agricultural wastes (Nuri et al. 2008; Parawira et al. 2008; Holm-Nielsen et al. 2009; Rao et al. 2010; Eze and Ojike 2012), or other organic material of agricultural origin such as dry or wet manure (Boysan et al. 2015; Zhang et al. 2013a, b; Khalid et al. 2011; Singh et al. 2011; Wu et al. 2010; Chae et al. 2008) which is appropriate for bacterial biodegradation (Fig. 8.9).

8.4.1.1 Anaerobic Digestion—Microbial Processes

Anaerobic digestion is the process by which organic material, including agricultural wastes, is converted to biogas through a series of biochemical processes in an oxygen free environment (Korres et al. 2010a). The microorganisms that grow under this anaerobic environment produce and reduce one each other's end products. The outcome of this complex process is methane and carbon dioxide (McCarty 1982). The consecutive biological processes through which organic polymers are breaking down biochemically include hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Fig. 8.10). During hydrolysis the organic material is converted into monomers by extra-cellular enzymes. During acidogenesis, these monomers are transformed into volatile fatty acids such as acetic, propionic, and butyric. Acetate, along with carbon dioxide (CO_2), and hydrogen (H_2), is then produced during acetogenesis which is finally converted into methane (CH_4) during methanogenesis (Bernet and Beline 2009).

8.4.1.2 Configuration of Anaerobic Digesters

The process of biogas production is not efficient unless it is conducted under controlled environmental conditions in an anaerobic digester a technology which, if correctly designed, will secure the optimization of organic material conversion to gaseous product (Demirbas and Ozturk 2005). The configuration of anaerobic digester depends on various factors which are related to solid content of the organic material, the number of digestion activities and phases, the method of material supply and its retention time in the digester, temperature, and finally the organic

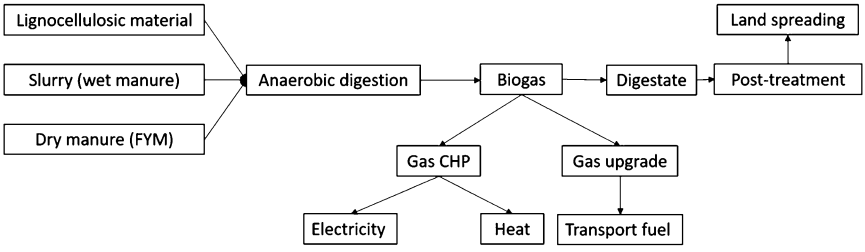

Fig. 8.9 Flow chart for the production of biogas from agricultural wastes through anaerobic digestion. *FYM* farmyard manure, *CHP* combined heat and power

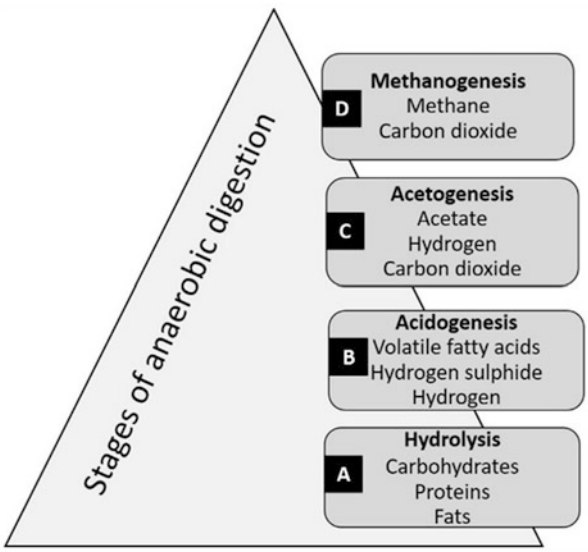

Fig. 8.10 Microbial processes during anaerobic digestion (end products from a predecessor process in one stage e.g. hydrolysis used as a source for the following immediate stage i.e. acidogenesis)

loading rate (Karagiannidis and Perkoulidis 2009; Nizami and Murphy 2010; Nizami et al. 2013). Various anaerobic digester configurations are shown in Fig. 8.11.

In a one-stage digester, hydrolysis, acidogenesis, and methanogenesis occur in the same tank whereas in the two-stage reactor configurations hydrolysis and acidogenesis occur in the first tank and acetogenesis and methanogenesis in the second tank. In batch digesters, the feedstock is inserted into the digester once for a certain period whereas in continuous digesters the feedstock is constantly or regularly fed into digester. In dry digesters, feedstock that contains dry matter between 20 to 50% is used as substrate. The feedstock is either sprinkled with recirculating water (dry batch digestion) or mixed with digestate (dry continuous). On the contrary, wet digesters, such as the continuously stirred tank (CSTR) digester, operate with feedstock that contains less than 12% dry solids. High solid content feedstock may be treated in a wet continuous system through homogenization to liquid state (Nizami and Murphy 2010).

8.4.2 Cellulosic Bioethanol

Cellulosic ethanol is chemically identical to first-generation bioethanol, but it is produced from different feedstock through a complex process known as cellulosic hydrolysis. Feedstock for the production of cellulosic ethanol can be agricultural residues and other lignocellulosic materials (EUBIA, 2012 European Biofuels Technology Platform, undated). Acidic or enzymatic hydrolysis transforms these materials to ethanol. In particular, the feedstock undergoes through a pre-treatment stage before the hydrolytic stage followed by fermentation, product separation,

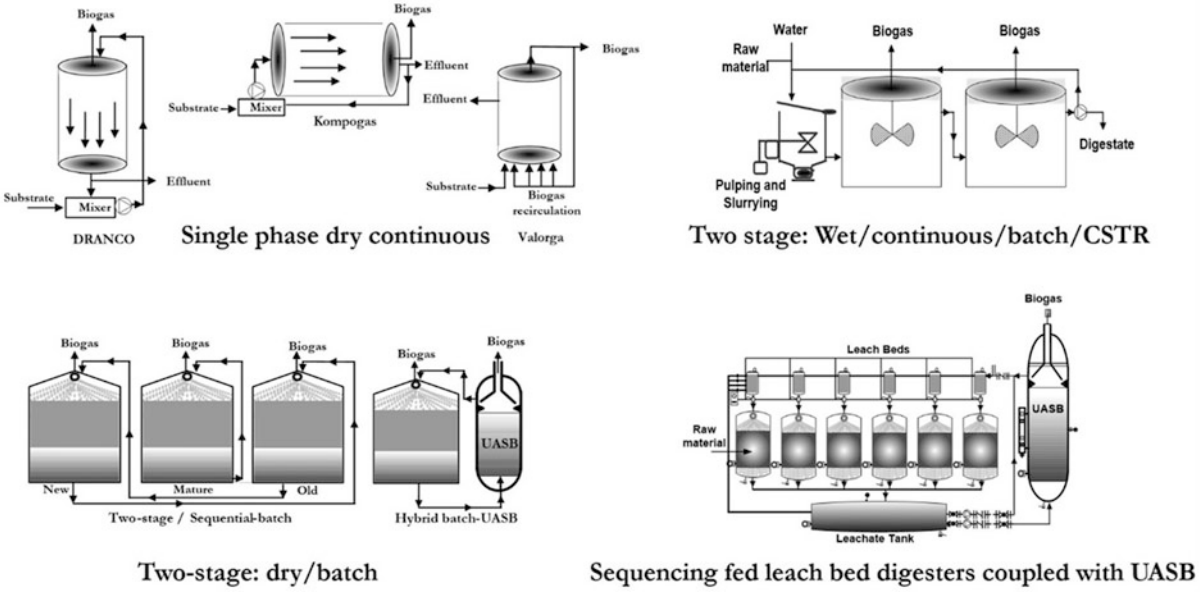

Fig. 8.11 Various anaerobic digester configurations. *Top left*: a single stage (phase) dry continuous digester; *Top right*: a two-stage (phase) wet, continuous, batch digester (CSTR, continuously stirred tank reactor); *Bottom left*: a two-stage (phase) dry batch digester and *Bottom right*: a sequencing fed leach bed digesters coupled with an upflow anaerobic sludge blanket (UASB). *DRANCO* DRy ANaerobic COnversion (based on Vandevivere et al. (2003), Nizami and Murphy (2010), Nizami et al. (2013))

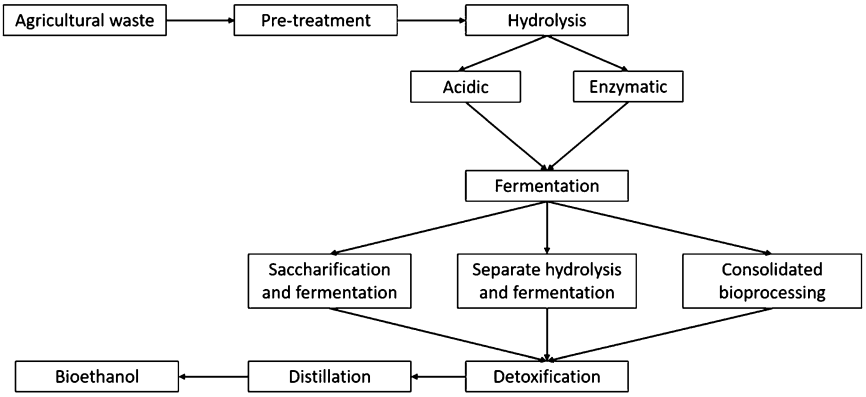

Fig. 8.12 Lignocellulosic bioethanol production (based on Korres et al. 2013)

distillation, and post-treatment of the liquid fraction (Fig. 8.12) (Balat et al. 2008; Hendriks and Zeeman 2009). According to Di Nicola et al. (2011), the classic method for the fermentation of the hydrolyzed biomass consists of two separate processes (i.e. hydrolysis and fermentation) which are conducted in two different units. Alternatively, a simultaneous saccharification and fermentation of lignocellulosic biomass (i.e. hydrolysis and fermentation are completed in the same unit) can be used (Di Nicola et al. 2011). Pre-treatment of initial feedstock is an important phase for high production rate and high yield of monomeric sugars (i.e. pentoses and hexoses) during the following hydrolysis phase. The remaining fractions of cellulose and hemicellulose from the pre-treatment phase are converted during hydrolysis (is facilitated by the addition of acids or enzymes such as cellulases) into monomeric sugars, which then can be fermented to bioethanol.

The fermentation of pentoses is a selective process which can only be accomplished by few organisms. Inhibitors to yeasts and bacteria, the fermenting vehicles of pentoses are ethanol itself along with furans, phenolic, carboxylic acids, and other compounds that are formed during fermentation (Hendriks and Zeeman 2009). Ethanol is recovered from the fermentation broth by distillation (Hendriks and Zeeman 2009) whereas the process residuals can be used to produce heat or chemicals through their conversion into octane boosters (Wyman 1994).

8.4.3 Biohydrogen Production

The role of biohydrogen as an energy carrier is considered vital (Balat 2008; Veziroglu and Sahin 2008) due to wide range of the available resources for its production (Perlack et al. 2005) including lignocellulosic material originating from agricultural wastes and residues (Balat and Kırtay 2010) particularly these with high content of soluble carbohydrates (Kapdan and Kargi 2006; Lay et al. 2003). These mainly include sugar cane or sweet sorghum bagasse, olive husks, rice washing

drainage fruit and vegetable wastes such as peeling extracts or vegetable pulp from cabbage and carrots or waste from processed potato and lettuce along with nut shells, rice husks, cereal straw, grain residues, sawdust, woodchip, wood bark, grasses (May-Tobin 2011; Tenca et al. 2011; Ntaikou et al. 2010; Venkata Mohan et al. 2009; Dong et al. 2009; Vijayaraghavan et al. 2006; Ni et al. 2006; Perlack et al. 2005; NRC-National Research Council (U.S) 2004; Okamoto et al. 2000), and animal manure which is usually used as co-substrate to other feedstock (Tenca et al. 2011; Wu et al. 2009; Zhu et al. 2009; Holm-Nielsen and Al Seadi 2004). However, the use of lignocellulosic material for hydrogen production depends on production method's cost (Kapdan and Kargi 2006) along with issues related to the logistics of agricultural wastes (Abedi et al. 2001).

8.4.3.1 The Process

Biochemical and thermochemical conversion of biomass to hydrogen are the two main pathways for biohydrogen production. The former includes anaerobic digestion and fermentation processes. Anaerobic digestion, as mentioned previously, uses bacteria in a free oxygen environment to break down a wide range of organic feedstocks, to produce biogas for electricity or transportation (Al Seadi et al. 2008; Korres et al. 2010a, b). Fermentation uses microorganisms (e.g. yeasts or bacteria) or enzymes at low pressure and temperature levels to enzymatically break down organic macromolecules to biohydrogen (Korres and Norsworthy 2017). It is a promising biological route due to the ability of the microorganisms involved to convert a wide range of biomass and organic wastes into valuable hydrogen energy (Korres and Norsworthy 2017).

8.4.3.2 Direct and Indirect Photolysis

Biological methods i.e. direct and indirect (bio)photolysis that involve cyanobacteria and algae, use light energy to produce hydrogen from water (Hallenbeck and Benemann 2002). During direct photolysis cyanobacteria and green algae break down water into oxygen and hydrogen through a PSII-dependent pathway that links water-splitting activity to H_2 (Show et al. 2019; Korres and Norsworthy 2017; Buitron et al. 2017). More particularly, the hydrogen production occurs through the direct absorption of light and transfer of electrons to two different enzymes i.e. hydrogenases and nitrogenases (Manis and Banerjee 2008).

The former catalyzes the reaction $2H^+ + 2X_{reduced} \rightarrow 6\ H_2 + 2X_{oxidized}$ in which water is the electron donor and ferredoxin, X, (a unique complex that one of the Fe atoms is complexed with CO and CN) acts as an electron carrier resulting in the transformation of H^+ to H_2 (Sorensen 2005; Turner et al. 2008) (Fig. 8.13).

Catalytic hydrogen production by nitrogenase, an enzyme mainly exists in prokaryotic organisms including cyanobacteria, occurs as a side reaction at a rate of one-third to one-fourth than that of nitrogen fixation (Miyamoto 1997). Indirect photolysis involves a PSII-independent pathway in which electrons derived from glycolysis enter the linear electron chain at plastoquinone pool before transferred to hydrogenase via the PSI-mediated electron flow (Buitron et al. 2017).

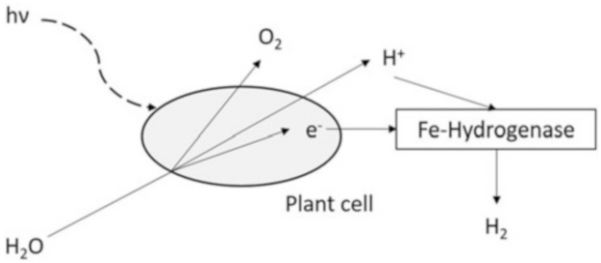

Fig. 8.13 Direct photolysis where O_2 and H_2 are produced simultaneously in aerobic direct photolysis that is involving water-oxidation and a light-dependent transfer of electrons to the [Fe]-hydrogenase and the reduction of H^+ to H_2 (based on Show et al. 2019)

8.4.3.3 Fermentation

Fermentation is another technology that involves several genera of bacteria capable of fermenting the carbohydrates (either structural or non-structural), proteins, and lipids as substrates to produce hydrogen. Various types of fermentation including dark-, photo-, or sequential dark- and photo-fermentation are also used for the production of biohydrogen from lignocellulosic materials and agricultural wastes.

8.4.3.4 Dark Fermentation

Hydrogen can be produced by anaerobic bacteria through dark fermentation such as those of genus *Clostridium* which are suitable for hydrogen production because of high production rate (Chong et al. 2009; Ferchichi et al. 2005; Krupp and Widmann 2009). Other bacteria species capable for hydrogen production include *Enterobacter* (Nath et al. 2006), *Bacillus* (Kotay and Das 2007), and *Thermotoga* spp. (Schroder et al. 1994). Simple sugars are metabolized by these microorganisms by converting glucose molecules to pyruvate which in turn is oxidized by pyruvate-ferredoxin oxidoreductase enzyme to acetyl CoA, CO_2, and reduced ferredoxin, the reoxidation of which produces hydrogen (Schroder et al. 1994; Hallenbeck and Benemann 2002). Dark fermentation, using the conversion of acetate to acetic acid CO_2 and H_2 as an example, can be described as follows (Zhang et al. 2018).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 6CO_2 + 12H_2$$

8.4.3.5 Photo-Fermentation

This approach is based on the ability of photoheterotrophic bacteria particularly the purple, non-sulfur bacteria such as those of the genus *Rhodobacter* to convert, in anaerobic conditions and the presence of light, organic acids (i.e. acetic, lactic, and butyric) or organic acid containing materials to CO_2 and H_2 (Rai Pankaj et al. 2012; Yongzhen et al. 2007; Kapdan and Kargi 2006; He et al. 2005; Fang et al. 2005; Maeda et al. 2003) (Fig. 8.14). Comparing with biophotolysis photosynthetic microbes in photo-fermentation convert solar energy into hydrogen with organic feed (i.e. organic acid containing materials).

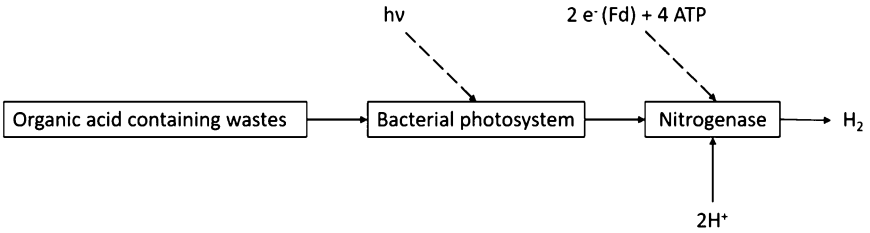

Fig. 8.14 Schematic representation of biohydrogen production through photo-fermentation. *Fd* ferredoxin (based on Korres and Norsworthy (2017))

Photo-fermentation using acetic acid (from acetate) to be converted to CO_2 and H_2 can be described as follows (Zhang et al. 2018).

$$2CH_3COOH + 4H_2O \rightarrow 4CO_2 + 8H_2$$

8.4.3.6 Sequential Dark- and Photo-Fermentation

Sequential dark- and photo-fermentation is a relatively new approach in biological hydrogen production (Kapdan and Kargi 2006) aiming for higher final hydrogen yields provided that the effluent produced under dark fermentation is adequate to cover the needs of the photoheterotrophic bacteria for organic acids under photo-fermentation (Yokoi et al. 1998; Kapdan and Kargi 2006). Nevertheless, the operational conditions of the system should be controlled as increased ammonia concentrations and C/N ratio in the effluent from dark fermentation could inhibit the action of the photosynthetic bacteria under photo-fermentative conditions (Lee et al. 2002).

The sequential dark- and photo-fermentation of hydrogen production, given the chemical reactions from dark- and photo-fermentation to converse acetate into H_2 as stated above, can be described as follows (Zhang et al. 2018).

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2$$

8.4.3.7 Thermochemical Hydrogen Production

The production of biohydrogen from lignocellulosic materials follows two main pathways namely the thermochemical method (i.e. conversion of organic material using heat (Salimi et al. 2016)) and the biochemical method (i.e. the conversion of organic material using living organisms) as it has already mentioned in previous sections of this chapter.

Gasification (i.e. the conversion of feedstock into fuel gases under high temperature and pressure but at low-oxygen environment) and pyrolysis (i.e. a similar process to gasification that is conducted under anaerobic conditions and produces liquids as the primary product) are the major thermochemical conversion processes for biohydrogen production (Korres and Norsworthy 2017). Nevertheless,

gasification in super-critical water media ($T > 374$ °C, $P > 22.1$ MPa), for example, is a modification of the former for the conversion of lignocellulosic materials into gaseous products that consists of CO, CO_2, H_2, and CH_4 (Salimi et al. 2016; Mehrani et al. 2015). The pyrolysis-catalytic steam, an approach that mimics the natural gas catalytic steam process, was employed by Akubo et al. (2018) for the conversion of agricultural biomass wastes. In general, these processes are characterized by faster conversion rates, compared to biological conversion, and best suited for lower-moisture feedstock at high temperatures (Korres and Norsworthy 2017). In addition, thermochemical routes can convert the entire organic (carbon) portion of the feedstock under treatment into energy (Korres and Norsworthy 2017) although environmental concerns and the need for treatment of air and waterborne pollutants and ash within the incineration facility can be the limiting factors (Ouda et al. 2016).

8.4.4 Use of Agricultural Wastes for Weed Management

8.4.4.1 Maize Gluten Meal

Maize gluten meal, a protein by-product of maize milling with a high nitrogen content (10%), used on turfgrass or high-yield crops can also be used for weed management. Although it does not act on the established and mature weed species, it has shown anti-germination action (Gough and Carlstrom 1999). Important factors that facilitate weed seed germination include adequate levels of moisture, temperature, and light which vary among species (Korres et al. 2018). The mechanism of maize gluten meal anti-germination action is most probably due to desiccation of the germinating seed (and surrounding micro-environment) because of the moisture absorbing characteristics of the gluten meal that acts as a desiccant (Chalker-Scott 2015). Although, it is relatively easy to control these conditions in the greenhouse (Chachalis et al. 2008), it is impossible to control them completely in the field. Seeds will germinate over the entire growing season, providing a continuous source of weeds (Korres et al. 2018). However, maize gluten meal is a fairly expensive product because it must be used at very high doses (even 2 t ha^{-1}). Recent prices range from \$30–\$40/50 lb bag not including shipping and handling (Chalker-Scott 2015). Nevertheless, as stated in PAN (Pesticide Action Network) Europe (2017) report maize gluten meal along with other natural products such as acetic acid, citric acid, clove oil have great potential as non-synthetic herbicides for controlling weeds and are used in natural-herbicide products available on the market.

8.4.4.2 Brassicaceae Seed Meal

Oilseed meals are by-products of the oil seed extraction process from the Brassicaceae plants including Indian mustard (*Brassica juncea*), rapeseed (*Brassica napus*), and yellow mustard (*Sinapis alba*) that contain glucosinolates (Hansson et al. 2008; Rice et al. 2007). Glucosinolates, are secondary sulfur- and nitrogen-rich metabolites that function in defense mechanism of plants against pathogens and herbivores (De Vos et al. 2008). They contain glycosides (Bone and Mills 2013) which, upon their enzymatic hydrolyzation by myrosinase, exhibit herbicidal

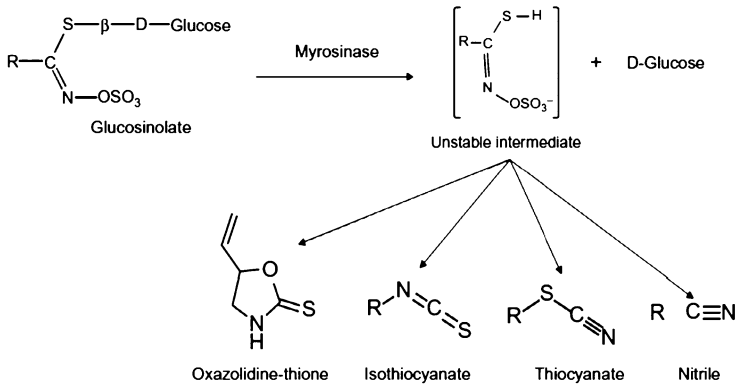

Fig. 8.15 Enzymatic hydrolysis of glucosinolate by myrosinase results in various autolytic products with herbicidal activity. *R* type of organic side chain (adopted from Korres et al. 2019)

activities (Fig. 8.15) (Hansson et al. 2008; Mithen 2001; Rice et al. 2007). Myrosinase is an enzyme that is normally stored in another compartment of the cell (Bone and Mills 2013) and is separated from the glucosinolates physically when plant tissues are crushed (Gimsing and Kirkegaard 2009). It has been reported that Brassicaceae seed meal provides adequate control on several weed species such as redroot pigweed (*Amaranthus retroflexus*), wild oat (*Avena fatua*), prickly lettuce (*Lactuca serriola*), and Italian ryegrass (*Lolium multiflorum*) (Handiseni et al. 2011; Hoagland et al. 2008). Brassicaceae seed meal could be used for weed control in vegetable production systems although weed species respond differently to these compounds (Hansson et al. 2008; Rice et al. 2007).

8.4.4.3 Weed Control by Abrasive Grit

According to Anonymous (1976) abrasive blasting is defined as a process of cleaning of materials by forceful direction of an abrasive media applied either dry or suspended in a liquid medium, against the surface of the workpiece. It is used today for various tasks e.g. to clean a surface by removing unwanted rust or scale, to deburr and others by foundries, shipyards, steel fabrication plants, gas transmission stations, steel mills, building cleaners, wineries, breweries, canneries, etc. Norremark et al. (2006) conceived the idea of using air-propelled organic grits to abrade tissue of small weeds. The grits are small particles that abrade small weed seedlings within the crop row without causing a damage to crop plants. Various grits derived from agricultural residues (e.g. maize cob, walnut shells, almond shell, grape seed, olive seed, poultry manure, sand, soybean meal, and walnut shell or some organically approved fertilizers) can be used for postemergence control of weeds (Wortman 2014, 2015; Perez-Ruiz et al. 2018). Forcella (2009, 2012) reported the efficacy of this approach in controlling small weed seedlings, and subsequent crop yield increases in corn, with two on-row applications of air-propelled maize cob grit combined with inter-row cultivation. Perez-Ruiz et al. (2018) recorded high control rates that in most cases were exceeding 80% on red root amaranth (*Amaranthus*

retroflexus), nettle-leaved goosefoot (*Chenopodium murale*), and garden cornflower (*Centaurea cyanus*) using various types of grit at two- to three-leaf stages. In addition, Wortman (2015) reported a weed biomass reduction 69–97% in tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) cropping systems compared with the weedy control by the application of air-propelled granulated walnut shells, maize cobs, greensand fertilizer (i.e. pelletized poultry manure), and soybean meal. The timing of grit application is critical as the highest levels of weed control were achieved during one- and five-leaf stages or one-, three-, and five-leaf stages of maize development (Korres et al. 2019). As it has been reported by Wortman (2015) the use of abrasive grit can contribute to crop fertilization by adding 35–105 kg N ha^{-1}.

8.4.5 Soil Amendments and the Use of Agricultural Waste Compost

8.4.5.1 Soil Amendments

Soil amendments such as steer manure, peat moss, compost, biochar, and other animal and vegetative origin materials would improve poor soils by increasing aeration, nutritional value, and water holding capacity. Biochar (i.e. charcoal derived from the pyrolysis of waste biomass under anaerobic conditions) for example has been found to increase soil physical and chemical properties such as moisture and nutrient retention (Jeffery et al. 2017; Abel et al. 2013; Spokas et al. 2012), especially in tropical soil with poor nutrient content (Jeffery et al. 2017). Nevertheless, applications of biochar in the soil can alter its hydrology by promoting minerals adsorption and increased soil aggregation, changes that may modify the water flow (Major et al. 2012). Reduced applications of chemical fertilizer by 25% combined with biofertilizer and vermicompost improved the morphophysiological and biochemical traits of mustard (*Brassica campestris*) (Mondal et al. 2017). In addition, Agegnehu et al. (2016) observed increases in maize biomass and yield when combined biochar and compost from agricultural residues were used. Soil physicochemical properties such as soil water content, total soil organic carbon, total nitrogen, available phosphorus, nitrate-nitrogen, ammonium-nitrogen, exchangeable cations and cation exchange capacity were also improved by the organic amendments. Nevertheless, wettability (hydrophilicity) of the soil in olive groves was slightly improved for the topsoil when olive mill pomace compost was used as soil amendment (Aranda et al. 2016). According to El-Naggar et al. (2015) the use of woody waste biochar (10 g kg^{-1} soil) to calcareous soils improved soil fertility.

Brasicacceae oilseed meals which are by-products of the oil seed extraction process with a relatively high nutrient content (Paul and Solaiman, 2004; Snyder et al. 2009) make these materials to be considered as soil amendments (Hollister et al. 2014). They contain 50% C, 5.3–5.9% N, and 1.3% P by weight (Rice et al. 2007; Snyder et al. 2009) and are applied at rates between 1M t and 2M t ha^{-1} which provide 53–59 kg N ha^{-1} in addition to weed control mentioned earlier. This amount of substituted N compensates for greenhouse gas emission reduction equal to approximately 200–220 kg CO_2 e ha^{-1} (based on Korres et al. 2010a, b).

8.4.5.2 Digestate from Anaerobic Digestion

Anaerobic digestion, as it has been already mentioned earlier, is one of the most effective approaches to recover value from agricultural wastes, mostly because energy, in the form of biomethane, is produced naturally as part of the digestion process (Champers and Taylor 2013). This conversion of lignocellulosic materials to biomethane results in the generation of residues known as digestate the recycling of which to land is considered as the best suitable and practicable environmental option in most circumstances, replacing both natural nutrient and carbon cycles (Champers and Taylor 2013; Seppala et al. 2009). Digestate is a valuable source of major plant nutrients that contains 2.1, 0.087, and 3.08 kg of nitrogen, phosphorous, and potassium ton^{-1} of digestate, respectively (Korres et al. 2010b; Smyth et al. 2009), which are essential nutrients for plant growth. Based on these values digestate from the anaerobic digestion of lignocellulosic biomass provides 102 kg N ha^{-1} $year^{-1}$, 4.2 kg P ha^{-1} $year^{-1}$, and 150 kg K ha^{-1} $year^{-1}$ assuming that 12 t of lignocellulosic feedstock ha^{-1} $year^{--1}$ are used for anaerobic digestion process which is producing approximately 49 ton of digestate ha^{-1} $year^{-1}$ (Korres et al. b; Smyth et al. 2009). In addition, as stated by Cherubini et al. (2009), the use of digestate as natural fertilizer that partially replaces conventional fertilizer promotes the environmental benefits of the entire process but also the efficiency and profitability of the production system due to lower production costs (Gerin et al. 2008; Matsunaka et al. 2006). Digestate also benefits soil structure and enriches the soil organic matter, which in turn increases the capacity of soils for carbon sequestration along with the ability of the soil to bind nutrients and regulate their supply to the crop (Champers and Taylor 2013). Soil physical properties, such as plant available water supply, aggregate stability and friability are also dependent on soil organic matter which can hold up to 20 times its weight in water, hence it can affect soil water retention (Dick and Gregorich 2004), therefore contributing to drought stress reduction under short or irregular rainfall regimes. Nevertheless, health and environmental protection issues regarding the nutrient content and the qualitative characteristics of digestate necessitate a hazard analysis and critical control point check to ensure that it satisfies the minimum requirements in terms of microbial pathogens, heavy metals, stability, and physical contaminants (Champers and Taylor 2013).

8.4.5.3 Use of Agricultural Wastes Compost as a Growing Medium

Composting of agricultural waste is an aerobic degradation process by which organic macromolecules of animal or vegetative origin are converted to smaller size molecular compounds which are more stable, disinfected, rich in humus content, beneficial to agricultural crops, and appropriate as soil amendments (Sequi 1996; Xu and Geelen 2018).

Compost tea, a liquid extract, is obtained when compost is steeped in water for an adequate period of time to produce a homogeneous solution consisting of organic materials, beneficial microbes, and nutrients (Mohd Din et al. 2017). Compost tea, based on the conditions of its preparation, can be classified as aerated tea (it is prepared under aerobic conditions) or non-aerated tea (it is prepared under anaerobic conditions) (Amos 2017). This rich in nutrient compost extract can stimulate plant

growth and improve soil fertility (Ahmad et al. 2017; Kim et al. 2015). In addition, there are many reports in which the potential effects of compost tea in suppressing plant diseases were investigated (Mengesha et al. 2017). Waqas et al. (2017) reported that the original feedstock, originating from organic wastes, for the production of the compost tea was prepared using an in-vessel compost bioreactor. Aerated and non-aerated compost tea could be then produced by mixing the initial compost material with water at a ratio 1:0 under 25 °C for 72 h. Using a standard brewing method for steeping and extraction period as described by Scheuerell and Mahaffee (2006) aerated compost tea was maintained by stirring the solution through a mechanical agitator for the entire extraction period. Non-aerated compost tea was produced by using a standard method of bucket fermentation (Diver 2002). During this process, the mixture was initially stirred and then left undisturbed under 25 °C for 3 days (Brinton et al. 2004; Weltzien 1991). Biostimulants, which are substances obtained from various organic or inorganic sources including composts and compost extracts, are able to improve plant growth, plant productivity, soil fertility, and alleviate the negative effects of abiotic stresses (Xu and Geelen 2018). Waqas et al. (2019), for example, found positive effects on percentage seed germination, germination index, mean germination time, and seed vigor of mung bean (*Vigna radiata*) when the seeds were exposed to compost extract (i.e. seed priming) produced by organic wastes. Aerated compost extract significantly enhanced mung bean seed vigor compared to vigor of the seed treated with non-aerated compost extract (Waqas et al. 2019). In addition, compost originating from agricultural wastes has been proved an important growing medium for ornamental plant production such as gerbera (*Gerbera jamesonii*), dahlia (*Dahlia coccinea*), and aglaonema (*Araceae* spp.) (Younis et al. 2007; Wilson et al. 2009; Tariq et al. 2012).

8.4.5.4 Miscellaneous Uses of Agricultural Wastes

Agricultural wastes are suitable for the production of activated carbon (Soleimani and Kaghazchi 2007) or for use in building industry as thermal insulating materials or in concrete industry with beneficial consequences on environment and natural resources protection (Aciou and Cobirzan 2013). Sugarcane vinasse, a cellulosic bioethanol production by-product, can be used as a nutrient source for microorganisms useful for bioremediation of the soil (Christofoletti et al. 2013). Vine shoots, a residue from crop pruning, contain phenolic, volatile, and mineral compounds that can be applied as grapevine biostimulant and foliar fertilizer (Sanchez-Gomez et al. 2014) whereas aqueous extracts of fennel, lemon, and barley grains processing by-products were shown potential to increase tomato yield and fruit quality (Abou Chehade et al. 2018).

8.5 Final Remarks and Future Suggestions

The agricultural waste management is developing rapidly due to microbiological and technological advancements but also because of the need for rapprochement of the entire agricultural production spectrum. Agricultural wastes and residues offer a

potential route to overcome the concerns over using food materials for non-food purposes. Issues related with the heterogeneity of agricultural wastes, policy concerns, and lack of outreach seem they are bypassing. What was formerly considered a liability, which had to be disposed of with least cost, now becomes an asset that needs to be mobilized and then transformed and utilized. This immediately means that "agricultural wastes" is a wrong terminology that creates wrong thinking for materials with alternative uses as it has already been mentioned above. This certainly applies to many agricultural wastes or residues like straw, or forest and wood processing "wastes." Rational use of resources dictates the allocation of "agricultural wastes" should be diversified between traditional and new uses such that the marginal revenue in each use is equated. Of course, the new uses are likely to start at a low level and generally speaking they involve processing large-volume low-value materials so economies of scale are likely. Hence, it may take some time before the new uses can compete on level terms with traditional uses. A SWOT (strengths, weaknesses, opportunities, and threats) in relation to agricultural waste management is shown in Box 8.2.

Box 8.2 SWOT Analysis for Agricultural Wastes Utilization

Strengths	Weaknesses
• Opportunity to turn waste streams into valuable resources resulting in improvements of agricultural and food production sustainability • Potential for the development of "green" jobs and non-farm income increases • Conversion technologies have been developed • Potential to produce simultaneously both bio-based chemicals and energy	• High costs, financing constraints, and a current lack of demand-pull effect • Availability of sufficient biomass constrained by logistical, technical, economic and environmental factors, and seasonality • Wastes and residues tend to be bulky, low value per ton, heterogeneous, and diffuse, hence their conversion in processing facilities therefore tends to be expensive, putting them at a cost disadvantage
Opportunities	**Threats**
• The potential to create jobs and economic growth makes the sector an attractive target for decision-making in times of economic downturns • Private sector initiatives to move toward bio-based sourcing (e.g. food packaging industry) • The bio-economy communication as a high-level policy initiative with the potential to stimulate decision-making by industry and policy makers	• Lack of technical standards for bio-based products may complicate market penetration • Lack of public awareness as regards bio-based products • The oil price is an important determinant of the profitability of many bio-based operations outside sector's control • Lack of sustainability criteria for biomaterials may undermine trust in the sector

For agricultural crop wastes utilization, the top priority should be avoiding the depletion of soil carbon and other nutrients. Appropriate limits and continued monitoring should be established ensuring that sourcing various industries with

agricultural residues does not impact negatively on soil carbon and other soil nutrients. Consequently, strengthening the environmental requirements in relation to soil organic matter would be a strong safeguard against unsustainable residue sourcing.

In conclusion, policy recommendations must be underpinned by clear evidence that the relevant bio-based pathways contribute toward meeting sustainability criteria that contributing to climate change mitigation targets by delivering GHG emission reduction or other defined environmental benefits compared to the traditional products they replace. Setting safeguards and monitoring policies should not be understood as an attempt to limit the development of a bio-based industry by imposing additional burdens. Instead, it should be seen as an effort to reduce uncertainty about necessary environmental performance.

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Plant Tissue Culture: Beyond Being a Tool for Genetic Engineering

9

Deepak Sehgal and Tanveer Khan

Abstract

Plant tissue culture has been in practice for more than 100 years since its conception by Haberlandt in 1902. The development of widely used media composition by Murashige and Skoog in 1962 forms the backbone of most of the tissue culture protocols. Besides micropropagation of economically important species by large- and small-scale companies worldwide, plant tissue culture is widely used to develop crops with economically important traits by transforming different explants and thereafter regenerating them under optimized culture conditions; it has been widely perceived and used as the workhorse for plant genetic engineering. This fitted the growing Plant Biotechnology arena in the late 1990s where commercial companies tried to develop protocols to deliver commercial traits into economically viable crops. However, plant tissue culture's untapped potential is getting revealed now during the changing climatic conditions and rising needs of the human population. Not only fulfilling the need to feed, plant tissue culture could also be used to develop a sustainable future under harsh conditions by multiplication of endangered plant species, developing heavy metal scavenging plant populations, replanting eroded lands and forests by tissue culture generated trees, developing viral free plant populations and establishment of ocean farms where one unit could be dedicated to plant tissue culture/hydroponic system. Thus, plant tissue culture has the potential to impact the future of mankind in many ways.

D. Sehgal (✉)
Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, Piracicaba, SP, Brazil

T. Khan
Centro de Tecnologia Canavieira, Piracicaba, SP, Brazil

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9.1 Introduction

Plants have pre-existed animals on earth; cyanobacteria and other photosynthetic eukaryotes existed 1 billion years ago. Flowering plants evolved around 200 million years ago; plants have endured and evolved through several epochs of harsh conditions and developed traits highly diverse and valuable. Plants have been a source of sustainability to aerobic life on earth; they have been used since ancient times as a source of valuable products: food, feed, fiber, oil, sugar, etc. for humans and animals. However, in last two centuries, due to ever-increasing population, urbanization, and industrialization, there has been an extreme pressure on this "Treasure of Earth." Earth has lost a considerable area of arable land, soil and water quality have deteriorated, forest areas have shrunk, and pollution levels are high, endangering many plant species. Increased demand for value crops like rice, wheat, cotton, sugarcane, etc. to sustain the economies have led to almost wiping out of some of the indigenous plant species. In this regard, plant tissue culture can offer a way to tackle the challenges mentioned above. There have been some successful examples of applied tissue culture to endangered species, economically important crops, fruits, flowers, and trees, but in today's time of climate change such efforts do not give a chance to sustain human race. Thus, plant tissue culture currently needs to be looked at from a different perspective and certainly above the economic gains. We need to understand the real basis of cellular totipotency of the plant kingdom and apply it to save and sustain the life on earth.

The term totipotency or the ability to regenerate plants from single cells was developed by Haberlandt in 1902. Now after more than 100 years of the first report and more than 58 years after the development of universally used Murashige and Skoog (1962) medium for culturing plants, plant tissue culture is still being used for generating transgenics and commercial micropropagation of some plant species.

The idea of totipotency itself is not utilized to its full scale mostly due to the plasticity of the plant system and unavailability of protocols to culture and regenerate plants from almost all the species. Plant developmental biology research is considerably slow due to long time taken for tissue culture protocol development. Protocols are highly dependent on explant type, species, medium for different phases for cell proliferation, embryo/organ formation, and regeneration. No two species are alike in terms of hormone regime, explant response, media requirements, etc. Large number of variables need long and steady optimization steps. Besides, lack of research funds for plant developmental research compared to the counterparts in animal biology and lack of highly reproducible and flexible protocols that can work across different plant species limit the horizons of plant tissue culture. There are several examples of tissue cultured plant species and genetic engineering products but mostly in cultivated crops/trees. Considering the size and diversity of the Plant Kingdom and inherent value traits available in plants growing in different geographies, in harsh or adverse conditions, might give us a glimpse into the untapped potential of plants.

In this chapter, we will cover these points and try to look beyond the normal process of plant propagation and see how academic and industrial collaboration and

investments in this industry can contribute towards saving environment, minimizing risks associated with genetic engineering and improving the livelihood of the farmers.

9.1.1 Plant Tissue Culture in Modern Agriculture

Micropropagation—tools and techniques, rapid protocols for multiplication of plantlets. Micropropagation is the method used to produce plantlets from a plant part or explant. It is the process to rapidly multiply explants material to regenerate many progeny plants. Micropropagation of plant material was started by Fredrick Campion Steward in 1950s and off late the technique has been extremely popular to bulk produce and regenerate plant material in species which happen to be difficult to produce seeds. It is also an effective way to regenerate tissues of genetically transformed material. The process involves isolation of a part of plant tissue e.g. leaf, bud, meristem under sterile condition and propagation under different source of hormone and media regime. This method happens to be fast and cost-effective as compared to conventional method of cell/tissue culture and has the potential to generate true to type plant material if meristem culture is involved. Below are the advantages of micropropagation:

a. Rapid multiplication of plants within a short period and on small space.
b. Plants are obtained under controlled conditions, independent of seasons.
c. Sterile plants or plants which cannot maintain their characters by sexual reproduction are multiplied by this method.
d. The rare plant and endangered species are multiplied by this method and such plants are saved.
e. Production of virus free plants like potato, banana, apple, papaya, etc.
f. Production of rare and endangered species like Taxus and other medicinal plants.

9.1.2 Source of True to Type Plant Material

Generally, tissue culture or micropropagation of species give rise to somaclonal variations especially when the plant tissue undergoes a callus phase. This is mainly due to the genetic or epigenetic changes that occur when the cells undergo a phase of dedifferentiation to form the callus (simple parenchymatous cells) and redifferentiation again when the callus cells give rise to complex tissue for regeneration. However, there are tissues or cell types that can give rise to true to type plants e.g. meristem cells. These specific cells are responsible for organ generation from simple cell types. In normal plants, meristematic zones are formed in areas of growth e.g. cambium tissue in stem and root, bud axis that can give rise to either a floral bud or branch. These cells are similar to the stem cell tissue of animals and have the potential to give rise to true to type plants under optimal conditions. However, optimizing those conditions whereby these cells directly regenerate into complete

plants in a short period is difficult and need to be optimized for each species and subspecies or varieties.

9.1.3 Generation of Disease-Free Plant Material

Micropropagation is a means to generate disease-free plant material. A two-step process is necessary in order to generate disease-free plants:

9.1.3.1 Selecting the Best or Disease-Free Source Material

This can be done by selecting vigorous or healthy source material and growing it in a nursery or controlled field or Greenhouse conditions in order to evaluate the growth and disease in the primary stock. Methods are available which utilize molecular kits to check the presence of disease-causing microorganisms e.g. bacteria, certain endophytes, etc. Assays for many viruses are not available so it is better to grow one generation of non-infected plant tissue in greenhouse and use it as a source to generate virus free or disease-free sapling.

9.1.3.2 Disease-Free Plantlet Regeneration Under Tissue Culture Conditions

In tissue culture aseptic conditions are utilized to multiply cells or tissues; these cells go through multiple cycles or multiplication and under certain conditions using antibiotics, etc. can be made disease-free. In another method, by using meristem culture, spreading of any viral disease could be avoided, as viruses cannot move through meristematic tissues. These tissues lack plasmodesmata or cell connectors and hence, movement of any infectious organisms from one cell to the other is impossible. Thus, selecting meristematic tissue and culturing it to develop into a complete plantlet is one of the popular means to make disease-free plants. Many micropropagation companies utilize this approach to bulk produce disease-free plants.

9.1.3.3 Cryopreservation for Generating Disease-Free Material

Recently storing plant parts or tissues under liquid nitrogen (−196 °C) for a short time has been found to be useful to render plant tissues virus free. Classical cryopreservation techniques involve slow cooling down to a defined pre-freezing temperature, followed by rapid immersion in liquid nitrogen. Some of the examples to use Cryopreservation for virus elimination are mentioned in Table 9.1.

9.1.4 Developing Improved Varieties via Wide Hybridization

A critical requirement for crop improvement is introduction of new genetic material into cultivated lines of interest, either by single or multiple genes through genetic engineering, through conventional hybridization or other tissue culture techniques. Normal fertilization process of embryo fusion or fusion of egg cell with pollen

Table 9.1 Representative examples of tissue culture technique used for virus elimination in selected woody and herbaceous plants (Source: Cruz-Cruz et al. (2013))

Species	Virus
Woody plant	
Grapevine	Grapevine fanleaf virus (GFLV) and grapevine leaf roll-associated virus-1 (GLRaV-1)
Banana	Banana bract mosaic virus
Citrus	Citrus psorosis virus
Cocoa	Cocoa swollen shoot virus
Rose	Rose mosaic virus
Herbaceous plants	
Sugarcane	Sugarcane mosaic virus (SCMV) and sugarcane yellow leaf virus (ScYLV)
Garlic	Leek yellow stripe (LYS) and onion yellow dwarf virus (OYDV)
Potato	Potato leafroll virus (PLRV) and potato virus (PVY)
Carnation	Carnation latent virus (CLV)
Chrysanthemum	Cucumber mosaic and tomato aspermy virus
Dahlia	Dahlia mosaic virus
Peanut	Peanut mottle potyvirus (PMV) and peanut stripe potyvirus (PStV)
Pumpkin	Zucchini yellow mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, bean yellow mosaic virus

nucleus could be hindered in some species due to multiple factors. These could be categorized either as pre-fertilization barriers e.g. failure of pollen to germinate or poor pollen tube growth or post fertilization barriers e.g. lack of endosperm development. Tissue culture presents an opportunity for another, pollen or embryo culture to overcome such barriers. When fertilization cannot be induced by in vitro treatments then protoplast fusion can be implemented to produce desired hybrids. Some of the techniques used are described below:

9.1.4.1 In Vitro Fertilization

IVF has been applied to produce many crosses by transferring pollen from one species to other leading to wide crosses. Interspecific and intergeneric hybrids of a number of agriculturally important crops have been successfully produced, including cotton, barley, tomato, rice, jute, Hordeum X Secale, Triticum x Secale, Tripsocumto and some Brassicas. However, with the advent of new editing technologies, these hybridizations would be possible in future whereby, recombination leading to the formation of an embryo by fusing egg and pollen nucleus would be done in vitro. One similar technique involves the fusion of protoplast. However, recovery of plants from this technique is pretty low suggesting that more optimizations are required to make it a reality similar to animal IVF techniques.

9.1.4.2 Embryo Culture

Recently embryo rescue has been utilized to overcome embryo fatality or abortion in some of the crop species. Some crops undergo normal fertilization but due to some genetic factors or due to poor embryo development the embryo is aborted thus

leading to no progeny. In this case, fertilized egg cell or embryo is removed from the plant and cultured under aseptic conditions leading to recovery of the plantlets. This technique has been successfully applied to crops like maize, rice, and wheat.

9.1.4.3 Protoplast Fusion

Any two plant protoplasts can be fused by chemical or physical means; production of unique somatic hybrid plants is limited by the ability to regenerate the fused product and sterility in the interspecific hybrids rather than the production of protoplasts. Although success of protoplast fusion has been limited mostly to Nicotiana and few other species, trials in other important species need to be optimized especially in relation to protoplast regeneration or formation of somatic hybrids after protoplast fusion. Protoplast fusion products are presently grown on approximately 42% of the flue-cured tobacco acreage in Ontario, Canada. This represents a value of approx. US$199,000,000.

9.1.4.4 Producing Homozygous Pure Breeding Lines for Hybrid Production

Demand of homozygous lines in breeding program has enhanced a lot. These homozygous lines have been achieved by producing doubled monoploid plants via haploid cultures. This is done by treating the plant cell with colchicine which causes doubling of chromosome number.

9.2 Plant Genetic Engineering

Plant genetic engineering refers to the process of genetic manipulation of any plant species. Over last 50 years, the field of genetic engineering has developed rapidly due to greater understanding of deoxyribonucleic acid (DNA) as the chemical double helix code from which genes are made. The term genetic engineering is used to describe the process by which the genetic makeup of an organism can be altered using "recombinant DNA technology." This involves the use of laboratory tools to insert, alter, or cut out pieces of DNA that contain one or more genes of interest. Genetic Engineering has been used to transfer or introduce new and valuable traits into plants. Unlike conventional breeding technology, where a trait is selected and bred into other varieties, genetic engineering allows direct transfer of one or just a few genes of interest between either closely or distantly related organisms to obtain the desired agronomic trait. Not all genetic engineering techniques involve inserting DNA from other organisms. Plants may also be modified by removing or switching off their own genes (ISAAA). A variety of genetic engineering techniques are described in the following text:

9.2.1 Agrobacterium Mediated Genetic Transformation

Agrobacterium tumefaciens is a naturally occurring soil microbe best known for causing crown gall disease on susceptible plant species. Agrobacterium has the potential to transfer its own DNA into the plant species. The transferred DNA is stably integrated into plant DNA, and the plant then reads and expresses the transferred genes as if they were its own. The transferred genes then direct the production of several substances that mediate the development of a crown gall. These tumor causing genes (opines and nopalines) are harbored onto the Ti plasmid of the *Agrobacterium*. In the early 1980s, strains of *Agrobacterium* were developed that lacked the disease-causing genes or engineered Ti plasmid DNA but maintained the ability to attach to susceptible plant cells and transfer DNA.

By substituting the DNA of interest for crown gall disease-causing DNA, scientists derived new strains of Agrobacterium that deliver and stably integrate specific new genetic material into the cells of target plant species. If the transformed cell then is regenerated into a whole fertile plant, all cells in the progeny will also carry and may express the inserted genes. Agrobacterium is the workhorse for delivering any desired genes into the plant and its flexibility to transform many plant species makes it a favorite natural plant genetic engineer.

9.2.2 Particle Bombardment

Since Agrobacterium has a limited host range in terms of plant species to be infected, an alternative was developed by Klein et al. (1987). They bombarded plant tissues with micro particles to which the DNA was precipitated and adhered using a technical device called as the Gene Gun. This device operates by compressing helium gas under vacuum and using this pressure to deliver the particles called as microprojectiles into the plant tissue. Since the projectiles carry the coated DNA with the gene of interest and are bombarded onto the plant tissue with great force, this method is widely utilized to transform tissues of recalcitrant species such as corn, rice, and other cereal grains which show a low transformation efficiency with Agrobacterium. The method has certain limitations as it is not very cost-effective, it also might cause DNA rearrangements or undesired insertions in the host. Several manipulations are required to optimize the efficient delivery of DNA and recovery of plants.

9.2.3 Electroporation

Electroporation involves the use of plant protoplasts (plant cells lacking the cell walls) to directly take up macromolecules/DNA from their surrounding fluid using an electrical pulse or other reagents. Plant protoplasts are generated by dissolving the cell walls using enzymes like pectinase, cellulase, and macerozyme; they are kept in a medium and then under an influence of electrical pulse or other reagents e.g. Polyethylene Glycol (PEG) that can alter the mechanics i.e. ion uptake of cell

membrane can take up macromolecules like DNA/RNA. Further these protoplasts are regenerated to form complete plants. This method is not widely used due to poor efficiency of macromolecule delivery and regeneration of the protoplast.

9.2.4 Microinjection

DNA can be injected directly into anchored cells. Some proportion of these cells will survive and integrate the injected DNA. However, the process is labor intensive and inefficient compared to other methods.

9.3 Non-transgenic Method for Genetic Manipulation

Use of virus to transiently deliver DNA that can result in genetic manipulation has been observed; however, this method needs more research to be used frequently. The other method includes epigenetic regulation of genes by transient delivery of proteins, RNA to manipulate the expression of some genes. This method has also been applied in animal studies and recently been tried in some of the plant species.

9.4 New Tools to Mitigate Environmental Risks Using Technologies Like CRISPR-Cas and Other Genome Editing Tools

CRISPR/Cas9 is a rapidly growing genome editing technology which has been successfully applied in many model and crop plants. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and Cas9 is a nuclease associated with CRISPRs. Lately the system has been modified as an application tool for genome editing in different organisms. The main components of this system are Cas9 protein and sgRNA (single guide RNA) which recognizes the PAM sequence in the genome and guides Cas9 to it, whereby it forms a complex and the Cas9 protein cuts the DNA in a highly specific manner. This technology is used to create simple additions/deletions, editing or replacement of nucleotide sequences, or insertions of large DNA sequences or genes.

9.4.1 Use of CRISPR Technology to Save Endangered Species

To meet the need of the growing population, mankind has focused on cultivating crops for food and feed; numerous species were rendered endangered or wiped out due to the expanding agricultural land use which included clearing of forest land. Also, an emphasis on cultivating economically important trees and other vegetable crop has put a pressure on the natural or indigenous varieties that were cultivated in the past and has led to loss of many such crop varieties over a period of time. Some

Table 9.2 Area in ha/village under different traditional crops in Kharif and Rabi seasons during 1970–1974 and 1990–1994 in central Himalaya (Source: Maikhuri, Rao & Semwal 2001)

Crop/cropping season	Area (ha/village)			Probable reasons for decline
	1970–1974	1990–1994	Area decline (%)	
Kharif season crops (April–October)				
Panicum miliaceum	14.2	4.9	65.5	Cultivation/introduction of high yielding varieties (HYVs)
Oryza sativa (Irrigated)	14.2	14.2	–	Cultivation/introduction of HYVs
Avena sativa	15.8	3.4	78.5	Cultivation/introduction of potato
Fagopyrum tataricum	8.6	1.5	82.5	Cultivation/introduction of potato + kidney bean
Perilla frutescens	1.3	–	100.0	Cultivation/introduction of soybean
Setaria italica	2.3	0.8	65.2	Cultivation/introduction of soybean
Oryza sativa (rainfed)	11.2	11.2	–	Cultivation/introduction of HYVs
Eleusine coracana	9.6	6.1	36.5	Cultivation/introduction of soybean + amaranth
Echinochloa frumentacea	2.5	0.7	72.0	Cultivation/introduction of pigeon bean
Vifna spp.	3.3	–	100.0	Cultivation/introduction of potato, amaranth +kidney bean
Rabi season crops (October–April)				
Triticum aestivum	14.2	14.2	–	Cultivation/introduction of HYVs + *Brassica* spp.
Hordeum himalayense	17.1	4.7	72.5	Cultivation/introduction potato, amaranth + kidney bean
Hordeum vulgare	7.0	1.1	84.3	Cultivation/introduction of HYVs
Brassica campestris	2.0	2.0	–	–

of these nutritious/other value trait varieties lost to cultivated varieties that farmers chose to boost their economic status. In the paper entitled 'Changing scenario of Himalayan agroecosystems: loss of agrobiodiversity, an indicator of environmental change in Central Himalaya, India', Maikhuri et al. (2001) described the loss of certain valuable crop species to other economically important species due to the changing ecosystem and socioeconomic status (Table 9.2).

India was once home to more than 100,000 indigenous rice varieties with valuable traits like high fiber, nutrient like Zn, Fe and other micronutrients, pests, disease resistance, drought and even lodging. However, most of these varieties were lost in last 30 years due to the cultivation of other economically viable varieties (The guardian, 2017). There have been some conventional methods tried in last few years

by the farmers to bring back these heirloom varieties. With the advent of new technology like CRISPR, it is possible to engineer native species or edit a particular trait to create value in an economically important species.

Since CRISPR based technology is highly precise as the guide RNAs are designed specific to the genomic sequences of the target organism, it presents high potential for improving genetic traits in a much precise manner as compared to other genetic engineering technologies. Also, there have been reports of engineering new traits in the target organism without leaving any traces of foreign DNA; this involves delivering Cas9 protein and sgRNA complex directly into the cell to edit, insert, or delete or epigenetically regulate certain genes by transiently upregulating or downregulating the gene expression. Recently many crops like rice, wheat, sorghum, millets, citrus, apple, etc. have been edited to improve economically important traits like herbicide tolerance, insect resistance, drought tolerance, and yield, using CRISPR technology. In the same way, this technique can be used to engineer crops for increasing metal uptake in soil polluted with heavy metals, mining areas, editing woody plants for virus and disease resistance and other crops for traits like water use efficiency, drought tolerance for arid region, improved root system for areas prone to soil erosion, high carbon assimilation, etc.

9.5 Plant Tissue Culture as an Industry

9.5.1 Value Crops

Large-scale production of medicinal, ornamental, and food crops—over the century, plant tissue culture has been established not only as a tool for genetic engineering but also for large-scale production of medicinal, ornamental, and food crops. These tissue culture grown ornamental plants form the bulk of large-scale production and have commercial value. Ornamental industry has applied immensely in vitro propagation approach for large-scale plant multiplication of elite/or superior varieties. As a result, hundreds of plant tissue culture laboratories have come up worldwide, especially in the developing countries due to cheap labor costs. There have been different methods applied for propagation of these value crops e.g. meristem culture, shoot tip culture, embryogenesis, etc. Of these, micropropagation has been most cost-effective in large-scale commercial cultivation of these crops. Majority of the pot plants such as *Begonia*, *Ficus*, *Anthurium*, *Chrysanthemum*, *Rosa*, *Saintpaulia*, and *Spathiphyllum* are produced in the developed countries and the Netherlands leads the export of ornamental and pot plants (Anonymous 2003). About 156 ornamental genera are propagated through tissue culture in different commercial laboratories worldwide. Four leading exporters (The Netherlands, Colombia, Italy, and Israel) constitute about 80% of the world market and the developing countries of Africa, Asia, and Latin America contribute only less than 20% (Rajagopalan 2000; Schiva 2000; Rout and Mohapatra 2006).

9.5.2 Medicinal Plants

The products derived from medicinal plants act as a source of drugs in many traditional and other medication systems. The percentage of people using traditional medicines has observed a decline in developed countries: 40–50% in Germany, 42% in the USA, 48% in Australia, and 49% in France (FAO). The global market for Herbal drugs is estimated to be about 72 billion USD, of which the revenue generated from extracts was reported to be USD 27.1 billion in 2016 and is expected to grow approximately by 65% and reach USD 44.6 billion by 2024. Species like *Dioscorea deltoidea*, *Papaver somniferum*, *Atropa belladonna*, *Rauvolfia serpentina*, *Hyoscyamus niger*, *Digitalis lanata*, *Datura metel*, *Digitalis purpurea*, *Pilocarpusa bonandi*, *Cinchona ledgeriana* are direct contributors to several prescribed medicines. This ever-increasing trend in use of medicinal herbs and herbal products in therapeutic purpose, research, and trade has created tremendous pressure on supply from their wild source. The increasing demand of chemical compounds from these species, indiscriminate extraction, and destruction of their wild habitat have put them at a risk of extinction. A viable alternative to overcome this unsustainability problem of medicinal plants would be the systematic cultivation of medicinal herbs from the wild source and an opportunity to optimize yield to achieve a uniform, high-quality product. Several drugs from species like cardamom, cannabis, cinnamon, ginger, cinchona, opium, linseed, and fennel are now obtained almost exclusively from cultivation source (Alamgir 2017). Since the species growing under different geographies and environmental conditions have different levels of chemical compounds, a profitable alternative could be cultivation of these species under uniform controlled conditions. However, micropropagation or tissue culture of medicinal plants is quite difficult due to high production of alkaloids and phenols which drastically affects the cell proliferation and regeneration of these plants. Unavailability of protocols for culture, long time to establish cell culture makes it a less lucrative system for commercial production. However, with the recent advances in cannabis extracts for medicinal use and the established market size (20 billion USD), medicinal marijuana has made new waves in last one year to look back at the medicinal plant industry and has opened new prospects in this area.

Plant tissue culture also provides interesting prospects for commercial production of food crops. Many fruit and vegetable crops like banana, tomato, potato, sweet potato, capsicum, strawberry, lettuce, etc. are being commercially propagated. Use of meristem culture, shoot-tips culture, cell or embryo culture is being deployed to disease-free cultivation of food crops. Maintenance of controlled conditions during the culture period for temperature, photoperiod, light spectra also has added advantage in maintaining nutrition, flavor, color, and texture for food crops. Such crops have high value in the consumer market as compared to conventionally grown crops. Recently many startup companies have invested in micropropagation of food crops to supply fresh fruits and vegetables to the consumer in big cities. Also, companies and government agencies are culturing and propagating these species in indoor farms to maintain the quality.

9.6 Land Reclamation Projects

Reclamation of Saline, drought stricken, and effluent contaminated lands and fields through propagation and planting of tolerant plant species—Over the period of time due to the increasing population and human activity, most of the forest and agricultural land has been rendered eroded or infertile. In order to reclaim the land, many projects have been started by NGOs and government agencies that involve planting of species that could be grown in adverse conditions; some of these species include N-fixing species of legumes, grasses, and trees.

9.6.1 Controlling Soil Erosion and Forest Lands

Propagation of woody and grass species for reforestation and controlling soil erosion. Soil erosion has been a problem due to the deforestation of land which then tends to be exposed to wind and water erosion. Species that have strong root system tend to hold the soil together. Half of the topsoil on the planet has been lost in the last 150 years. In addition to erosion, soil quality is affected by other aspects of agriculture like compaction, loss of soil structure, nutrient degradation, and soil salinity. Sustainable land use can help to reduce the impacts of agriculture and livestock, preventing soil degradation and erosion and the loss of valuable land to desertification (WWF). Bodies like WWF and other national agencies along with the government are running projects like zero net deforestation to save the current forest species and control erosion; however, for the already eroded land, selection of plant species that can hold the soil and bulk production of the same depending on the geographic location is also an alternative that needs to be considered. Cover crops, such as vetch, rye, and clover are the excellent plants for erosion control. These hardy easy to grow plants send out nets of roots that help hold topsoil in place while also reducing competitive weeds. Some small-scale efforts in this area are known but the true potential of tissue culture to either propagate or engineer such species is yet to be realized.

9.7 How Can We Use Plant Tissue Culture to Address Climate Change?

Propagation of endangered or rare plant species—The study, conducted by the World Wildlife Fund, University of East Anglia and the James Cook University, warned that rising temperatures could have a disastrous impact on areas such as the Amazon, Galapagos islands, and Madagascar, as well as European coasts and the Caribbean. Various plant species in the different geographies have been classified as endangered. Botanical garden conservation international (BGCI) describes temperature and rainfall to be the main determinants of the loss of change in behavior of many plant species. Some isolated or disjunct species are particularly vulnerable, as they may have “nowhere to go” e.g. Arctic and alpine species, and Island endemics,

Coastal species which will be "squeezed" between human settlements and rising sea levels. Plant genetic composition may change in response to the selection pressure of climate change. Some plant communities or species associations may be lost as species move and adapt at different rates.

As conditions become more suitable for exotic species and less well suited for the native species, an increase invasion trend by alien species may be observed (for example, Bromus is more invasive in wet years (Smith et al. 2000)). This is especially true given human interventions which have deliberately and accidentally facilitated the spread of species across the globe. Many plant communities act as "sinks" (store carbon), which helps to offset carbon emissions. However, over the next 70 years, the effects of climate change on plants may result in many terrestrial sinks turning into sources.

9.7.1 Conservation of Plant Species Using Tissue Culture Practices

Tissue culture is the technique to culture plant parts or tissues under in vitro conditions. As mentioned earlier in the chapter, research in this area is limited and most of the efforts have been directed towards micropropagation or culture of economically important plant species. The other issues include lack of expert manpower to deal with complicated plant species, lack of complete protocols, and genetic complexity of the plant species itself, some of the species happen to be recalcitrant or do not respond to tissue culture.

Advances in plant biotechnology provide new options for collection, multiplication, and short- to long-term conservation of plant biodiversity, using in vitro culture techniques. Significant progress has been made for conserving endangered, rare, crop ornamental, medicinal, and forest species, especially for non-orthodox seed and vegetatively propagated plants of temperate and tropical origin. Cell and tissue culture techniques ensure the rapid multiplication and production of plant material under aseptic conditions. Medium-term conservation by means of in vitro slow growth storage allows extending subcultures from several months to several years, depending on the species.

Cryopreservation (freezing in liquid nitrogen, −196 °C) is the only technique ensuring the safe and cost-effective long-term conservation of a wide range of plant species (Table 9.3). Cryopreservation of plant germplasm has obvious advantages over in vitro storage in terms of space saving and improved phytosanitation (Towill 1991; Engelmann 1997). Cryopreservation banking for long-term germplasm storage can be applied to a variety of propagules, including seeds, embryos, spores, pollen, gametophytes, shoot tips, and embryogenic callus cultures. However, long-term storage and culture of plant tissues often present a problem of somaclonal variation (Scowcroft 1984); variation have been reported in *Musa* spp. (Vuylsteke et al. 1990), *Solanum tuberosum* L. (Harding 1991), *Vitis vinifera* L. (Harding et al. 1996), and others. The risk of genetic instability may be minimized through the selection and optimal use of organized tissues such as meristems or shoot tips (Kartha 1985). Over 110 accessions of rare or threatened species are stored under

Table 9.3 Efforts in cryopreservation of plant samples (Source: Cruz-Cruz et al. 2013)

Plant material	Gene bank/country
Seeds of 1200 accessions from 50 different species mainly of endangered medicinal plants	The National Bureau for Plant Genetic Resources (NBPGR). New Delhi, India
Seeds of more than 110 accessions of rare or threatened species	Kings Park and Botanical Garden, Perth, Australia
Seeds of coffee involving 450 accessions	IRD Montpellier, France
Dormant buds of apple involving 2200 accessions	National Center for Genetic Resources (CNGR), Fort Collins, USA
Dormant buds of mulberry involving 420 accessions	National Institute of Agrobiological Resources (NIAR), Yamagata, Japan
Shoot-tips of banana involving 630 accessions	INIBAP International Transit Center, Catholic University of Leuven, Belgium
Shoot-tips of cassava involving 540 accessions	International Center for Tropical Agriculture (CIAT), Call, Colombia
Pollen of 13 pear cultivars and 24 *Pyrus* species	National Center for Genetic Resources (CNGR), Fort Collins, USA
Pollen of more than 700 accessions of traditional Chinese flower species	College of Landscape Architecture, Beijing Forestry University, Beijing, China
More than 1000 callus strains of species of pharmaceutical Interest	Phytera, Sheffields, UK
Several thousand conifer embryogenic cell lines for large-scale clonal planting programs	Sylvagen, Vancouver, Canada
Embryogenic cell lines of coffee and cacao	Biotechnology Laboratory of the Nestle Company, Notre Damedoe, France

cryopreservation at the Perth Royal Botanic Garden in Australia (Touchell and Dixon 1994) and the Cincinnati Botanic Garden in the USA conserves seeds of rare and endangered native species in liquid nitrogen (Pence 1999).

9.7.2 Bio Cleaners or Development of Plants Scavenging Heavy Metals, Metabolizing Herbicide and Chemicals

Metal tolerant plants can be effective for acidic and heavy metals bearing soils—a drawing of combined effect of human activity and climate change affecting the plants. Excessive human activity, changes in agricultural practices, and industrialization has led to decrease in soil quality, erosion of the top layer often leads to infertile or barren land. With modern day urbanization and industrialization, heavy metal (HM) contamination has become a prime concern. The heavy metals (HMs) and metalloids, including Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, Pb, among others, can result in significant toxic impacts. Metals/metalloids concentrations in the soil have been raising at an alarming rate and affect plant growth, food safety, and soil microflora. Heavy metals are extracted from their ores during mineral processing. During this process, some parts are left in the open and transported to other places via wind and flood, causing severe threats to the environment (Lenntech 2004;

Nagajyoti et al. 2010). The biological and geological reorganization of heavy metal depends mainly on green plants and their metabolism. Metal toxicity has direct effects to flora that forms an integral component of ecosystem. Altered biochemical, physiological, and metabolic processes are found in plants growing in regions of high metal pollution and some of the plants can either detoxify or accumulate metals in their organs like roots, stem, or leaves thus removing excess metals from the soil. For example, under Al-stress, the roots of buckwheat secrete oxalic acid to form non-toxic Al-oxalate complexes which get transferred into the leaves (Ma et al. 1998). A Chinese brake fern (Pteris vittata) can hyper-accumulate As (more than 1000 mg As/kg shoot dry weight, DW). This plant has the capacity to transform As (V) to As (III) and transports it via xylem as an As (III)-S compound along with water and minerals, and gets accumulated in the fronds as As (III) (Ma et al. 2001). The other plants that can volatilize heavy metals include Thlaspi caerulescens for Zn, Cd, Pb, Achillea millefolium for Zn, Alyssum sp. for Ni, cabbage for Pb and Cd (Argint 2003).

In case of eroded soil, drought-resistant, fast growing crops or fodder are chosen which can grow in nutrient deficient soils. Selected plants should be easy to establish, grow quickly, and have dense canopies and root systems. Grasses, particularly C4, can offer superior tolerance to drought, low soil nutrients, and other climatic stresses. Roots of grasses are fibrous that can slow down erosion and their soil forming tendencies eventually produce a layer of organic soil, stabilize soil, conserve soil moisture, and may compete with weedy species. Nitrogen fixing species have a dramatic effect on soil fertility through production of readily decomposable nutrient rich litter and turnover of fine roots and nodules. Reclamation of soil involves introduction of both microbial and plant species into the damaged soil. Plant cell and tissue culture is considered as an important technique for fundamental studies that provide information about the plant–contaminant relationships, which helps to predict plant responses to environmental contaminants, and improve the design of plants with enhanced characteristics for phytoremediation. Callus, cell suspensions, hairy roots, and shoot multiplication cultures are used to study the interactions between plants and pollutants under aseptic conditions (Couselo et al. 2012). Besides being a source of basic research to study phytoremediation, genetically engineered plants with traits for metal chelating, transport, deep root system, etc. are the step forward. Recently, a new transgenic for cotton has been developed at Texas A&M by a research group led by Dr. Keerti Rathore that can detoxify phosphite residues from the soil (The News & Observer 2018). More such research would involve understanding biochemical and molecular basis of phytoremediation, drought, or stress tolerance in native or wild species and genetically engineer these traits in plants that could be used for planting in contaminated or eroded soil. Micropropagation of such plants that possess native traits to scavenge metals, herbicides, and other chemicals is an option that could be considered for future research, besides engineering plants with these traits and improving the genetics of such species involves the use of tissue culture thus presenting the true potential this technique possesses.

9.7.3 Ocean Farms

It is estimated that by 2050, the world population will be close to 9.1 billion and the demand for food will rise by 70%. To tackle these problems, some of the companies are coming up with the idea of Floating Farms. The design suggests building light weight, multilevel, buoyant rigs floating in the oceans. The farms will harvest fish, crops, and sunlight and will be located near areas where food is most needed or there is scarcity of cultivable land. This kind of concept is interesting from environmental perspective as it runs on clean energy i.e. solar energy. The farms will require much less fresh water and fertilizer for producing crops, as the nitrogenous excreta from fish could be used to fertilize crops. The "Smart Floating Farm (SFF)" concept was the brainchild of Barcelona based designers Javier Ponce and Jacob Dycha, who look forward to work with traditional farmers on this (www.dailymail.co.uk). Nearly 40% of the world's oceans are severely affected by human activities, resulting in pollution, exhausted fisheries, and loss of coastal habitats. Here are some of the pictures of Floating Farms (Picture Credit: Mr. Javier Ponce, Founder, Floating Farms) (Figs. 9.1, 9.2, 9.3, and 9.4).

Another company "Green Wave" came up with similar idea for ocean farms as systems of underwater vertical gardens, it builds 3D vertical ocean farms, where kelp, scallops, and mussels grow on floating ropes, stacked above oyster and clam cages. Green Wave's ocean systems produce healthy and local foods, while capturing carbon and providing a source for biofuel. That is because its farms can grow between 10 to 30 tons of sea vegetables and 250,000 shellfish per acre per year crops that can be used to produce food, fertilizer, animal feed, and sustainable biofuels. According to the company, the kelp produced absorbs five times more carbon than land-based plants, and the crops do not require any fertilizers, fresh water,

Fig. 9.1 Top level: solar panels for renewable energy (Photo courtesy: Mr. Javier Ponce, Founder, Floating Farms)

Fig. 9.2 Level 2 for hydroponics of vegetables and other crops (Photo courtesy: Mr. Javier Ponce, Founder, Floating Farms)

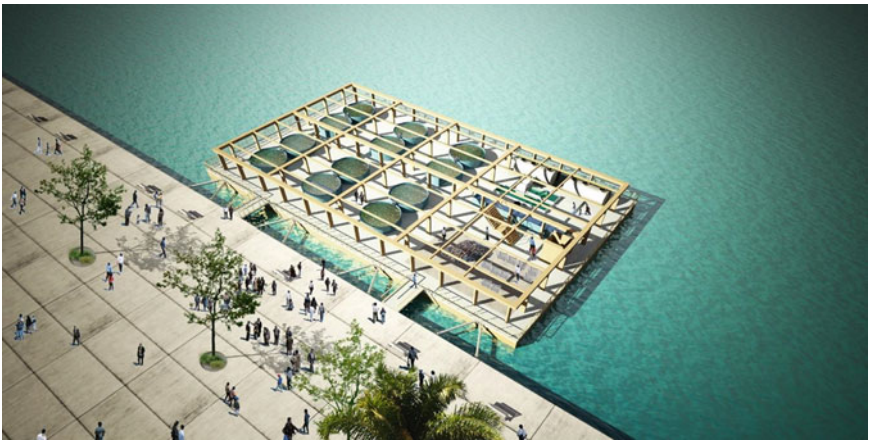

Fig. 9.3 Level 1 is used for fish farming (Photo courtesy: Mr. Javier Ponce, Founder, Floating Farms)

antibiotics, or pesticides (https://www.virgin.com/virgin-unite/how-vertical-ocean-farming-could-restore-marine-ecosystems). This design has other benefits as well e.g. instead of growing vulnerable monocultures, they create biodiverse ecosystems using the entire water column in production. They also help in rebuilding natural reef systems, using native and restorative species and sequester the nitrogen runoff from farms, factories, and homes, and help prevent oxygen-depleted marine dead zones. Such sustainable units combined with tissue culture units for marine bio-algae or seaweeds could be used in future ocean farms. Some of the chemicals produced by marine bio-algae are biologically active and some possess potent pharmacological

Fig. 9.4 Interior of level 2 of floating farm (hydroponic cultivation of vegetable crops) (Photo courtesy: Mr. Javier Ponce, Founder, Floating Farms)

activity. For example, the tropical red alga Portieria hornemannii contains halomon, a halogenated monoterpene with potent anti-tumor activity (Fuller et al. 1992). Another example is temperate brown alga Laminaria saccharina, which contains eicosanoid and oxylipin compounds involved in the mediation of inflammation (Gerwick and Bernart 1993; Rorrer et al. 1997). Some of advantages and limitations of Seaweed tissue culture and description of marine algal culture in bioreactor units have been described (Rorrer and Cheney 2004; Baweja et al. 2009).

9.7.4 Sustainable PTC Units Running on Solar Panels or Wind (Green Energy)

Commercial Plant Tissue Culture units are operated around the globe by public and private companies. Normally for the developed countries the energy cost for running such labs is low due to the low cost of electricity and uninterrupted power supply; however, for developing nations where the energy cost is high and power supply is interrupted frequently, the cost for running a commercial tissue culture unit could be high. The other alternatives could be use of solar panels or solar roofs; most of the developing nations like India, China, Brazil, Colombia, and some African countries have abundant sunlight for most part of the year. Solar cells are an alternative in these nations as a renewable source of energy. With the new developments in improved solar panels, tiles, or roofs whereby the total cost has been reduced significantly over last 3 years running such laboratories is cost-effective.

Recently University of British Columbia Researchers developed a Bacteria Powered Solar Cell; they engineered E. coli cells to produce high levels of lycopene to act as a light harvesting molecule and coated the bacterial cells with a mineral that

acts as a semiconductor and applied to the glass surface that acted as an anode. They harvested the current density of 0.6 mA/cm^2 which is higher than others in the field. This innovation with further optimizations in engineering and cost production for these bacterial cells could be used in countries that receive less amount of solar energy per year e.g. Europe, Canada, North America. Overall, combining the innovation and improvement in energy sector, material research and engineering can make these PTC units run at very low cost in near future and could be a cost-effective alternative to traditional farming that involves large fields or land, chemicals, and water to produce crops. With these applications, in next 20 years commercial crop production can see a dramatic switch.

9.7.5 The Rainmakers

Trees for cloud seeding in deserts—Greenery can have a number of effects on a local climate. Plants are thought to transfer moisture from the soil into the air by evaporation from their leaves, and hold water in the soil close to the surface, where it can also evaporate. What is more, the darker surfaces of plants compared to sandy deserts also absorb more solar radiation, which, along with their rough texture, can create convection and turbulence in the atmosphere. This might create more—or less—rainfall (Noorden 2006). It has been shown that vegetation effects account for 30% of annual rainfall variation in Africa's Sahel region (Los et al. 2006). Trees are capable of seeding or cloud formation, and clouds are made of microscopic droplets of liquid water or, in some cases, of small ice crystals. But in the atmosphere, water vapor cannot simply turn into a cloud: it needs solid or liquid particles, known as aerosols, on which to condense. Some of the research studies done in Amazon forests describe that trees help seed the cloud or rain by releasing salt or other chemicals from their leaves; this helps in aerosol formation that acts later as the cloud seed and thus resulting in rain. In one of the studies published in Nature, it was reported that aerosols can form and grow to the size needed to seed a cloud from compounds emitted by trees—without any sulfuric acid and accelerated by simulated cosmic rays.

In another study, it was revealed that Amazonian rainforest not only help in cloud formation, but they also trigger the shift in wind pattern drawing moisture from the ocean and actually causing the rainy season. It was observed that vapor rising from the sea is light as the isotope of H2 deuterium is left behind in the ocean, while in rainforests, high temperatures result in high rate of transpiration during high heat or dry season, the vapors released during transpiration during this period has deuterium which is heavy as compared to the water vapors from the sea. This deuterium acts as the seeder resulting in cloud formation, as these clouds get heavier, it rains causing the atmosphere to heat up resulting in circulation and drawing the movement of water vapors from the sea towards the forest and resulting in rainy season (Wright et al. 2017). This action could be replicated in deserts by growing small patches of vegetation involving seeds with higher rate of transpiration.

Along with the artificial cloud seeding these areas can further be transformed into green oasis. Culturing such plants could be possible by micropropagation; on the other hand, new technologies in genetic engineering could lead to development of tree species that can survive arid or semi-arid conditions. Overall, natural cloud seeding by trees is an exciting phenomenon and with the studies mentioned above, it is proven that trees or vegetation can bring about not only the change in rainfall but can drive changes in the season. The matter is thought provoking and calls for projects and plans involving multidisciplinary applied research to save land from desertification.

9.8 Policies, Guidelines, and Connectivity Between Environmentalists and Plant Biotechnologists

Environmental Biotechnology is a multidisciplinary branch of environmental science that includes applied research in microbiology and biotechnology with a view to address the issues related to environmental pollution. However, it needs to be extended to areas like plant sciences and agriculture as well. As we have discussed in the above topics, agriculture and forest lands are one of the main affected areas due to increasing population and industrialization. Thus leading to increasing pollution in land, natural water, oceans and air. We have also discussed about some of the technologies like plant tissue culture and genetic engineering that could be applied to address some of the issues e.g. phytoremediation by plants for mined areas, reforestation for eroded soil, land revival by planting suitable species. This calls for establishing more connectivity between environmental scientists, plant biologists, and microbiologists. Instead of conducting research in silos or individual areas, a networked research spanning the discussed areas would lead to faster solutions for such problems.

In an excerpt from The Economic and Social Council of the United Nations (ECOSOC), diffusion of both endogenous and exogenous innovations is a key factor for agricultural growth, hunger eradication, and poverty reduction. The Economic and Social Council of the United Nations (ECOSOC) underscored in 2004, that most developing countries are unlikely to meet the internationally agreed Millennium Development Goals (MDGs) of reducing poverty and hunger without a clear political commitment to making science and technology top priorities in their development agenda and increasing the related budget up to at least 1% of the gross domestic product (GDP).

Government agencies along with Universities and Industries need to develop and launch programs related to multidisciplinary research in these areas of environmental biotechnology and agriculture science or plant biotechnology. In Europe, the kBBE concept has been translated in kBBE specific European Technology Platforms (ETP) and the implementation of several European Research Area (ERA) networks to reduce fragmentation and improve the coherence and coordination of national research programs. Along with this, several European Commission expert groups have been established. The rising need for a sustainable supply of food, raw

materials and energy, together with tremendous progress in the life sciences has led to the concept of the knowledge-Based Bio-economy (kBBE) (2007) or "bio-economy" with emerging key technologies as major drivers of innovation Research in the different areas of the kBBE has been promoted and financed through the Commission's Framework Programme 7 (FP7) and several Member State initiatives (The knowledge Based Bio-Economy kBBE in Europe: Achievements and Challenges 2010). Some interesting examples of government agencies driving the implementation of such programs are seen in Africa e.g. in Gabon, Centre national dela recherche scientifique et technologie (CENAREST) was established in 2002 and is the biggest in vitro propagation facility in Gabon. By 2004, this laboratory was already producing micropropagated pineapples, bananas, and plantains that were expected to be distributed to farmers by 2005. In Nigeria, vegetatively propagated crops (cassava, yams, sweet potato, pineapple, plantain, banana, etc.) have a significant relevance for food security and poverty reduction. The Federal government is making efforts to rapidly apply biotechnologies for the propagation of some of these important crops, especially cassava, the staple food for most Nigerians. A national program code named "Presidential Initiatives for Cassava Production in Nigeria," aims at replacing local cultivars of cassava with improved ones. Such initiatives are going to drive the socioeconomic development for the countries and reduce the expenditure on research by investing in overlapping research areas towards a common goal.

9.9 Societal Impact and Future Path

Science has provided solutions to some of the most challenging problems of the century. The last few decades of investment in biotechnology, agriculture, and environmental sciences research has created awareness in the masses towards scientific solutions to some of the most pressing problems of today e.g. increasing population and decreasing food security, degradation and reduction of agricultural land, pollution of water bodies, land, and oceans due to increased industrialization and urbanization, climate change, etc. All these problems have a direct impact on human livelihood, health, and pose a risk to sustainability of human civilization in near future. However, some of the programs and scientific research have made a positive impact on the society by either providing short term and long-term solutions to some of these issues. We will cover the impact of tissue culture or micropropagation of some of the economically important plants had on raising the economic status of the farmers in developing poor countries and how this can impact other future programs to drive humanity towards a better and sustainable future. In Shandong Province of China, the economic impact of micropropagated virus free sweet potato has been assessed and results indicated that 80% of the farmers have taken up the technology because of its proven ability to increase yields by up to 30%; the IRR was estimated to be 202%, with a NPV of USD 550 million (assuming a 10% real discount rate). By 1998, the annual productivity increases were valued at USD 145 million, with an increase in agricultural income of the province's seven

million sweet potato growers by 3.6 and 1.6%, in relatively poor and better-off districts, respectively (Fuglie et al. 1999). In Kenya, the commercial micropropagation of disease-free bananas had been adopted by over 500,000 farmers (Wambugu 2004) and was predicted (Qaim 1999) and shown (Mbogoh et al. 2003) to offer relatively higher financial returns than traditional production. In Vietnam, the introduction of improved high yielding and late blight resistant potato varieties and the subsequent adoption of micropropagation by farmers have seen potato yields increasing significantly from 10 to 20 tons/hectare. The self-supporting plantlet production by the farmers has made the seed more affordable and the rate of return on investment in this new seed is system highly favorable.

Micropropagation not only increased farmer's yields and incomes, but also led to the creation of rural micro-enterprises that have specialized in the commercial provision of disease-free seed (Uyen et al. 1996). In India the "Revolving Fund Scheme for Potato Breeders Seed Production" integrated micropropagation and virus detection in the initial stages of potato breeders seed production, leading to two- to threefold improvement of health standards of the seed produced. The scheme generated total revenue of over USD 4 million, over a period of 10 years, with a cumulative balance of USD 0.735, deducting the total expenditures for the development of infrastructure and for the recurring costs (Naik and Karihaloo 2007). Adoption of micropropagated sweet potato varieties in Zimbabwe has brought about a change in the socioeconomic status of the farmers livelihood. Improvements associated with adoption of new sweet potato varieties include diet diversification, food security, increased capacity of investing in other agricultural activities (inputs, etc.), purchasing equipment and animals, paying school fees, and coping with vulnerability factors (drought, disease, inflation). The success in these projects depended on the increase in yield with low inputs thus decreasing the cost of production. Policy decisions e.g. subsidies provided by the government and the service package in terms of not only the dissemination of the technology but also enablement of the implementation of a new technology by providing guidance and solution to short term problems arising during the implementation phase of such projects also made these projects successful. Adaptation of these technologies depends on the ease associated with the above-mentioned factors and the government policies and support. A synergistic approach from the scientific experts, government agencies, policy makers, and farmers can truly bring about a huge benefit to not only the individual farmer or the farming communities but to poor and developing countries, leading to stability and progress of the society.

9.10 Conclusion

Though plant tissue culture has been practiced for more than a century now and significant advances have been made over the last 50 years in research and commercial development of this area, its full potential has still not been realized. There are some roadblocks in the field of plant tissue culture due to reasons discussed earlier in the chapter that happen to have a significant impact on the development and

application of this area. Most of the academic research in Plant Tissue Culture is conducted with a view to publish the findings of the research rather than with a vision to apply the findings of the research to benefit the unmet needs of the farmers. Hence most of the published protocols from plant tissue culture research are hardly reproducible or lack enough data in terms of fine observations of the tissue or cell growth that could lead to have a stabilized protocol or could become a process. More than this, most of the research involving genetic engineering of plants whereby plant tissue culture is used just as a tool to test the effect of the newly engineered trait in plant cells deploys the use of easy to transform tissue or variety. The wild species cultivated indigenously or elite varieties that are cultivated by conventional means are normally left out even from tests and only few research institutes or companies focus on such varieties. These significant gaps hinder in the application of this technology to a broad range of varieties or species. However, with the right kind of scalable protocols that work for more than one variety, the technology demonstrates high potential to be used for areas in commercial production of fruits, vegetables, cereals, and woody plant species. The topics covered in the chapter about large-scale production of woody plants or trees for reforestation, eroded lands, phytoremediation, etc. can be realized by investing in research in this area leading to cost-effective large-scale production of such species.

Another important need is to break the silos i.e. instead of providing funds and having multiple research programs for each scientific discipline due to the expertise, it would be valuable to have network research programs in multidisciplinary areas towards a common goal; these programs need to be launched by Government Organizations, Industries, and other private funding agencies e.g. engineering "Poplar" for phytoremediation could be a network project with experts from genetic engineering, environmental science, physiology, and forestry. Such sporadic initiatives are launched but the numbers of such network programs are too few and often involve experts located in far off places. This can be curbed by short term transfer of experts to the relevant area or country to facilitate the project. This kind of initiatives will cut down the costs and timeline of a project.

Technology transfer or implementation of large-scale propagation of plants that could be viable for farmers or other industries involves not only a robust scalable technology but also support in form of subsidies from the government. Farmer awareness programs by Industry experts or government officials are also important to make this a success. Examples of commercial micropropagation of crops in Africa involved initiatives and support from the government, funds from different agencies, and awareness of the farmers made these initiatives successful and led to an upliftment of the socioeconomic status of the farming communities. Initiatives like Floating Farms are building the commercial agriculture platforms for the future. Investment in such technologies and spreading awareness about building a sustainable ecosystem and guaranteeing food security at low cost by intelligent use of clean energy and technology is the need of the hour. Overall, despite many challenges, tissue culture is not only a workhorse for genetic engineering, but it also presents tremendous opportunities for other disciplines e.g. environmental sciences and forestry, and farming communities. In this time of climate change, decreasing food

security and sustainability of the human kind investments in tissue culture can provide a means of livelihood as an alternative to conventional agriculture that involves land, high input of chemicals, and overall cost.

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Links

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Microbial and Biotechnological Approaches in the Production of Biofertilizer

10

P. S. Renjith, K. R. Sheetal, Sandeep Kumar, Jairam Choudhary, and Shiv Prasad

Abstract

The biofertilizers are formulations containing live or latent cells of effective microbial strains cultured in the lab and packed in appropriate carriers. When applied to seeds, soil, or plant surfaces, they enhance the availability of plant nutrients and growth stimulus to target crops. Biofertilizers are known to deliver many benefits, including plant nutrition, disease resistance, and tolerance to adverse climatic conditions. During the past few decades, notable progress has been made to explore microbes' potential and for biofertilizer production to enhance agricultural productivity. All biofertilizers are known to be environment-friendly and valuable inputs for the farmers. Their application has been considered an essential component of integrated nutrient management and a potential alternative to chemical-based agriculture due to its vital role in food security and sustainable crop production. Currently, biofertilizer demand and production are gaining momentum, as there is burgeoning passion for organically grown food among the health-conscious societies. Various initiatives and affirmative regulations laid by government institutions and agencies would further be fueling the extension of the biofertilizer market worldwide. Application of these eco-friendly and cost-effective inputs would not only promote growing healthy food, but also help to maintain a sustainable environment and holistic human well-being.

P. S. Renjith · K. R. Sheetal
ICAR-Central Arid Zone Research Institute (CAZRI), Regional Research Station, Bikaner, Rajasthan, India

S. Kumar · S. Prasad (✉)
Centre for Environmental Sciences and Climate Resilient Agriculture, ICAR-Indian Agricultural Research Institute, New Delhi, India

J. Choudhary
ICAR-Indian Institute of Farming Systems Research, Meerut, Uttar Pradesh, India

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10.1 Introduction

The biofertilizers are defined as the formulations containing live or latent cells of active microbial strains, which increases the availability of nutrients to crop plants. It comprises of mycorrhizal fungi, blue-green algae, and bacteria already present and available in nature (Itelima et al. 2018). When applied to seeds, plant surfaces, or soil, they accelerate the biological N-fixation, solubilization/mobilization of P, K, Zn, and many other nutrients and thus enhance the availability of plant nutrients and growth stimulus to target crops (Bhattacharjee and Dey 2014). Biofertilizers were developed with the discovery of biological nitrogen fixation (BNF). BNF is a natural source of nitrogen and plays a vital role in the sustainable production of leguminous and even non-leguminous crops. The most striking relationship that these have with plants is symbiosis, in which the partners derive benefits from each other. Plants have many connections with fungi, bacteria, and algae, the most common of which are with Mycorrhiza, Rhizobium, and Cyanophyceae (Sinha et al. 2014; Itelima et al. 2018). These are known to deliver many benefits, including plant nutrition, disease resistance, and tolerance to adverse climatic conditions.

Plants nutrients are essential for crop production and healthy food production for the world. Biofertilizers, when applied, add nutrients to the soil through the natural processes (Vessey 2003; Sinha et al. 2014). They also add plant growth-promoting substances like phytohormones and enzymes, thus increasing the productivity of crops (Barman et al. 2017; Itelima et al. 2018). As we know, India is overwhelmingly reliant on imports for meeting its fertilizer needs. Therefore, the Government of India is trying to explore innovative ideas to build self-reliance on environment-friendly fertilizers to reduce the dependence on imports. The Indian government is also encouraging the domestic fertilizer sector, primarily to promote the use of biofertilizers across the country (Gupta 2018). Biofertilizer has been considered as an alternative to chemical-based crop production due to its potential role in food safety and sustainable agriculture (Bhattacharjee and Dey 2014).

10.2 Microbes Used in Biofertilizers and Their Suitability for Crops and Benefits

Globally, the commercial journey of biofertilizer began with the introduction of the rhizobium "Nitragin" by Nobbe and Hiltner in 1895. Currently, many biofertilizers are available on a commercial scale (Kribacho 2010). Table 10.1 shows the classification of biofertilizers based on the different types of microorganisms used in their formulations. According to the recommended package of practices, farmers typically require to apply 120 kg of N per hectare for crops such as rice and wheat. However, the N-fertilizer use efficiency is generally below 40%, meaning that most applied fertilizer either wash out or is lost to the environment. Similarly, plants utilize P-fertilizer to the tune of only 10–15% of the phosphate applied. The balance 85–90% remain in insoluble form in the soil, which can be solubilized by applying P-solubilizing biofertilizers such as *Bacillus megaterium*. According to Zulaika et al.

Table 10.1 Examples of various types of microbes used in biofertilizer production (*Source:* Bhattacharjee and Dey (2014))

Microbial groups	Examples of microbial species
Biological N-fixing microbes	
Free-living microbes	*Anabaena*, *Nostoc*, *Azotobacter*, *Beijerinckia*, *Clostridium*, and *Klebsiella*
Symbiotic microbes	*Anabaena*, *Azolla*, *Rhizobium*, and *Frankia*
Associative symbiotic microbes	*Azospirillum*
P-solubilizing biofertilizer	
Bacterial strains	*Bacillus subtilis*, *Bacillus circulans*, *Bacillus megaterium var. phosphaticum*
Fungal strains	*Aspergillus awamori* and *Penicillium bilaiae*
P-*mobilizing biofertilizers*	
Arbuscular mycorrhiza	*Vesicular-arbuscular mycorrhiza (VAM) like Gigaspora* spp., *Acaulospora* spp. *Glomus* spp., *Scutellospora* spp., *and Sclerocystis* spp.
Ectomycorrhiza	*Pisolithus* spp, *Boletus* spp., *Laccaria* spp., and *Amanita* spp.
Orchid mycorrhiza	*Rhizoctonia solani*
Ericoid mycorrhiza	*Pezizella ericae*
Potash biofertilizers	
K-BF	*Bacillus mucilaginous*, *Frateuria aurantia* and *Aspergillus niger*
Biofertilizers for micronutrients	
Zinc solubilizers	*Bacillus and Thiobacillus* spp.
Sulfur solubilizers	*Thiobacillus spp. oxidizing sulfur to sulfates*
Manganese solubilizer	*Penicillium citrinum*
Silicate solubilizers	*Bacillus* spp., *mobilize the inaccessible form of soil potassium (silicates)*
Plant growth-promoting rhizobacteria	
PGPR	*Pseudomonas fluorescens and Bacillus spp.*

(2017), the application of biofertilizers can increase the yield by around 25–50%, while reducing the application of chemical fertilizers up to 25–50% for N and about 25% for P. Suitability of biofertilizers for crops and its benefits are given in Table 10.2.

10.3 Biofertilizer Formulations

Biofertilizers are also known as cultures, most commonly attributed to as particular beneficial microbial strains cultured in a lab and packed in appropriate carriers (Hari and Perumal 2010; Itelima et al. 2018). The biofertilizer preparations are commercially available as carrier-based, as well as liquid-based formulations (Swarnalakshmi et al. 2016). In general, there are 6-steps involved in making carrier-based biofertilizer formulation and its production (Fig. 10.1). These are exploration and identification of active microorganisms, isolation and choosing of

Table 10.2 Suitability of biofertilizers for crops and benefits

Biofertilizers	Suited for crops	Benefits
Rhizobium strains	Legumes crops: pulses, groundnut, soybean	10–35% yield increase, 50–200 kg N ha^{-1}
Azotobacter	Soil treatment for non-legume including dryland crops	10-15% yield increase, adds 20–25 kg N ha^{-1}
Azospirillum	Nonlegumes crops: maize, barley, oats, sorghum, millet, sugarcane, rice, etc.	10–20% yield increase
Phosphate solubilizers	Soil application for all crops	5–30% yield increase
Blue-green algae and Azolla	Rice/wet/flooded lands	20–30 kg N ha^{-1}, Azolla can give biomass up to 40–50 tons and fix 30–100 kg N ha^{-1}
Mycorrhizae (VAM)	Horticultural trees, crops, and ornamental plants	30–50% yield increase, enhances uptake of P, Zn, S, and water

Fig. 10.1 Steps involved in making carrier-based biofertilizer

beneficial target microbes, selection of suitable method and carrier, selection of best propagation method, phenotype (quality) testing, and field trials at a diverse environment to examine its effectiveness and limitations (Khosro and Yousef 2012; Youssef and Eissa 2014). The necessary steps involved in making carrier-based biofertilizer are shown in Fig. 10.1.

Carrier-based preparations are primed with powdered charcoal or lignite (10^6 CFU/g) with 3–6 months' shelf-life and are commonly available in the market (Swarnalakshmi et al. 2016). However, vermiculite, press mud, farmyard manure, peat soil, and soil mixture can also be used as carrier materials. The use of lignite is sometimes found to have harmful effects on the workers involved in biofertilizer production. Contamination of cultures is often caused by high moisture. It may either compete with biofertilizers or cause antagonistic interaction. Sometimes retail traders and shopkeepers do not prefer to sell biofertilizers because of short

shelf-life, limited demand by farmers, and non-existence of storage facilities (Mazid and Khan 2014). The carrier-based formulation is available in various forms such as powder, slurry, and granules. The powder form is usually used as seed coating before planting. The smaller the particle size of powder, the better the inoculants will adhere to the seeds. Standard size varies from 0.075 to 0.25 mm. The amount of inoculants required or used is around 200–300 g ha^{-1}. The powder-type inoculants suspended in liquid or slurry are directly applied to the furrow, or the seeds are dipped just before sowing. The granular form is used directly to the furrow together with seeds. Size ranges are from 0.35 to 1.18 mm. The granular form is better than powder inoculants for rhizobia under stressful planting conditions, but costlier owing to more required quantity (Trimurtulu and Rao 2014).

Liquid biofertilizers (LBFs) are aqueous, oil, or polymer-based liquid formulations or suspensions carrying desired microorganisms and their nutrients along with cell protectants and additives that promote the formation of resting spores or cysts for extended shelf-life and tolerance to adverse conditions after application to seed or soil (Brahmaprakash and Sahu 2012). They are neither the water suspension of carrier-based biofertilizers nor the usual broth culture obtained by fermentation. LBFs consist of beneficial microbial cultures suspended in liquid medium rich in cell protectors and nutrients. The cell protectors and additives improve the inoculant quality by stabilizing the product, preventing osmolysis, giving better binding to seed, enhancing the microbe's survival during storage, and protecting the inoculum after exposure to extreme environmental conditions upon inoculation into seed or soil (Sahu and Brahmaprakash 2016). LBFs are now considered a great alternative to conventional carrier-based biofertilizers in current agriculture. Carrier-based biofertilizers are susceptible to high temperatures and UV rays, and most importantly, the population density of the microbes reduces gradually to almost nil by the end of 6 months from its time of production (Mahdi et al. 2010). Liquid-based preparations are coming up with a high microbial mass (10^9 CFU/mL), with longer shelf-life of 12–24 months and additionally, they can be applied in drip irrigation and as a component of organic farming (Swarnalakshmi et al. 2016; Vyas and Shelat 2019). LBFs can help to boost crop yields, rescue soil health, and sustainable food production as they offer several advantages. Advantages of LBFs over carrier-based biofertilizers are as follows:

- Negligible contamination during the production process
- Comparatively longer shelf-life (12–24 months)
- The higher microbial load of the desired microorganism (10^9 Cells/ml) which can be maintained during storage
- Easy to identify the biofertilizer by the typical fermented smell
- Cost-saving on preparation (pulverization, sterilization, and packing) and transportation of carrier material
- Easy to follow and maintain the quality control standards
- Higher survival on seed and soil
- Farmers friendly as it is easy to apply in the field
- Low dosage compared to carrier-based biofertilizers

- Tolerant to high temperatures and ultra-violet radiations
- Compatible with modern agriculture machinery for its application
- They allow alternative applying methods such as fertigation and spray application to soil

10.4 Biofertilizer Application

Currently, both solid-carrier based and liquid-suspension based biofertilizers widely used by the farmer. Liquid inoculants can be based on broth cultures, mineral or organic soils, or on oil-in-water suspensions. In the case of solid carriers, powder, granules, or beads are the ideal forms utilized. The application of both the carrier and liquid-based biofertilizers is discussed below.

10.4.1 Application of Carrier-Based Biofertilizers

10.4.1.1 Seed Treatment or Seed Inoculation

Seed treatment is the crucial practice used for various types of bio-inoculants, in which the inoculant is mixed with water to take the form of slurry and then mixed with seeds (Narayanasamy et al. 2012). The seed treatment can be done with more than one type of bio-inoculant. It is an effective and economical method. The carrier-based biofertilizer seed treatment process is shown in Fig. 10.2. Inoculants of

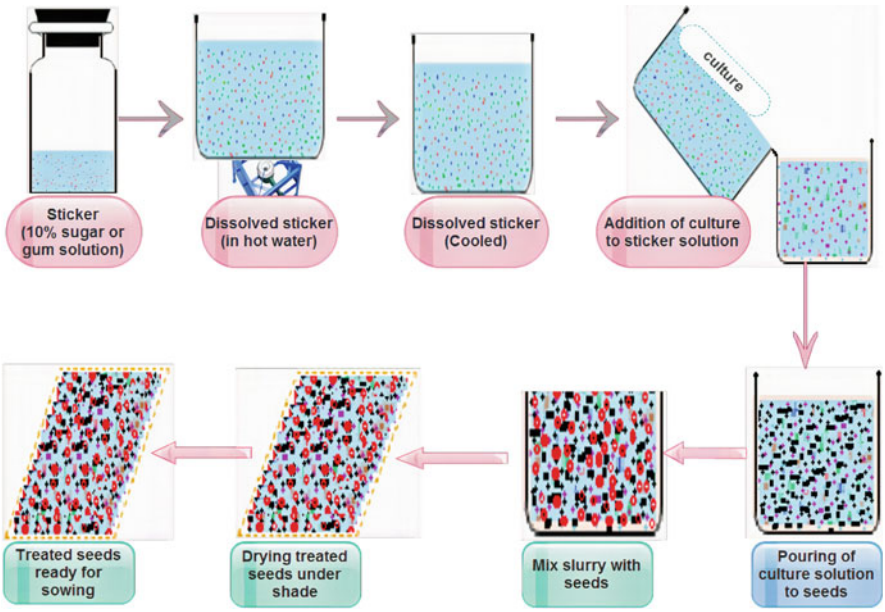

Fig. 10.2 Carrier-based biofertilizer application process

Table 10.3 Application and doses of carrier-based biofertilizers for various crops (adapted from Narayanasamy et al. 2012)

Name of organism	Host crops for which used	Method of application	Rate of inoculant
Rhizobium	Legumes like pulses, soybean, groundnut	Seed treatment	200 g/10 kg seed
Azotobacter	Cereals, millets, cotton, vegetable	Seed treatment	200 g/10 kg seed
Azospirillum	Nonlegumes like maize, barley, oat, sorghum, millet, sugarcane, rice, etc.	Seed treatment	200 g/10 kg seed
Phosphate solubilizers	Soil application for all crops	Seed treatment	200 g/10 kg seed
Blue-green algae (BGA)	Rice	Soil application	10 kg/ha
Azolla	Rice	Soil application	1 ton dried material/ha
Mycorrhiza (VAM)	Many tree species, wheat, sorghum, ornamentals	Soil application	–

Rhizobium, Azotobacter, Azospirillum, and phosphate solubilizing bacteria are generally applied through seed treatment. In general, 150–200 g of inoculant is enough to treat seed for an acre of land. For the purpose of making stickers, mainly adhesive, like sugar (gur), gum, rice-gruel water, sucrose solutions are used. The total quantity of water to be taken should be just sufficient to moisten the seeds, and 10% solution of jaggery or sugar is to be prepared. Boil the solution and let the solution be cooled completely. Biofertilizers are mixed thoroughly in the cooled solution, and the slurry thus prepared is poured on seeds and shaken continuously so that a uniform layer of biofertilizer is coated onto each seed. After coating, the inoculated seeds are shade-dried for 20–30 min and to be sown immediately. For acidic and alkaline soils, it is always advisable to use 1 kg of slacked lime or gypsum powder, respectively, for coating the wet biofertilizer treated seeds. Precautions must be taken carefully to avoid direct exposure of the inoculant packet and the inoculated seeds to sunlight, contact with chemical fertilizers, and pesticides. Application and doses of carrier-based biofertilizers for various crops are shown in Table 10.3.

10.4.1.2 Soil Application or Main Field Application

The second technique is direct soil application by the broadcasting of biofertilizer on plantation or during sowing or before sowing. It is mostly practiced for plantation crops, fruit crops, sugarcane, and other crops where the localized application is required. Generally, for one acre, depending upon the total plant density, 2–4 kg of the recommended biofertilizer is mixed with 50–100 kg of compost or FYM, and the mixture is kept overnight with adequate moisture for conditioning. Then, the mixture is applied in the rows or during the leveling of soil or sowing. In the case of standing crops, it is to be applied to soil near the roots.

10.4.1.3 Root/Seedling/Sett/Tuber Inoculation

This method is mainly used for transplanted crops, seed-setts of sugarcane, cut pieces of potato, the base of banana suckers, and forest nurseries. In the case of seedlings, the seedlings required for one acre should be inoculated using 2–2.5 kg of recommended biofertilizers. The inoculant has to be mixed with just sufficient quantity of water (10–15 l), and the root portion of the seedlings has to be dipped in the mixture for a minimum 30 min before transplantation. It has been found well-suited for crops like tomato, onion, cole crops, and flowers. For transplanted rice in low land, a sufficient size bed should be made in the field, and it is filled with 3–4 inches of water. Recommended biofertilizers (5 kg/ha) are to be mixed thoroughly in this water, and roots of seedlings are dipped in this bed for 8–10 h and then transplanted. For sett treatment, the ratio of biofertilizer to water is approximately 1:50. Culture suspension is prepared by mixing 1 kg of biofertilizer in 40–50 l of water, and cut pieces of planting material are kept immersed in the suspension for 30 min. Then, the cut pieces are shade-dried before planting.

10.4.2 Application of Liquid-Based Biofertilizers

Application of LBFs in the field is easy and very simple. Like carrier-based biofertilizers, they are also applied through seed and soil, root/seedling treatment on the plantation, at sowing or before sowing, but the dosages of LBFs are usually 10 times lesser than that of former. In addition to the above-mentioned methods of application, alternative application methods, such as fertigation, in-furrow, foliar sprays, and direct spraying to the soil, can also be employed (Campo et al. 2010; Fukami et al. 2016; Moretti et al. 2018). They are also compatible with modern agricultural machinery and can be applied using hand sprayers, power sprayers, fertigation tanks, and drip irrigation. A bottle containing a 200 ml culture of Azotobacter/Azospirillum is sufficient for one acre area of cereal crops like rice, wheat, oat, barley, maize, and sorghum; pulses like chickpea, pea, groundnut, soybean, beans, lentil, alfalfa, berseem clover, green gram, black gram, cowpea, and pigeon pea; oilseeds like mustard, sesame, linseeds, sunflower, castor, etc. Some of the most important LBFs and their application method and the ideal amount required for various agricultural crops and agroforestry/fruit/trees are given in Table 10.4.

10.5 Biofertilizers: Global and Indian Scenario

The expanding use of biofertilizers is showing its potential for sustainable farming. The growing concern about food safety is expected to drive more interest in the industrial production of biofertilizers globally as well in the Indian scenario.

10.5.1 Biofertilizers: Global Scenario

The global biofertilizer market size was at US $787.8 million in the year 2016. That stood at US $1.34 billion during the year 2018. It is projected to witness a compound

Table 10.4 Liquid biofertilizers and their method of application and doses used for different crops

Crop	Liquid biofertilizer	Application method	Dose/quantity used acre (ac)
Pulses	Rhizobium	Seed treatment	200 ml ac^{-1}
Cereals	Azotobacter/ Azospirillum	Seed treatment	200 ml ac^{-1}
Oilseeds	Azotobacter	Seed treatment	200 ml ac^{-1}
Forage and grasses	Azotobacter	Seed treatment	200 ml ac^{-1}
Plantation crops	Azotobacter	Seedling treatment	500 ml ac^{-1}
Tea, coffee	Azotobacter	Soil treatment	400 ml ac^{-1}
Rubber and coconut	Azotobacter	Soil treatment	2–3 ml/plant
Agroforestry/fruit trees	Azotobacter	Soil treatment	2–3 ml/plant at the nursery
Leguminous trees	Rhizobium	Soil treatment	1–2 ml/plant

Note: Dosages recommended when the inoculum count is 1×10^8 cells ml^{-1}. Besides, the above-said rate of 200 ml ac^{-1} could be applied for all crops

annual growth rate (CAGR) of 14.08% during 2018–2025, thereby reaching US$3.3 billion by the end of 2025 (TechSci 2019). The worldwide biofertilizers market growth can be attributed to the rising adoption of cutting-edge technologies by farmers to increase soil fertility and inclusive crop productivity and sustainable farming (Itelima et al. 2018). Besides this, a growing passion for organically produced food is driving force for biofertilizers demand over chemical fertilizers across the world (Santos et al. 2012; Itelima et al. 2018). As a result, vast quantities of cultivated produce to cater to the demand from the growing population are subsequently stimulating the use of biofertilizers on a large scale. Various initiatives and affirmative regulations and action policies laid by government organizations would further be fueling the extension of the worldwide biofertilizer market.

10.5.2 Biofertilizers: Indian Scenario

In India, a systematic study on biofertilizers was started by NV Joshi in 1920 (Barman et al. 2017). Rhizobium was isolated from various cultivated legumes, and the first commercial production of Rhizobium biofertilizer in India began in 1956, and by the late 1960s, when soybean was introduced, large scale production began (Panda 2011; Bhattacharjee and Dey 2014; Barman et al. 2017). The use of mycorrhizae as biofertilizer is a recent development. The use of microbial strains in biofertilizers re-establishes the natural nutrient cycle, maintains the optimal nutrients level, increases organic carbon contents and fertility of the soil (Sinha et al. 2014; Shelat et al. 2017). The production of biofertilizer in India during 2010–11 was 37997.6 metric tons. However, during 2014–15, the total biofertilizer production reached 80696.5 metric tons (Table 10.5).

Table 10.5 Zone wise carrier-based biofertilizer production in India (2010–15) compiled by NCOF (data provided by production units'/state government/RCOFs and presented by the Ministry of Agriculture in Lok Shaba)

Sl. No.	State	2010–11	2011–12	2012–13	2013–14	2014–15
South zone (in metric ton)						
1.	Andaman & Nicobar Islands	0.0	0.0	0.0	0.0	0.0
2.	Andhra Pradesh	999.6	1126.4	1335.7	2714.2	2668.8
3.	Daman & Diu	0.0	0.0	0.0	0.0	0.0
4.	Karnataka	6930.0	5760.3	7683.7	9907.3	16462.6
5.	Kerala	3257.0	904.2	1045.6	3520.7	4917.0
6.	Lakshadweep	0.0	0.0	0.0	0.0	0.0
7.	Pondicherry	783.0	509.5	621.0	517.0	561.0
8.	Tamil Nadu	8691.0	3373.8	11575.7	14104.8	15373.3
Total		20660.6	11674.1	22261.8	30764.0	39982.6
West zone						
1.	Chhattisgarh	0.0	276.3	501.6	712.1	1024.7
2.	Gujarat	6318.0	2037.4	978.5	6411.4	3667.9
3.	Goa	443.4	0.0	370.0	66.3	802.5
4.	Madhya Pradesh	2455.6	2309.1	1408.1	4824.2	2638.0
5.	Maharashtra	2924.0	8743.7	5897.9	6218.6	14847.4
6.	Rajasthan	819.8	199.8	982.0	1315.0	599.9
7.	Dadra &Nagar Haveli	0.0	0.0	0.0	0.0	0.0
Total		12960.7	13566.2	10138.1	19547.6	23580.4
North zone						
1.	Delhi	1205.0	1617.0	0.0	396.0	104.5
2.	Chandigarh	0.0	0.0	0.0	0.0	0.0
3.	Haryana	6.5	914.4	5832.6	1146.5	873.0
4.	Himachal Pradesh	9.0	1.3	0.0	26.1	0.8
5.	Jammu & Kashmir	0.0	0.0	0.0	45.3	0.0
6.	Punjab	2.5	692.2	2311.3	2124.9	6305.5
7.	Uttar Pradesh	1217.5	8695.1	1310.0	2682.2	4099.1
8.	Uttarakhand	45.0	263.0	2758.2	5493.9	2130.0
Total		2485.5	12183.0	12212.2	11914.8	13512.7
East zone						
1.	Bihar	136.3	75.0	52.4	52.4	64.9
2.	Jharkhand	0.0	8.4	35.3	14.2	9.1
3.	Odisha	357.7	590.1	407.1	1097.6	1074.5
4.	West Bengal	393.4	603.2	1110.0	1682.7	2061.8
Total		887.3	1276.7	1604.8	2846.9	3210.3
North East zone						
1.	Arunachal Pradesh	0.0	0.0	0.0	59.0	59.0
2.	Assam	130.0	68.3	89.0	149.0	88.0
3.	Manipur	0.0	0.0	0.0	0.0	0.0

(continued)

Table 10.5 (continued)

Sl. No.	State	2010–11	2011–12	2012–13	2013–14	2014–15
4.	Meghalaya	0.0	0.0	0.0	0.0	0.0
5.	Mizoram	2.0	0.0	0.0	4.0	3.6
6.	Nagaland	21.5	13.0	7.5	7.5	7.5
7.	Sikkim	0.0	0.0	9.5	10.1	12.4
8.	Tripura	850.0	1542.9	514.0	225.0	240.0
Total		1003.5	1624.2	620.0	454.6	410.5
Grand total		37997.6	40324.2	46836.8	65527.9	80696.5

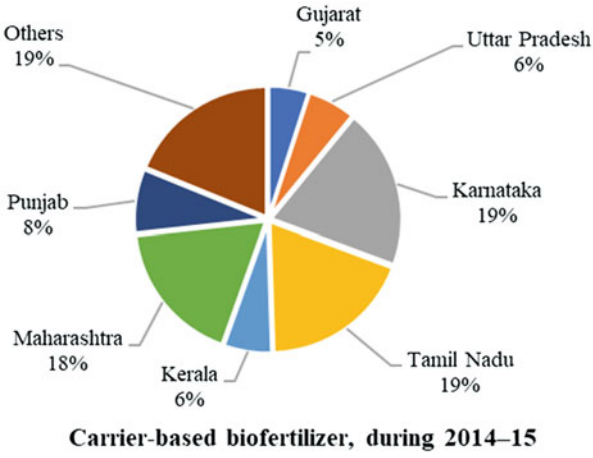

Fig. 10.3 State-wise contribution to the production of carrier-based biofertilizers

It was also noticed that the maximum utilization capacity of biofertilizer is in the south zone at 49.5% and west zone at 29.5%, while the North East Zone recorded the lowest capacity production at <1% during 2014–2015 (NCOF 2015). Zone wise carrier-based biofertilizer production in India is given in Table 10.5. Based on crop area in India, the requirement of biofertilizers is estimated at around 5,50,000 metric tons (Pindi and Satyanarayana 2012). However, the total production of biofertilizers in the country is much less than the need. This signalizes the inevitability of an increase in biofertilizer production (Barman et al. 2017).

The state-wise contribution in the production of carrier-based biofertilizer in India during 2014–15 (Fig. 10.3) indicates that Karnataka ranks first in the production of carrier-based formulation, followed by Tamil Nadu and Maharashtra. However, Gujarat is leading in the production of liquid-based biofertilizers, followed by Maharashtra (Fig. 10.4). Approximately 225 biofertilizer production units have been installed with a production capacity of around 98,000 million tons/annum.

Recently, under the NAIP-ICAR project, Anand Agricultural University, Gujarat has developed a liquid biofertilizer formulation, a safe and eco-friendly substitute for chemical fertilizers. They fix atmospheric N and solubilize insoluble P and make it

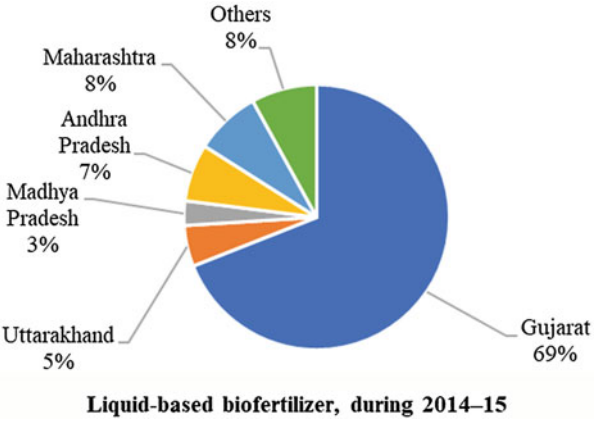

Fig. 10.4 State-wise contribution to the production of liquid-based biofertilizers

available to the crops. It is sold to farmers under the brand name "Anubhav liquid Biofertilizers." It is produced from native bacterial cultures, viz. *Azospirillum lipoferum*, *Azotobacter chroococcum*, and *Bacillus coagulans*. Anand Agricultural University has licensed the LBF technology to commercialize under the Public-Private Partnership to Business Planning and Development Unit, Gujarat. Anand Agricultural University has provided around 50,000 l LBFs to the Gujarat government. It is mostly used in cotton, potato, turmeric, rose, banana, and papaya and showed better yield and quality of these crops (Vyas and Shelat 2019).

The Indian government has taken several initiatives to harness the potential of microbes by introducing as biofertilizers along with chemical fertilizers to the farmers (Barman et al. 2017). In 2006, the Government of India had enforced BIS certification to assess the quality control of biofertilizers such as Rhizobium, Azotobacter, Azospirillum, and PSB under the ambit of Fertilizer (control) Order (1985) during 2006. Still, very few manufacturers opt for quality standards (Swarnalakshmi et al. 2016). Central and state governments are promoting the biofertilizer production and use both at the farmer and the investor level through (1) farm level extension and promotion programs, (2) financial support to investors for setting units, (3) subsidies on sales, and (4) direct production in public and cooperative sector, and through universities and research institutions (Mazid and Khan 2014). Presently, the Government is helping to improve productivity of biofertilizer industries in India. The Government of India is also promoting the use of biofertilizers across the country. Much financial support has been provided to establishment of biofertilizers production unit as back ended subsidy, at 25% of total financial outlay up to a maximum of Rs. 40 lakh. National Bank for Agriculture & Rural Development (NABARD) also awards a national productivity award to the efficient biofertilizer production unit in India (Borkar 2015; Barman et al. 2017).

10.6 Biofertilizers: Microbial and Biotechnological Approaches

Today, microbial techniques and biotech-tools are assisting in solving many challenges, especially in the unexplored potential of microbes. The integration of the microbial and biotechnological approaches in the field of biofertilizers can help in improving microbe's efficiencies, and making better use of natural resources. It will also help to increase agriculture production and reduce the use of chemical fertilizers, pesticides, and insecticides on crops.

10.6.1 Bacterial Biofertilizers

Most of the bacteria added in biofertilizers have a close association with crop roots and the ability to fix atmospheric N. Many symbiotic and free-living bacteria are involved in biological nitrogen fixation (Table 10.1). The annual biological N-fixation is assessed to be nearly 175 million tons, of which almost 79 % is considered by terrestrial N-fixation (Ali et al. 1998). Soil bacteria drive the majority of plant growth-promoting activities. For example, biological N-fixation performed by *Rhizobium*, *Azotobacter*, *Azospirillum*, *Acetobacter*, *Klebsiella*, etc.; P-solubilization is performed explicitly by *Bacillus megaterium* and *Pseudomonas striata*; while K and Zn solubilization is mostly by *Bacillus* species. Some of the soil microbes, such as *Azotobacter*, *Pseudomonas, are* capable of producing growth hormones, while some of them are involved in antibiotic production like *Pseudomonas* and *Bacillus*, etc.

10.6.2 Mycorrhizal Biofertilizers

Mycorrhiza is a symbiotic relationship between the fungus and green plants. The fungus colonizes with the host plant roots. It may be either intracellularly (arbuscular mycorrhizal fungi) or extracellular (ectomycorrhizal fungi). The fungus is dependent for food on plant, basically for glucose and fructose, which are translocated from leaves to roots and then on to fungus (Maillet et al. 2011). In return, the plant gets the benefit of fungal mycelium's high absorptive capacity for nutrients (especially phosphorus) and water uptake due to the large surface area of hyphae. Fungal hyphae cover the plant roots, hence protecting desiccation. Most of the higher plants form mycorrhizal associations, except the members of Brassicaceae and Chenopodiaceae. The most common type association is arbuscular mycorrhiza, which is found in 70% plant species. Mycorrhizal associations are divided into two broad categories. First is ectomycorrhiza, in which fungal hyphae do not penetrate the cell wall. Second is endomycorrhiza, in which fungal hyphae penetrate the cell wall, and invaginates the cell membrane. Endomycorrhiza is further sub-divided into arbuscular, ericoid, and orchid mycorrhiza. Another category called arbutoid mycorrhiza is also known as ecto-endo mycorrhiza.

10.6.3 Mechanism and Mode of Actions of Bacterial and Mycorrhizal Biofertilizers

The biofertilizer mode of action depends on the groups or types of microbes they contain. Plant growth-promoting rhizobacteria (PGPR) improves plant growth and resistance to abiotic stresses through several mechanisms. These mechanisms of action are: (1) the synthesis of plant nutrients or phytohormones as growth promoter, (2) mobilization of soil nutrients to plant, (3) plant protection under stress conditions, and countering its injurious effects, (4) defense against plant pathogens, combating the plant diseases or death (Paula et al. 2015). The mechanisms of PGPR are shown in Fig. 10.5.

Among the PGPR species, *Azospirillum* is capable of secreting gibberellins and auxins. Some plant-associated bacteria help in phytohormone synthesis. For example, lodgepole pine, when inoculated with *Paenibacillus polymyxa*, increases Indole-3-Acetic Acid (IAA) in plant roots. *Rhizobium* and *Bacillus* were cited for synthesizing IAA in different cultures, pH, and temperature in agro-waste material as a carrier.

Nitrogen-fixing microbes, especially Rhizobium and *Azospirillum* species and blue-green algae fix the atmospheric N and convert it to ammonium (NH^{4+}) or nitrate (NO^{3-}) ions in the soil and root nodules of legume crops, in that way making it available and usable to crop plants (Choudhury and Kennedy 2004).

Phosphorus exists in the soil typically as insoluble phosphate form, which cannot be readily absorbed by crops (Gupta 2004). Phosphate solubilizers in biofertilizers,

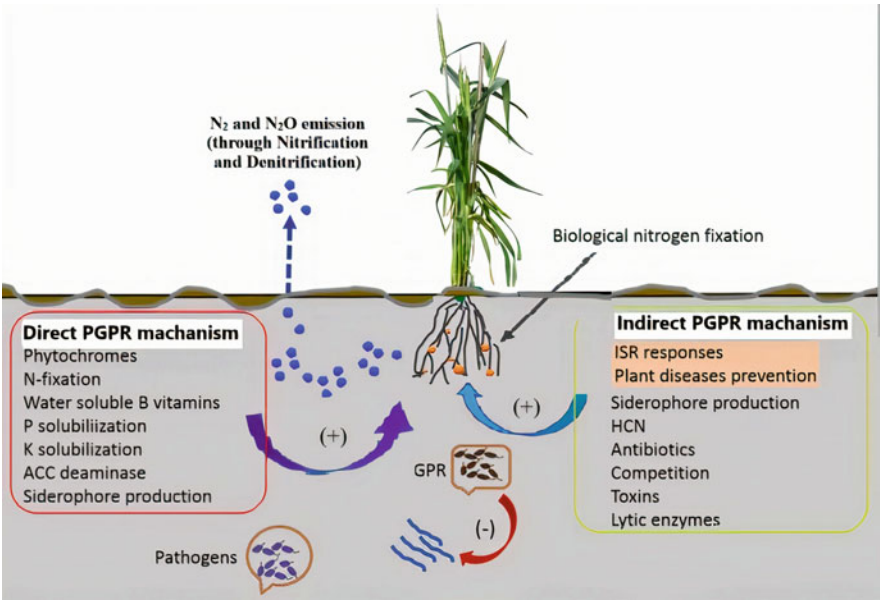

Fig. 10.5 Mechanisms of plant growth-promoting rhizobacteria

especially *Bacillus*, *Pseudomonas*, and *Aspergillus*, solubilize insoluble phosphate to orthophosphate. This solubilized phosphate can then be easily used by crop plants (Chang and Yang 2009). Potassium solubilizing bacteria (KSB) solubilize K-bearing insoluble silicate minerals by producing organic acids. KSB also help in metal ions removal, thereby making them accessible to crops (Itelima et al. 2018). Zinc and sulfur solubilizers (*Thiobacillus*) and manganese solubilizer (*Penicillium citrinum*) are also recognized for commercial operations (Borkar 2015). PGPR and *Pseudomonas* in biofertilizers produce hormones and anti-metabolites, which promotes root growth, decomposing the organic matter. They also help in the soil mineralization process, in that way increasing the availability of plant nutrients and increasing crop yield.

Abiotic stress in plants is the primary cause of crop loss worldwide (Wang et al. 2003). Pseudomonas strains in biofertilizers were found to enhance asparagus seedling growth and seed germination under water-stress conditions (Liddycoat et al. 2009). *Pseudomonas fluorescens* MSP-393 strains act as a PGPR for many crops grown in the saline soils of coastal ecosystems (Paul and Nair 2008). *Pseudomonas putida* Rs-198 supports cotton seedling growth under salt stress. It also increases germination and protects against salt stress through growing absorption for Mg^{2+}, K^{+}, Ca^{2+}, reducing Na^{+} uptake, and production of endogenous indole acetic acid (Yao et al. 2010). Strains of *Paenibacillus alcaligenes*, *Bacillus polymyxa*, and *Mycobacterium phlei* produce calcisol and improved maize growth and nutrient uptake under high-temperature conditions as well as under salinity (El-Akhal et al. 2013).

Some PGPR synthesizes antibiotic substances to inhibit the growth of plant pathogens. For example, *Pseudomonas* sp. produces antibiotics to inhibit *Gaeumannomyces graminis* var. tritici, a necrotrophic soilborne pathogen, the causal organism of take-all of wheat (Mazzola et al. 1995). *Bacillus cereus* UW85 was identified as a biocontrol agent of alfalfa damping-off caused by *Pythium* spp (Silo-Suh et al. 1994). Kumar et al. (2010) found that *Sinorhizobium fredii* KCC5 and *Pseudomonas fluorescens* LPK2 produce chitinase and β-glucanases that inhibit fungal growth and control the fusarium wilt caused by *Fusarium udum*. The siderophores of *Pseudomonas* have been found to control the *Fusarium oxysporum*, the causal organism of wilt disease in potato (Schippers et al. 1987). *Pseudomonas* and *Bacillus* species produce siderophores that inhibit fungal pathogens in maize crops (Pal et al. 2001).

PGPRs are accepted worldwide as bio-agents for agricultural sustainability, and alternatives to chemical agents. Apart from acting as a plant growth promoter, they are providing tolerance against pathogens by producing various metabolites (Backman and Sikora 2008). *Bacillus subtilis* GBO can induce defense-related pathways. *Bacillus subtillis* N11, along with digested compost and farmyard manure was found to control *Fusarium* infestation in the banana root. Generally, they are exploited in dealing with spotted wilt viruses in tomato, pepper, cucumber mosaic, and banana bunchy top disease. In some circumstances, it is witnessed that mycorrhizae and bacterial species can also provide resistance against fungus and inhibit the progress of root pathogens such as *Pythium* and *Rhizoctonia solani* spp. (Itelima et al. 2018).

10.7 Producers of Biofertilizers

In India, Rhizobium based biofertilizer production was started in 1934 by M. R. Madhok (Yadav and Raychaudhary 2004). However, the commercial Rhizobium culture production was started in 1956 at the IARI, New Delhi and ACRI, Coimbatore in the mid-sixties, Tamil, Nadu. Nitragin inoculant shipped from the USA in 1964, along with soybean. It was a significant event in the biofertilizer history of India. That fortified Rhizobium success and Nitragin inoculant replaced locally produced rhizobium inoculants for soybean. A letter on this was extended to other pulses and legume oilseeds.

In the years 1965–1990, around 30 biofertilizer production and research units were set up to meet the country's demand. The 1990s saw a histrionic growth in the industry by the addition of new biofertilizers such as *Azotobacter*, *Azospirillum*, PSBs (Essays 2018). The current positive trend has significantly changed the biofertilizer industry in India. Currently, Agro Industries Corporation (AIC) is the largest producer of biofertilizers, followed by the State Agriculture Department. National Biofertilizer Development Centre, State Agriculture Universities also contribute significantly (Pindi and Satyanarayana 2012). Tamil Nadu, Madhya Pradesh, Utter Pradesh, Gujarat, and Maharashtra are the leading states in biofertilizer's production capacity.

Globally, Novozymes (Denmark), Madras Fertilizers Ltd. (India), Gujarat State Fertilizers and Chemicals Ltd. (India), National Fertilizers Ltd. (India), Rizobacter Argentina S.A. (Argentina), T. Stanes and Company Ltd. (India), Camson Bio Technologies Ltd.(India), Rashtriya Chemicals and Fertilizers Ltd. (India), Lallemand Inc. (Canada), Nutramax Laboratories, Inc. (the USA), Biomax (Singapore), Symborg (Spain), Ajay Bio-Tech (India) Ltd. (India) and Agri Life (India), and CBF China Bio-Fertilizer AG (Germany) are critical players in control of global biofertilizers marketplace.

10.8 Future Prospective

Uncontrolled chemical fertilizer use has led to excess nutrient buildup in soils, which sometimes causes soil sickness. As we know, the availability of plant nutrients depends primarily on soil-based microbes. Several microbes are involved in N-fixing, P, and K solubilization and mobilization. Apart from these, many of them play a vital role in the cycling of Ca, Fe, Mn, Zn, S, and Mo. The use of biofertilizers leads to enhanced soil biological processes such as nutrient cycling and minerals transformation and soil fertility. Its application also stimulates the plant growth, and provides resistance to plants under abiotic and biotic stresses. Biofertilizers are eco-friendly and profitable technology for the farming communities. Hence, future crop production strategy should be a combined use of chemical, organic, and biofertilizer, rather than sole chemical fertilizers. Progress of the biofertilizer industry cuts the demand for chemical fertilizer and helps in moving towards eco-friendly agriculture and farmer's prosperity and food safety.

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A Prelude of Plant Strategies to Deal with the Peril of Salinity: An Archive of Regulatory Responses

11

Suruchi Singh, Bhavna Jaiswal, S. B. Agrawal, and Madhoolika Agrawal

Abstract

Salinity is a global concern and profoundly impacts many aspects of plant processes. Sodium ions in the soils have many negative implications on plants thus adversely affecting enzymes, photosynthesis, potassium nutrition, and metabolism. Microbes help the plants in withstanding salinity stress through enhancing many adoptive traits. Salinity affects plants at many levels which complicate the whole nexus but the regulations are highly coordinated and finely tuned especially in tolerant species. The different proteins involved in regulatory processes belong to diverse functional groups and as a part of regulations, the metabolic pathways are reprogrammed. This updated compilation will give an overview of salt tolerance mechanisms, thus paving way for raising salt tolerant crops, through targeted breeding strategies.

11.1 Introduction

The unpredictable climate variability resulted under climate extremes such as scanty rainfalls, reduction in freshwater availability, saltwater intrusion, storms, etc. may lead to the loss of productivity (Table 11.1).

Many direct and indirect effects cause soil salinization. Soil salinization develops by a variety of sources. Primary sources are salt water intrusion, evapotranspiration, while secondary sources are mainly anthropogenic such as improper land use, irrigation with saline water, etc. (Fig. 11.1). Soil salinity is directly related to the rainfall, evapotranspiration, and ground water depth (Liu et al. 2019). Higher evapotranspiration leads to increase in soil salinity. Saturated water content in

S. Singh · B. Jaiswal · S. B. Agrawal · M. Agrawal (✉)
Laboratory of Air Pollution and Global Climate Change, Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, India

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Table 11.1 Yield losses under moderate to higher salinity

Plant	Yield loss (%)	References
Mung bean	20–68	Ahmed et al. (2010)
Wheat	8–12	Shafi et al. (2010)
Rice	50–13	Gan et al. (2010)
Wheat	46–90	Rasouli et al. (2013)
Pea nut	7–53	Abd El-RheemKh and Safi-naz (2015)
Wheat	25–66	Kalhoro et al. (2016)
Corn Wheat Cotton	55 28 15	Satir and Berberoglu (2016)
Cotton	55	Satir and Berberoglu (2016)
Miscanthus x giganteus	50	Stavridou et al. (2017)

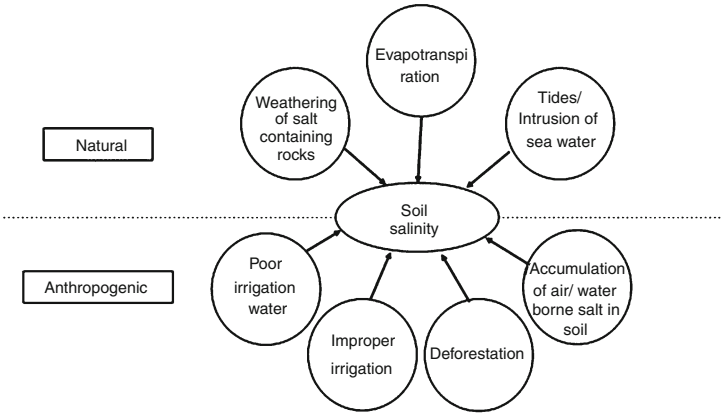

Fig. 11.1 Multiple sources of salinity in soil

riparian wetlands causes increase in salinity (Liu et al. 2019). Salt water intrusion increases the soil salinity as well as it disturbs the soil biogeochemical cycle (Servais et al. 2019).

Salinity may impact plants in many diverse ways. High levels of salinity causing ions in soil solution disturb the water potential at the interface of root cells and surrounding soil water, which manifested in the form of water loss. Higher concentrations of salts also facilitate passive movement of salt ions through plasma membrane and thus lead to deposition of salt ions in cell cytoplasm thus inhibits enzymatic activities. Stress tolerance is attributed to the ability of plants in actively reprogramming their entire metabolic pathways, right through growth/ development.

Although interspecies differences prevail against salinity tolerance and in general tolerant and sensitive plants show differences in genomic, transcriptomic, and proteomic responses:

1. Genomic level: Specific stress responsive genes are expressed in tolerant species which may lack in sensitive plants.
2. Transcriptomic level: Changes in regulation of gene expression of genes regulating key traits responsible for stress tolerance.
3. Proteomic level: altered activities in proteins involved in stress response in tolerant plants.

Keeping in view the severity of the problem, the factors contributing in enhancing salinity formation and spread and how the sensitive and tolerant plants differ in their ability to regulate the genomic, transcriptomic, and proteomic responses are discussed in this chapter.

11.2 Role of Microbes in Alleviating the Adversity of Salinity Stress

Microbes particularly plant growth promoting rhizobacteria (PGPR) help the plant to withstand under salt stressed condition. The help is provided by many ways to the plants from microbes in terms of enhanced nutrient uptake, increased enzyme activity, maintaining water balance, regulating ion channels, enhancing photosynthetic pigments, osmolytes and antioxidants, etc. (Radhakrishnan and Baek 2017). There is mutual interaction among microbes and plant growth (Radhakrishnan and Baek 2017) and they have the capability to modify the response of the plant against a particular stress. Many bacterial species such as *Pseudomonas fluorescens*, *P. migulae*, *Azospirillum*, *P. syringae*, *P. putida*, *Enterobacter aerogenes*, *P. mendocina*, *Rhizobium*, *Streptomyces* sp., etc. are reported by many researchers to alleviate the salt stress by various mechanisms (Gupta et al. 2020).

Phosphate solubilizing microbes solubilize the unavailable P to available form that is easily taken up by plant (Bal et al. 2013). Egamberdieva et al. (2019) reported that similar to plants, microbes also in salt stress accumulate proline, glycinebetaine, polyamines, and other quaternary ammonium compounds to cope with osmotic stress. Many halo-tolerant microbes induce trehalose synthesis as an osmoprotectant against salt stress (Shim et al. 2019).

ACC deaminase activity of microbes has been reported by many workers to alleviate salinity stress (Gupta et al. 2020; Egamberdieva et al. 2019; Bal et al. 2013). The mechanism involves the downregulation of ethylene stress for plants as it degrades ACC which is the precursor for ethylene. It also promotes the growth and thus productivity of the plants as well as help in membrane stability, synthesis of osmolytes, pigments, etc.

Tiwari et al. (2018) studied ACC deaminase activity from 37 bacteria on the growth and bioethanol production from Panicum maximum. They found that application of ACC deaminase producing bacteria under salt and drought stress improved plant growth, water retention capacity, prevent membrane damage, photosynthetic pigment degradation, enhanced the synthesis of proline, sugars, soluble protein and phenol. Cell wall composition (cellulose and lignin) of the plant was also found to be

improved, even better than control under ACC deaminase activity. Overall, better growth, biomass, and enhanced bioethanol production was obtained.

Exopolysaccharide synthesis is also reported as a protective barrier against salt stress for roots. Under unfavorable condition, microbes secret huge amount of exopolysaccharides. Secretion reduces the uptake of Na^+ ions by roots and thus reducing the ionic toxicity to plant cells (Ashraf et al. 2004). Antioxidant enzymes produced by microbes help the plant to grow under salt stress (Egamberdieva et al. 2019; Radhakrishnan and Baek 2017). Sen and Chandrashekhar (2015) studied the response of two rice cultivar under salinity stress with the use of *Pseudomonas* strains. Catalase, peroxidase, and nitrate reductase activities were observed to be enhanced under the treatment of *Pseudomonas*, which helped the rice plant to cope up with stress.

11.3 Factors Contributing in Salinity

11.3.1 Irrigation

Irrigation with poor quality water is the primary source of soil salinization. Surface water utilized for irrigation contributes in some amount of salts which get deposited on soil over time and cause the degradation in its quality. Although the amounts of salts get leached, but the process is quite slower.

Method of irrigation also affects the soil salinity (Isidoro and Grattan 2011). Irrigation is mainly done in three ways—surface, sprinkler, and drip or trickle irrigation. These irrigation systems have many advantages and disadvantages and drainage is necessary to remove extra water so that accumulation of salt does not occur. In India, border, basin, sprinkler, and drip irrigations are usually practiced. Sprinkler method is one of the best methods of irrigations that cause least salinization as water application rate is lower than infiltration rate thus leaching of salts occurs properly. In drip irrigation method, water is directly provided to the vicinity of roots so the rate of evaporation is lower and excess salt accumulation does not occur. Lowest salt accumulation occurs at the vicinity of roots (Shahid 2013). Furrow irrigation is one of the most suitable way to protect plants from accumulated salts as the salt will move as a result of evaporation to the ridges.

Many countries nowadays are utilizing saline water for irrigation so as to maintain the fresh water storage (Yuan et al. 2018). This leads to salt accumulation in soil on surface and even in deep layers gradually year after year. Although the salt in the root zone gets reduced with the growth of plants (Yuan et al. 2018). Farmyard manure when applied under saline water irrigation condition, the growth of plant is maintained whereas when poultry manure is applied, growth is reduced (Ahmed et al. 2010). Isidoro and Grattan (2011) through a model reported that sprinkler irrigation helps plants to avoid stress by providing enough water. The same is with surface irrigation but in fine textured soil, surface irrigation causes the soil salinity to get lower than sprinkler irrigation (Isidoro and Grattan 2011).

Poor drainage also results in high salinity in soil. Shallow water table is source of salt accumulation in root zone as the water is taken by capillary action and evaporation also takes place, thus causing salt deposition. Water diffusivity decreases as the soil salinity increases (Gan et al. 2010).

11.3.2 Fertilizer

Fertilizer and manure applications can also contribute in soil salinization. Xiang et al. (2008) reported that animal manure of chicken and pigeon enhanced the total soluble salts in soil. Concentration of K, Na, SO^{4-}, Mg, and Cl^{-} were found higher. Many indirect consequences result due to excess use of fertilizers and pesticides due to surface run off, leaching, etc.; many hazardous chemicals reach into ground water and rivers thus causing serious health issues to the living organisms. Fertilizers such as *N* and *P* also cause eutrophication. It is also reported to deteriorate the soil structure (Savci 2012). Soil pH is affected by the use of fertilizers. In due course of time, acidity or alkalinity develops in soil which disturbs the ionic balance so the plant uptake of nutrients gets interrupted (Savci 2012).

11.3.3 Texture

Texture being a very important physical property of soil decides various other characters of soil. These characters directly regulate soil permeability, water infiltration, water holding capacity, and exchange of ions. Leaching is a widely used method for salinity reclamation and is largely dependent on soil texture. Feigin et al. (1991) reported that high sodium concentration in saline water cause reduction in soil porosity by soil particle contraction, breaking of soil colloids, and swelling. Yuan et al. (2018) also observed decreases in soil porosity and saturated hydraulic conductivity with increasing salinity lead to increase in bulk successively. Fine textured soil usually has higher salinity than course textured soil that has low available water (Isidoro and Grattan 2011). Fine textured soil has high osmotic potential as it can hold higher amount of water than the fine texture soil and thus affects microbes (Setia et al. 2011). High Na ions cause swelling of clay particles when get wet, thus destroying the structure of soil by swelling, slaking, and dispersion of clay resulting in blockage of water movement (Dongli et al. 2015).

Soil texture is very important determinant for soil hydraulic property (Dongli et al. 2015). Evapotranspiration leads to salt accumulation which is also dependent upon soil texture and upward pull of water. Soil layering is equally taking part in this process and can be used as soil reclamation method (Li et al. 2013a, b).

11.3.4 Cropping Pattern

Devkota et al. (2015) suggested permanent skipped furrow irrigation to reduce the salt deposition on the surface of the seed bed. Raised bed cropping benefits are based

on the soil texture and the climatic conditions like rain and wind (Bakker et al. 2010). In coarse textured soil, raised bed planting technique causes alterations in salt dynamics. Sand mulching affects the movement of salts in soil and influences the productivity positively (Bakker et al. 2010). Monoculturing of rice reduced the soil salinity effectively compared to different land shaping (Mandal et al. 2019). Mandal et al. (2019) practiced three farming systems, farm pond , deep furrow and high ridge system, and paddy cum fish system and all the methods were effective in reducing the soil salinity. Alternate furrow irrigation and double row planting on raised bed can be used to avoid the exposure of salt (Fipps 2003).

Salinity induces osmotic and ionic stresses but the regulatory pathways are specific under both the stresses. Subsequently the pathways, regulations, and manifestations are discussed which impart selective tolerance against salinity.

11.4 Salinity Tolerance and SOS Pathway

Complex genetic network regulated by multiple genes contributes in inducing salt tolerance. Based on concentration of salt ions, the implications of salinity may be via both osmotic and ionic stresses. The effect of salt by lowering the soil water potential causes osmotic stress thus making water uptake by roots a difficulty. Accumulation of salts in due course of time in plant tissues causes ionic stress (Munns and Tester 2008). The survival chances under saline conditions depend on plant's ability to detect changes in ion levels and show appropriate responses. To further check the death of cell due to salt stress, accumulation of toxic sodium (Na^+) is restricted and cellular ion homeostasis is maintained (Tester and Davenport 2003). Ion homeostasis is under the control of well-orchestrated salt overly sensitive (SOS) signaling pathway. SOS pathway maintains ion homeostasis by ion exclusion at the cellular level (Zou et al. 2008). Various independently operative Na^+-permeable transporters maintain Na^+-uptake by cells (Tester and Davenport 2003). Three proteins constituting SOS signaling pathway are SOS1, SOS2, and SOS3 that work to attain ion homeostasis and thus impart plant's tolerance against high salt (Hasegawa et al. 2000). Upon salt stress perception, a spike in Ca^{2+} concentration was recorded in cytoplasm which further activates the SOS signal transduction events which is a signal to protect the cells from ionic stress (Chinnusamy et al. 2005).

SOS3 encodes a myristoylated protein which binds with calcium and perceives the increase in cytosolic Ca^{2+} which is induced by large amount of Na^+ that has entered the cytosol. On Ca^{2+} binding, SOS3 interacts and activates SOS2, which is a serine/threonine protein kinase of SnRk3 family (Hrabak et al. 2003). SOS3-like calcium binding protein 8 (SCaBP8, commonly known as calcineurin B-like CBL) is one of the regulators of SOS2 which functions mainly in Arabidopsis shoot while in roots, SOS3 is functional (Quan et al. 2007). SOS2 phosphorylates SCaBPB which stabilizes the protein complex (Lin et al. 2009). Phosphorylation of SOS3-like proteins by the interacting protein kinases is the most common regulatory mechanism of CBL/SCaBP-CIPK/PK5 modules (Du et al. 2011). Either SOS3-SOS2 or SCaBPB-SOS2 interactions through SOS2 initiates activation of the downstream

target of SOS1, a Na^+/H^+ antiporter, which causes exclusion of excessive Na^+ from cytosol (Quintero et al. 2011). Thus, with the overexpression of SOS1, tolerance against salinity in plants can be achieved (Yang et al. 2009a, b). Chinnusamy et al. (2004) reported that SOS2 and some other related Ca^{2+}-activated protein kinases initiate a cascade of downstream phosphorylation mediated through mitogen-activated protein (MAPK) kinases. Teige et al. (2004) identified role of MAP kinase kinase 2 (MKK2) and two MAP kinase (MPK4 and 6) under salt stress.

Another strategy for maintaining the balance of ion concentration in cytoplasm involves controlling the cellular levels of Na^+/K^+. Mechanisms involved in the transport of both the ions are same therefore they compete with each other for the entry into the cytoplasma. Under elevated salinity levels, Na^+ amount increased which leads to decrease in uptake of K^+ (Munns and Tester 2008). This change enhances the expression of transporters such as Histidine kinase transporters (HKT) and NHX (Rajagopal et al. 2007), which facilitate uptake of K^+. Although under low to no saline condition, Na^+ uptake is through HKT transporters family. High throughput techniques like biophysical transport and phylogenetic analysis identified two subgroups of HKT transporters, based on the preferences for Na^+ uniport (class I) or Na^+-K^+ symport (class 2) (Yao et al. 2010). When Na^+ concentration increases in the vicinity of roots, Na^+ enters inside the plant through broad spectrum cation channels present on the plasma membrane or Na^+ may also enter passively through the cracks and wounds into the endodermis of roots (Munns and Tester 2008). Remarkable increase in cytoplasmic Na^+ disturbs the enzymatic functions and is dangerous initially to individual cells and then to the whole plant. Plants are inbuilt with three mitigation machineries against high cytosolic Na^+ which includes: (1) minimizing uptake of Na^+ by the cell; (2) maximizing the compartmentalization of Na^+ into the vacuole; and (3) increasing the efflux of Na^+.

11.5 Regulation of Gene Expression Under Salinity Stress

The response to salt stress comprises activation of a multiple genes along with suppression in large number of housekeeping genes (Sahi et al. 2003). Remarkable variations were observed at transcriptional and post-translational levels to acclimatize plants against salt stress.

Many transcription factors (TFs) such as bZIP, WRKY, AP2, NAC, C2H2 zinc fingers gene, MYB and DREB alter and regulate the gene expression under suboptimal conditions. Differences in transcriptional regulation under different environmental conditions contribute in variations in developmental and functional outcomes.

11.5.1 Basic-Leucine Zipper (bZIP)

The bZIP family is one of the largest TF family in higher plants. It comprises proteins having DNA-binding domain which reside next to a leucine zipper dimerization domain. bZIP proteins are capable of attaching to the DNA sites through

formed homodimers and heterodimer via the coiled-coil region (Hai and Curran 1991). On the basis of region specificity for DNA binding and the similarities between the sequences among bZIP domains, these bZIP TFs are further divided into 11 groups, i.e., I–XI (Nijhawan et al. 2008) or 13 groups, i.e., A,B,C,D,E,F,G,H, I,J,K,L, and S (Corrèa et al. 2008). Group A bZIP TFs in Arabidopsis is ABI5 and it binds with the homologous ABA-responsive elements (ABRE). Thus the Group A members are also known as the ABRE binding factors (ABFs/AREBs) which play key role in plant responses to dehydration and salt stress (Uno et al. 2000; Kang et al. 2002). Another Group A members from rice, such as TRAB1 (Hobo et al. 1999), OsbZIP23 (Xiang et al. 2008), OsbZIP46 (Tang et al. 2012), OsbZIP12/OsABF1 (Hussain et al. 2011), and OsAB15 (Zou et al. 2008) also play pivotal roles in ABA signaling transduction and osmotic stress responses. Group S is again divided into three subgroups S1, S2, and S3, based on the homology of bZIP domains (Ehlert et al. 2006).

The cue for salinity is perceived at plasma membrane (Christmann et al. 2013) which is followed by a hike in NADH oxidases (Zhang et al. 2008) which causes an increase in cytosolic levels of Ca^{2+} (Laohavisit et al. 2013) and abscisic acid (ABA) synthesis (Huang et al. 2012). bZIP interacts with G-ABRE known as ABFs (ABF1-ABF4) or AREBs (AREB1-AREB3) (Choi et al. 2000; Uno et al. 2000), which again play dual roles of binding and regulating ABA/stress response (Furihata et al. 2006). ANF3 or ABF4 overexpression in Arabidopsis confers ABA hypersensitivity and drought tolerance (Kim et al. 2004), while their knockout mutants display partial insensitivity to ABA and susceptibility to drought (Finkelstein et al. 2002). Cheng and Long (2007) confirmed that ABFs/AREBs can be modulated by several kinases.

Stress signaling pathways mediated by ABA (Fujii et al. 2009) are also sensed by the PYR/PYL/RCAR (pyrabactin resistance 1/PYR1-like/regulatory component of ABA receptor1) co-receptors (Park et al. 2009). ABA bounded to its receptors activates members of the redundant PP2C (protein phosphatase 2C) family, thereby checks their inhibitory action over kinases like the one belonging to SnRK2 (sucrose-nonfermenting1-related protein kinase 2) family (Fujita et al. 2009). Furihata et al. (2006) reported phosphorylation of ABA-responsive element binding 1 (AREB1) by activated SnR2. AREB1, AREB2, and AREB3 are related bZIPs which regulate ABA-dependent transcription via binding to promoter cis-element of ABA-responsive gene (Yoshida et al. 2010).

Under stressed condition when the energy level is generally low, the C/S1 network of bZIP TFs regulates metabolic reprogramming (Hanson et al. 2008). Under starved condition, bZIP1 targets amino acid metabolism related genes, namely asparagine synthetase 1 (ASN-1) and proline dehydrogenase 1 (Dietrich et al. 2010). Sugars, in addition to their function as energy supply, are also important signaling molecules. Interestingly, bZIP1 transcription in seedling is represented by glucose and depends on HXK1 (Dietrich et al. 2010). bZIP also interacts with genes that encode the transposable elements with many possible implications (Fig. 11.2) and the details about the interacting factors are given in Table 11.2. The nexus of bZIP and other processes is given as ancestral chart in Fig. 11.3.

Fig. 11.2 The coexpression network of bZIP

11.5.2 WRKY

The WRKY family of TFs is marked by conserved amino acid sequence of WRKYGQK presence and can be classified into three groups (Rushton et al. 2010). Crucial regulatory roles are played by WRKY factors in modulating defense responses (Pandey and Somssich 2009). WRKY proteins act specifically on the W-box elements containing a TGAC core sequence present in downstream target-gene promoters. Hu et al. (2013a) reported predominance of WRKY8 gene expression in roots under NaCl treatment. They observed that in nucleus, WRKY8 interacted with VQ9 which is a protein having VQ motif. Hu et al. (2013b), however suggested that VQ reduced the DNA-binding affinity of WRKY8, thus negatively regulated responses against salinity stress.

The functional disparity between WRKY8 and VQ9 is important for maintaining a balance of signaling pathways mediated by WRKY8 so that salt stress tolerance can be established. Function of Arabidopsis WRKY33 transcription factor is to target the salt-responsive downstream genes which detoxify ROS and help in regulation of LOX1 (lipoxygenase), GSTU11 (glutathione-S-transferase), and peroxidases (Jiang and Deyholos 2009). Zheng et al. (2013) elaborated protective responses of cells against salinity through ThWRKY4 by bringing ROS below toxic levels. ZmWRKY33 mediated tolerance against salinity stress in Arabidopsis thaliana was demonstrated by Li et al. (2013a, b). Cai et al. (2014) found that WRKY58 which is a group II transcription factor increased the level of salt tolerance. Two other WRKY factors, WRKY25 and WRKY33 increase salinity induced stress tolerance in Arabidopsis thaliana (Jiang and Deyholos 2009). Stomatal closure was affected which indicated the contribution of WRKY factors in ABA-dependent salinity and drought adaptation pathways (Ren et al. 2010).

Table 11.2 Different interacting factors of bZIP coexpression network

Locus	Short description
At2g12900	Basic-leucine zipper (bZIP)transcription factor family protein • Sequence specific DNA-binding transcription factor activity
At5g09360	Putative laccase; a member of laccase family of genes
At5g58850	Myb domain protein 19. Encodes a putative transcription factor, member of the R2R3 factor gene family (MYB119) • Sequence specific DNA-binding transcription factor activity
At3g43320 At2g06230 At2g15810 At3g31430 At2g15520 At2g06820 At2g14640	Transposable element
At4g38590	Beta-galactosidase 14 • Beta-galactosidase activity
At5g11080	Ubiquitin-like superfamily protein
At3g42140	Zinc ion binding; nucleic acid binding
At4g07720 At3g27590 At2g11010 At2g30380	Unknown protein
At5g6620	Isomerase • Chalcone-flavanone isomerase family protein
At5g20560	Glycosyl hydrolase superfamily protein
At5g15380	Domain rearranged methylase 1 (DRM1) • Encodes methyltransferase involved in the de novo DNA methylation and maintenance of asymmetric methylation of DNA sequences

11.5.3 NAC 2

NAC abbreviated for NAM-ATAF1/2-CUC2 is one of the largest TF families (Olsen et al. 2005). NAC TFs regulate many developmental processes and control processes like floral development (Sablowski and Meverowitz 1998), apical meristem formation (Hibara et al. 2003), stress signaling (Balazadeh et al. 2010), and germination (Kim et al. 2008) under salinity stress (Table 11.3).

ANAC092/AtNAC2/ORE1 was initially reported to regulate lateral root development under salinity by acting downstream of two signaling pathways, i.e., ethylene and auxin. Balazadeh et al. (2010) analyzed the microarray data and observed elevated levels of ANAC092 transcript in roots under salt stress and shoot of Arabidopsis. Salt stress induce the expression of NAC genes such as ANAC092 and AtNAP as revealed by microarray data which suggests NAC role in promoting senescence under salt stress. miRNA164 also controls ANAC092 under natural and salinity induced senescence (Kim et al. 2008; Balazadeh et al. 2010).

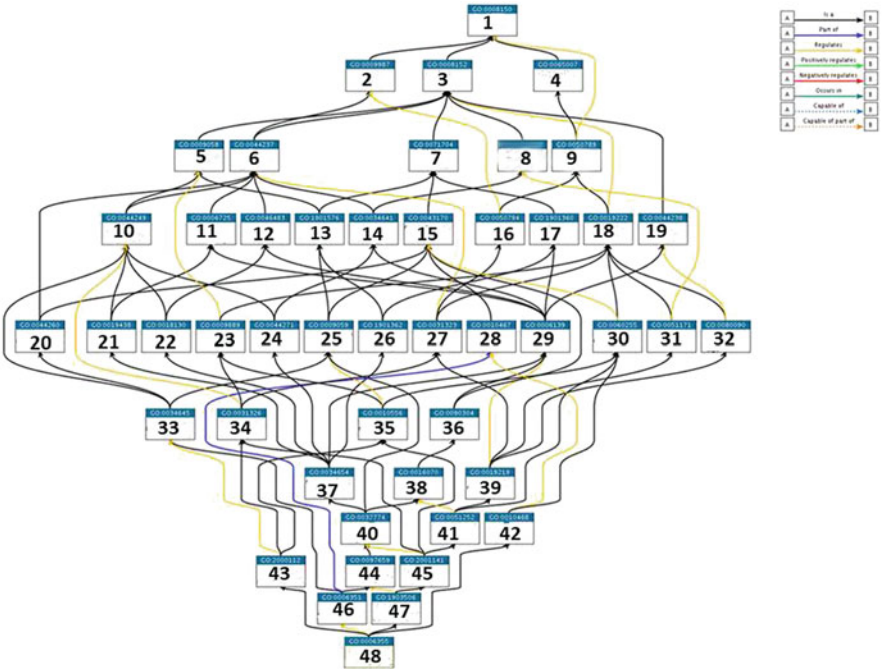

Fig. 11.3 The ancestral chart of bZIP depicting the complex nexus. The number denotes 1. biological process, 2. cellular process, 3. metabolic process, 4. biological regulation, 5. biosynthetic process, 6. cellular metabolic process, 7. organic substance metabolic, 8. nitrogen compound metabolic, 9. regulation of biological process, 10. cellular biosynthetic process, 11. cellular aromatic compound, 12. heterocyclic metabolic process, 13. organic substance biosynthetic, 14. cellular nitrogen compound, 15. macromolecule metabolic process, 16. regulation of cellular process, 17. organic cyclic compound metabolic, 18. regulation of metabolic process, 19. primary metabolic process, 20. cellular macromolecule metabolic, 21. aromatic compound biosynthetic, 22. heterocycle biosynthetic process, 23. regulation of biosynthetic process, 24. cellular nitrogen compound, 25. macromolecule metabolic process, 26. organic cyclic compound biosynthetic, 27. regulation of cellular metabolic, 28. gene expression, 29. nucleobase containing compound, 30. regulation of macromolecule metabolic, 31. regulation of nitrogen compounds, 32. regulation of primary metabolic, 33. cellular macromolecule biosynthetic, 34. regulation of cellular biosynthetic, 35. regulation of macromolecule biosynthetic, 36. nucleic acid metabolic process, 37. nucleobase containing compounds, 38. RNA metabolic process, 39. regulation of nucleobase containing, 40. RNA biosynthetic process, 41. regulation of RNA metabolic process, 42. regulation of gene expression, 43. regulation of cellular macromolecule, 44. nucleic acid templated transcription, 45. regulation of RNA biosynthetic, 46. transcription-DNA template, 47. regulation of nucleic acid-templated, 48. regulation of transcription-DNA templated

11.5.4 MYB

MYB TFs at N-terminus has highly conserved domain in entire eukaryotes, whereas the trans-acting C-terminus is highly variable and regulates multiple functions (Chen et al. 2017). MYB proteins consist of highly variable repeats of 52 amino acid residues in three MYB domains (Stracke et al. 2001). MYB family is further classified into MYB1-R, 4R-MYB, R2R3-MYB, and R1R2R3-MYB, which is

Table 11.3 Interacting proteins of NAC2

S. No	Locus	Short description	Function
1	At3g10800	BZIP28	Basic-leucine zipper (bZIP) transcription factor family protein
2	At3g57880	Transferase	Calcium-dependent lipid-binding (CaLB domain) plant phosphoribosyltransferase family protein
3	At5g03340	CDC48C	ATPase, AAA-type, CDC48 protein
4	At5g62620	Transferase	Galactosyltransferase family protein
5	At3g53230	CDC48B	ATPase, AAA-type, CDC48 protein
6	At4g22740	Glycine-rich	Glycine-rich protein
7	At2g20560	DNAJ	DNAJ heat shock family protein
9	At1g31280	AGO2	Argonaute family protein
10	At1g61140	EDA16	SNF2 domain-containing protein/helicase domain-containing protein/zinc-finger protein-related
11	At5g22060	J2	DNAJ homologue 2
12	At3g12580	HSP70	Heat shock protein 70
13	At1g78420	RING/U-box	RING/U-box superfamily protein
14	At5g52640	HSP83	Heat shock protein 90.1
15	At3g07090	Peptidase	PPPDE putative thiol peptidase family protein
16	At3g44110	J3	DNAJ homologue 3
17	At2g46500	UBDK GAMMA 4	Phosphoinositide 4-kinase gamma 4
18	At2g33700	PP2CG1	Protein phosphatase 2C family protein
19	At1g32870	NAC13	NAC domain protein 13
20	At3g62260	Protein phosphatase 2C	Protein phosphatase 2C family protein
21	At1g55530	RING/U-box	RING/U-box superfamily protein
22	At2g41160	UBA	Ubiquitin-associated (UBA) protein
23	At3g10500	NTL4	NAC domain-containing protein 53
24	At1g75400	RING/U-box	RING/U-box superfamily protein
25	At4g30490	ATPase	AFG1-like ATPase family protein
26	At3g05670	RING/U-box	RING/U-box protein
27	At3g24500	MBF1C	Multiprotein bridging factor 1C
28	At2g25140	CLPB4	Casein lytic proteinase B4
29	At1g74310	HSP101	Heat shock protein 101
30	At5g64510	TIN1	
31	At1g07350	SR45a	RNA-binding (RRM/RBD/RNP motifs) family protein
32	At4g15420	UFD1	Ubiquitin fusion degradation UFD1 family protein
33	At3g09350	Fes1A	Fes1A
34	At3g08970	TMS1	DNAJ heat shock N-terminal domain-containing protein
35	At1g01720	ATAF1	NAC (no apical meristem) domain transcriptional regulator superfamily protein

(continued)

Table 11.3 (continued)

S. No	Locus	Short description	Function
37	At4g11740	SAY1	Ubiquitin-like superfamily protein
38	At4g02890	UBQ14	Ubiquitin family protein
39	At2g40950	BZIP17	Basic-leucine zipper (bZIP) transcription factor family protein
40	At2g26150	HSFA2	Heat shock transcription factor A2
41	At3g04240	SEC	Tetratricopeptide repeat (TPR)-like superfamily protein
43	At1g10170	NFXL1	NF-X-like 1
44	At1g62740	Hop2	Stress-inducible protein, putative
45	At3g09440	Heat shock 70	Heat shock protein 70 (Hsp 70) family protein
46	At1g07510	ftsh10	FTSH protease 10
47	At5g42050	DCD domain	DCD (development and cell death) domain protein
48	At1g07870	kinase	Protein kinase superfamily protein
49	At3g15430	RCC1	Regulator of chromosome condensation (RCC1) family protein
50	At3g14200	DnaJ-domain	Chaperone DnaJ-domain superfamily protein
51	At5g61510	Dehydrogenase	GroES-like zinc-binding alcohol dehydrogenase family protein
52	At1g09140	SR30	Serine-arginine protein 30
53	At4g27680	Hydrolase	P-loop containing nucleoside triphosphate hydrolases superfamily protein
54	At4g03430	STA1	pre-mRNA splicing factor-related
55	At2g40830	RHC1A	RING-H2 finger C1A
56	At2g24100	ASG1	
57	At2g44970	Alpha/beta-hydrolases	Alpha/beta-hydrolases superfamily protein
58	At4g12400	Hop3	Stress-inducible protein, putative
60	At3g15880	WSIP2	WUS-interacting protein 2
61	At4g31860	Protein phosphatase 2C	Protein phosphatase 2C family protein
62	At5g06960	TGA5	OCS-element binding factor 5
63	At4g11660	HSFB2B	Winged-helix DNA-binding transcription factor family protein
64	At5g24810	ABC1	ABC1 family protein
65	At5g05410	DREB2A	DRE-binding protein 2A
66	At5g53120	SPMS	Spermidine synthase 3
67	At5g02490	Hsp70-2	Heat shock protein 70 (Hsp 70) family protein
68	At1g29150	RPN6	Non-ATPase subunit 9
69	At1g49710	FUT12	Fucosyltransferase 12
70	At3g23920	TR-BAMY	Beta-amylase 1
71	At2g21940	SK1	Shikimate kinase 1
72	At1g20110	Zinc finger	RING/FYVE/PHD zinc-finger superfamily protein
73	At4g22820	Zinc finger	A20/AN1-like zinc-finger family protein

(continued)

Table 11.3 (continued)

S. No	Locus	Short description	Function
74	At1g76390	PUB43	ARM repeat superfamily protein
75	At5g01960	RING/U-box	RING/U-box superfamily protein
76	At4g16760	ACX1	Acyl-CoA oxidase 1
77	At5g45900	ATG7	ThiF family protein
78	At1g32230	RCD1	WWE protein-protein interaction domain protein family
79	At4g05020	NDB2	NAD(P)H dehydrogenase B2
80	At4g03320	Tic20-IV	Translocon at the inner envelope membrane of chloroplasts 20-IV
81	At4g28600	NPGR2	No pollen germination related 2
82	At4g28480	DNAJ	DNAJ heat shock family protein
83	At3g14075	Lipase	Mono-/diacylglycerol lipase, N-terminal; Lipase, class 3
84	At3g12050	Aha1	Aha1 domain-containing protein
85	At1g77450	NAC032	NAC domain-containing protein 32
86	At4g00550	DGD2	Digalactosyl diacylglycerol deficient 2
87	At4g30600	Signal recognition particle receptor	Signal recognition particle receptor alpha subunit family protein
88	At2g04350	LACS8	AMP-dependent synthetase and ligase family protein
89	At4g31670	UBP18	Ubiquitin-specific protease 18
90	At1g67360	REF	Rubber elongation factor protein (REF)
91	At5g52580	RabGAP/TBC	RabGAP/TBC domain-containing protein
92	At3g13330	PA200	Proteasome activating protein 200
93	At5g42220	Ubiquitin-like	Ubiquitin-like superfamily protein
94	At3g22370	HSR3	Alternative oxidase 1A
96	At5g51740	M48	Peptidase family M48 family protein
97	At2g22010	RKP	Related to KPC1
98	At1g22020	SHM6	Serine hydroxymethyltransferase 6
100	At2g33590	CRL1	NAD(P)-binding Rossmann-fold superfamily protein
101	At5g58070	TIL	Temperature-induced lipocalin
102	At2g43320	Transferase	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
103	At3g09840	CDC48A	cell division cycle 48
104	At1g14200	RING/U-box	RING/U-box superfamily protein
105	At1g72770	HAB1	Homology to ABI1
106	At3g49530	NTL6	NAC domain-containing protein 62
107	At3g14070	CCX3	Cation exchanger 9
108	At5g51440	Chaperonin	HSP20-like chaperones superfamily protein
109	At2g17220	Kin3	Protein kinase superfamily protein
110	At5g12030	HSP17.6A	Heat shock protein 17.6A

(continued)

Table 11.3 (continued)

S. No	Locus	Short description	Function
111	At5g11730	Transferase	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein
112	At5g59420	ORP3C	OSBP(oxysterol binding protein)-related protein 3C
113	At4g11800	Phosphoesterase	Calcineurin-like metallo-phosphoesterase superfamily protein
114	At5g20910	AIP2	RING/U-box superfamily protein
115	At3g12480	NF-YC11	Nuclear factor Y, subunit C11
116	At5g25450	Cytochrome bd ubiquinol oxidase	Cytochrome bd ubiquinol oxidase, 14kDa subunit
117	At4g15410	PUX5	Serine/threonine protein phosphatase 2A 55 kDa regulatory subunit B prime gamma
118	At3g23540	Alpha/beta-hydrolases	Alpha/beta-Hydrolases superfamily protein
119	At3g28210	SAP12	Zinc-finger (AN1-like) family protein
120	At1g10050	Hydrolase	Glycosyl hydrolase family 10 protein/ carbohydrate-binding domain-containing protein
121	At2g35510	SRO1	Similar to RCD one 1
122	At4g26080	ABI1	Protein phosphatase 2C family protein
123	At2g26920	EF1B	Ubiquitin-associated/translation elongation factor EF1B protein
124	At5g52530	Dentin sialophosphoprotein	Dentin sialophosphoprotein-related
125	At3g46230	Heat shock 17.4	Heat shock protein 17.4
126	At3g19240	Vid27	Vacuolar import/degradation, Vid27-related protein
127	At5g13200	GRAM domain	GRAM domain family protein
128	At1g27760	SAT32	Interferon-related developmental regulator family protein/IFRD protein family
129	At3g03440	ARM repeat	ARM repeat superfamily protein
130	At4g26750	EXT-like	Hydroxyproline-rich glycoprotein family protein
131	At3g08720	S6K2	Serine/threonine protein kinase 2
132	At5g04720	PHX21	ADR1-like 2
133	At3g55730	MYB109	Myb domain protein 109
134	At4g17530	RAB1C	RAB GTPase homolog 1C
135	At2g17760	Protease	Eukaryotic aspartyl protease family protein
136	At5g07920	DGK1	Diacylglycerol kinase1
137	At3g46930	Kinase	Protein kinase superfamily protein
138	At4g05050	UBQ11	Ubiquitin 11
139	At2g01600	ENTH/ANTH/VHS	ENTH/ANTH/VHS superfamily protein
142	At2g46240	BAG6	BCL-2-associated athanogene 6
145	At5g20730	TIR5	Transcriptional factor B3 family protein / auxin-responsive factor AUX/IAA-related

(continued)

Table 11.3 (continued)

S. No	Locus	Short description	Function
146	At2g46270	GBF3	G-box binding factor 3
147	At4g11220	RTNLB2	VIRB2-interacting protein 2
148	At5g48570	ROF2	FKBP-type peptidyl-prolyl cis-trans isomerase family protein
149	At2g02800	Kin2	Protein kinase 2B
151	At3g25800	PR 65	Protein phosphatase 2A subunit A2
152	At5g59820	ZAT12	C2H2-type zinc-finger family protein
153	At3g47990	SIS3	Sugar-insensitive 3
154	At1g63840	RING/U-box	RING/U-box superfamily protein
155	At4g16440	NAR1	Ferredoxin hydrogenases
156	At3g53810	Kinase	Concanavalin A-like lectin protein kinase family protein
157	At4g09150	T-complex 11	T-complex protein 11
159	At1g42990	BZIP60	Basic region/leucine zipper motif 60
160	At3g46450	SEC14 cytosolic factor	SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein
161	At1g25280	TLP10	Tubby like protein 10
162	At2g27580	Zinc finger	A20/AN1-like zinc-finger family protein
163	At2g43500	RWP-RK	Plant regulator RWP-RK family protein
164	At2g11520	CRCK3	Calmodulin-binding receptor-like cytoplasmic kinase 3
165	At1g63720		
166	At3g62590	Alpha/beta-hydrolases	Alpha/beta-hydrolases superfamily protein
167	At1g08050	Zinc finger	Zinc-finger (C3HC4-type RING finger) family protein
168	At1g77890	RNA polymerase	DNA-directed RNA polymerase II protein
169	At1g34260	FAB1D	Forms aploid and binucleate cells 1A
170	At2g34660	MRP2	Multidrug resistance-associated protein 2
171	At5g51070	SAG15	Clp ATPase
172	At5g08560	Transducin	Transducin family protein/WD-40 repeat family protein
174	At3g07700	Kinase	Protein kinase superfamily protein
175	At2g18090	GYF	PHD finger family protein/SWIB complex BAF60b domain-containing protein/GYF domain-containing protein
176	At4g32070	Phox4	Octicosapeptide/Phox/Bem1p (PB1) domain-containing protein/tetratricopeptide repeat (TPR)-containing protein
177	At5g12020	HSP17.6II	17.6 kDa class II heat shock protein
178	At3g51250	Senescence/ dehydration-associated	Senescence/dehydration-associated protein-related
179	At4g34390	XLG2	Extra-large GTP-binding protein 2
180	At1g68620	Alpha/beta-Hydrolases	Alpha/beta-Hydrolases superfamily protein
181	At2g40140	ZFAR1	Zinc-finger (CCCH-type) family protein

(continued)

Table 11.3 (continued)

S. No	Locus	Short description	Function
182	At2g32120	HSP70T-2	Heat shock protein 70T-2
183	At5g66730	IDD1	C2H2-like zinc-finger protein
184	At1g56560	A/N-InvA	Plant neutral invertase family protein
185	At1g07530	SCL14	SCARECROW-like 14
186	At1g22930	T-complex 11	T-complex protein 11
187	At5g27600	LACS7	Long-chain acyl-CoA synthetase 7
188	At5g63320	NPX1	Nuclear protein X1
189	At4g24200	TFIIS	Transcription elongation factor (TFIIS) family protein
190	At1g16030	Hsp70b	Heat shock protein 70B
191	At3g17611	RBL14	RHOMBOID-like protein 14
192	At5g63370	kinase	Protein kinase superfamily protein
193	At1g15430	DUF1644	Protein of unknown function (DUF1644)
194	At3g05360	RLP30	Receptor like protein 30
195	At4g02940	Oxygenase	Oxidoreductase, 2OG-Fe(II) oxygenase family protein
196	At3g48070	RING/U-box	RING/U-box superfamily protein
197	At5g51770	Kinase	Protein kinase superfamily protein
199	At2g16900	Lipase	Arabidopsis phospholipase-like protein (PEARLI 4) family
200	At3g26910	Glycoprotein	Hydroxyproline-rich glycoprotein family protein
201	At3g17000	UBC32	Ubiquitin-conjugating enzyme 32
202	At3g08690	UBC11	Ubiquitin-conjugating enzyme 11
203	At4g24160	Alpha/beta-hydrolases	Alpha/beta-hydrolases superfamily protein
204	At3g17800	DUF760	Protein of unknown function (DUF760)
205	At1g70590	F-box	F-box family protein
206	At2g38340	DREB19	Integrase-type DNA-binding superfamily protein
207	At1g23780	F-box	F-box family protein
208	At5g17380	Decarboxylase	Thiamine pyrophosphate dependent pyruvate decarboxylase family protein
210	At1g29340	PUB17	Plant U-box 17
211	At3g51890	Clathrin	Clathrin light chain protein
212	At5g23850	DUF821	Arabidopsis thaliana protein of unknown function (DUF821)
213	At5g62020	HSFB2A	Heat shock transcription factor B2A
214	At5g60410	SIZ1	DNA-binding protein with MIZ/SP-RING zinc finger, PHD finger and SAP domain
215	At5g11650	Alpha/beta-hydrolases	Alpha/beta-Hydrolases superfamily protein
216	At5g22220	E2FB	E2F transcription factor 1
217	At4g17895	UBP20	Ubiquitin-specific protease 20
218	At1g01650	SPPL4	Signal peptide peptidase-like 4

(continued)

Table 11.3 (continued)

S. No	Locus	Short description	Function
219	At4g11350	DUF604	Protein of unknown function (DUF604)
220	At1g16010	MRS2-1	Magnesium transporter 2
222	At4g00900	ECA2	ER-type Ca^{2+}-ATPase 2
223	At2g27090	Protein of unknown function	Protein of unknown function (DUF630 and DUF632)
224	At5g52200	I-2	Phosphoprotein phosphatase inhibitors
225	At5g24590	TIP	TCV-interacting protein
226	At1g69270	RPK1	Receptor-like protein kinase 1
227	At3g62240	RING/U-box	RING/U-box superfamily protein
228	At3g22910	Hydrolase	ATPase E1-E2 type family protein/haloacid dehalogenase-like hydrolase family protein
229	At5g47120	BI1	BAX inhibitor 1
230	At5g51640	YLS7	Plant protein of unknown function (DUF828)
231	At5g63790	NAC102	NAC domain-containing protein 102
232	At1g64610	Transducin/WD40 repeat-like	Transducin/WD40 repeat-like superfamily protein
233	At5g45130	RHA1	RAB homolog 1
234	At3g19970	Alpha/beta-hydrolases	Alpha/beta-Hydrolases superfamily protein
235	At5g24870	RING/U-box	RING/U-box superfamily protein
236	At1g60420	DC1	DC1 domain-containing protein
237	At1g79610	NHX6	Na^+/H^+ antiporter 6
238	At4g18050	PGP9	P-glycoprotein 9
239	At4g28390	AAC3	ADP/ATP carrier 3
240	At5g65140	TPPJ	Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
241	At4g23570	SGT1A	Phosphatase-related
242	At5g54730	G18F	Homolog of yeast autophagy 18 (ATG18) F
243	At4g25520	SLK1	SEUSS-like 1
244	At1g69790	Kinase	Protein kinase superfamily protein
245	At1g17440	TAF12B	Transcription initiation factor TFIID subunit A
246	At5g09330	VNI1	NAC domain-containing protein 82
247	At2g42950	CorA-like	Magnesium transporter CorA-like family protein
248	At5g02310	PRT6	Proteolysis 6
249	At2g44200	Splicing	CBF1-interacting co-repressor CIR, N-terminal; Pre-mRNA splicing factor
250	At3g51620	PAP/OAS1 substrate-binding domain	PAP/OAS1 substrate-binding domain superfamily
251	At4g25390	Kinase	Protein kinase superfamily protein
252	At3g16940	Transcription	Calmodulin-binding; transcription regulators
253	At3g12740	ALIS1	ALA-interacting subunit 1
254	At2g20010	DUF810	Protein of unknown function (DUF810)
255	At4g24560	UBP16	Ubiquitin-specific protease 16

(continued)

Table 11.3 (continued)

S. No	Locus	Short description	Function
256	At1g04960	DUF1664	Protein of unknown function (DUF1664)
257	At1g76970	Target of Myb 1	Target of Myb protein 1
258	At2g30110	UBA1	Ubiquitin-activating enzyme 1
259	At5g13160	PBS1	Protein kinase superfamily protein
260	At3g19640	MRS2-3	Magnesium transporter 4
261	At1g11100	Zinc finger	SNF2 domain-containing protein/helicase domain-containing protein/zinc-finger protein-related
262	At4g03260	Outer arm dynein 1	Outer arm dynein light chain 1 protein
263	At3g17770	Kinase	Dihydroxyacetone kinase
264	At4g12120	SEC1B	Sec1/munc18-like (SM) proteins superfamily
265	At5g53130	CNGC1	Cyclic nucleotide gated channel 1
266	At4g11860	DUF544	Protein of unknown function (DUF544)
267	At5g57050	ABI2	Protein phosphatase 2C family protein
268	At5g04930	ALA1	Aminophospholipid ATPase 1
269	At4g08180	ORP1C	OSBP(oxysterol binding protein)-related protein 1C
271	At1g53540	Chaperonin	HSP20-like chaperones superfamily protein
272	At4g37370	CYP81D8	Cytochrome P450, family 81, subfamily D, polypeptide 8
273	At1g04780	Ankyrin repeat	Ankyrin repeat family protein
274	At2g28320	PH	Pleckstrin homology (PH) and lipid-binding START domains-containing protein
275	At4g34990	MYB32	Myb domain protein 32
276	At5g59220	SAG113	Highly ABA-induced PP2C gene 1
277	At4g23630	RTNLB1	VIRB2-interacting protein 1
279	At1g06780	GAUT6	Galacturonosyltransferase 6
280	At2g20320	AEX-3	DENN (AEX-3) domain-containing protein
282	At4g39850	PXA1	Peroxisomal ABC transporter 1
283	At3g11880	Protein of unknown function DUF2359	Protein of unknown function DUF2359, transmembrane
284	At2g21470	SAE2	SUMO-activating enzyme 2
285	At2g31260	APG9	Autophagy 9 (APG9)
286	At2g37970	SOUL-1	SOUL heme-binding family protein
287	At4g23850	LACS4	AMP-dependent synthetase and ligase family protein
288	At1g34220	Regulator of Vps4 activity in the MVB pathway	Regulator of Vps4 activity in the MVB pathway protein
289	At5g10270	CDKC;1	Cyclin-dependent kinase C;1
290	At1g02890	ATPase	AAA-type ATPase family protein
291	At1g73730	SLIM1	Ethylene-insensitive3-like 3
292	At5g14640	SK13	Shaggy-like kinase 13

(continued)

Table 11.3 (continued)

S. No	Locus	Short description	Function
293	At5g16680	Zinc finger	RING/FYVE/PHD zinc-finger superfamily protein
294	At2g19710	Regulator of Vps4 activity in the MVB pathway	Regulator of Vps4 activity in the MVB pathway protein
295	At3g47730	ATH1	ATP-binding cassette A2
296	At1g21080	N-terminal	DNAJ heat shock N-terminal domain-containing protein
297	At3g15180	ARM repeat	ARM repeat superfamily protein
298	At2g42270	Helicase	U5 small nuclear ribonucleoprotein helicase
299	At1g52560	Chaperonin	HSP20-like chaperones superfamily protein
300	At1g21450	SCL1	SCARECROW-like 1

based on the repeats number in the MYB domain in Arabidopsis (Stracke et al. 2001). Many members of MYB family have been isolated and recognized from Arabidopsis thaliana, Oryza sativa, Zea mays, and Glycine max that play many roles in regulating variety of cellular processes, for instance cell-cycle and -morphogenesis, stress tolerance (Lippold et al. 2009), hormonal signaling (Abe et al. 2003), secondary metabolite biosynthesis (Cavallini et al. 2015), and regulation of development (Xie et al. 2018).

Under drought and salt stress, AtMYC2 and AtMYB2 genes also contribute in TFs which regulate ABA-dependent gene expression (Abe et al. 2003). In other researches, AtMYB102 was observed to be playing a crucial role in wounding and osmotic related pathways in Arabidopsis (Denekamp and Smeekens 2003). Besides this, Lippold et al. (2009) suggested that AtMYB41 coordinates the transcriptional responses for short term against osmotic stress. Overexpression of OsMYB2 increases the osmolytes like soluble sugars and proline formation via the upregulation of target gene for synthesis and transport of proline, and overcoming the oxidative injury caused by excessive accumulation of H_2O_2 and MDA contents in cells under salt stress (Yang et al. 2012). Proline also stabilizes the proteins thus acts as a molecular chaperon as well as involves in plant's defense system (Székely et al. 2008). These osmolytes are formed due to overexpression of ScMYBAS1-3 genes in Saccharum officinarum which is a MYB TF and provides tolerance against dual stress of drought and salt stress (Prabu and Prasad 2012). Similarly TaODORANT1 overexpression led to enhanced resistance in transgenic plants against salt stress (Wei et al. 2017). PbrMYB21 gene through ADC expression regulation modulated polyamine synthesis and thus enhanced drought (Li et al. 2017). The CaMYB85 protein conferred tolerance against drought and salt via enhancing the expression of stress related genes in transgenic Arabidopsis.

11.5.5 AP2 (Activating Protein 2)

Another group of TFs which are plant specific includes AP2 (activating protein 2)/ERFBP (ethylene responsive element binding factor) family and have highly conserved DNA-binding domain. It binds directly with GCC box and can also bind with DRE/C-repeat element (dehydration responsive element CRT) at promoter region of downstream genes (Riechmann and Meyerowitz 1998). In *Oryza sativa*, ERF/AP2 transcription factor consists of 163 members which helps plant growth and differentiation and also responds to change in environmental cue and the specificity of response depends on 60 amino acid conserved sequences of AP2 domain of protein (Nakano et al. 2006; Sharoni et al. 2011). Sakuma et al. (2002) further classified AP2/ERF TFs into subfamilies-dehydration responsive element (DRE) binding protein, ERF, AP2 associated ABI3 (abscisic acid insensitive 3)/VP1 (viviparous 1), and others on the basis of similarity and number of DNA-binding domains. ERF/AP2 (APETALA2) plays crucial role against various biotic and abiotic stresses like salinity, UV, drought, pathogens, heat, and cold (Mizoi et al. 2012).

Moreover, many plant responses like hormone responses, cell proliferation, reproductive, and vegetative development are governed by AP2/ERFBP (Licausi et al. 2010; Sharoni et al. 2011; Nakano et al. 2006). Their overexpression increased osmotic stress tolerance in *Nicotiana tobacum* (Park et al. 2009). Five types of drought responsive element binding (DREBs) named as DREB1A, DREBB, DREBC, DREBD, and DREB2A are known (Dubouzet et al. 2003). Constitutive expression of DREB1A in *Oryza sativa* and *A. thaliana* led to growth and development compromise and the resource is diverted for increasing the cold and drought tolerance. Another inducible promoter, Rd29 helps in attaining stress tolerance without compromising the growth of plants (Kasuga et al. 2004). Overexpression of OsDREB1A led to salinity tolerance in *A. thaliana* (Dubouzet et al. 2003). Overexpression of OsDREB1B enhanced the salinity stress tolerance in *Oryza sativa* var. Indica (Datta et al. 2012).

11.5.6 C_2H_2-Zinc Finger

Zinc-finger proteins are the transcription factors that facilitate stress tolerance. They are further classified on the basis of the number and order of cysteine (C) and histidine (H) residues that is responsible for the binding activity of the zinc-finger domains. Among all other zinc-finger proteins, C_2H_2 zinc-finger proteins are one of the most dominating which contain an ethylene responsive element binding factor in association with amphiphilic repression (EAR) motif, which can act as an active repressor (Hichri et al. 2014). There are many known zinc-finger proteins which are important regulators for inducing stress tolerance, for instance ZAT12 from Arabidopsis (Davletova et al. 2005) and S12F2 from tomato (Hichri et al. 2014). Under NaCl treatment the expression of ZAT12 was induced which suggested that the overexpression of ZAT12 improves osmotic stress tolerance in Arabidopsis.

Although ZAT12 appears to regulate APX1, but this regulatory mechanism is not fully understood. Under abiotic stresses, the coexpression of ZAT12 with APX1 implicates that this zinc-finger protein is involved in the regulation of AsA metabolism (Rizhsky et al. 2004).

11.6 Post-transcriptional Regulation of Gene by MicroRNA (miRNA) or Small Interfering RNA (siRNA) During Salinity Stress

Several complex mechanisms can perceive salt stress and evoke tolerance and the regulation of tolerance is mainly at transcription level. The significance of gene regulation at post-transcriptional got momentum with small RNAs such as siRNA (small interfering RNA) and miRNA (micro RNA) discovery. siRNA or RNAi carried out post-transcriptional genes regulation. Transcription factors like Myb, NAC1, and HD-ZIP (homeodomain-leucine zipper proteins) are the major targets of zma-miR159a/b, zma-miR164a/b/c/d, and zma-miR1661/m, respectively. miRNA targets MADS-box proteins and zinc-finger proteins which are also salt stress responsive factors in plants (Fang et al. 2006; Xu et al. 2008).

A cis-acting ABA-responsive element (ABRE) lies on the upstream region of miR169m, which is regulated by abscisic acid, a stress hormone. A salt tolerant (NC286) and sensitive (Huangzoa4) maize genotypes were compared which showed that miR-156, -164, -167, and -396 families were downregulated, while miR-162, -168, -395, and -474 families were upregulated. Salt-responsive genes which include NADP-ME and Cyt. oxidase are miRNAs targets under salinity stress (Cheng and Long 2007; Yan et al. 2005).

A basic-helix-loop helix (bHLH) family proteins and F-box proteins are induced by salt stress and are regulated by miR393a (Jones-Rhoades and Bartel 2004). During stress responsive processes, the responsive targets of miRNAs are monitored post-transcriptionally. High levels of NaCl increase the expression of laccase genes (Cai et al. 2006; Liang et al. 2006). Laccases are multicopper-containing glycoproteins, present in plants. A target responsive to salt, aspartic proteinase 1, APA1 was found to be regulated by cca-novel-18, a miRNA. Minute decrease in superoxide dismutase (SOD) expression was observed with concomitant increase in the expression of miR398 in Arabidopsis under NaCl treatment (Attia et al. 2008). Argonaute1 (AGO1) gene encodes the RNA slice enzyme used in the miRNA pathway and is regulated by miR168 (Li et al. 2012). Salt tolerance is controlled through complex genetic regulatory networks.

11.7 Salinity and Proteomic Responses

Pivotal roles are being played by proteins in plant stress response since they directly participate in adjustment of physiological traits against altered environment, thus reshape the phenotype. Proteins function both as structural and functional proteins

and functional regulation involves managing plant epigenome, transcriptome, and metabolome. Molecular structure alone does not decide the function of protein but also by its cellular position, post-translational modification, and interacting partners (Jorrín-Novo et al. 2009; Kosová et al. 2011). Effects of salinity on proteins of different cellular parts are summarized in Table 11.4. To cope, plant adopt two strategies, i.e., either undergo ion exclusion or compartmentalize ions into vacuoles thus lead development of tolerance. Both the processes are energy intensive and are associated with several ATP-dependent ion-transporters such as Na^+/H^+-ATPases, V-ATPases, and ionorganic pyrophosphatase. Osmotic effect is caused by all stresses which cause dehydration; contrary to this ionic effect is only caused by salinity stress (Munns and Tester 2008).

Plant stress tolerance as a part of salt acclimation involves profound diverse changes in protein metabolism leading to both degradation and biosynthesis of proteins. During acclimatory phase, gene expression alteration leads to remarkable changes in many metabolic pathways as well as also leads to an accumulation of stress related proteins such as chaperones and proteins with protective functions. Changes in energy metabolism are to attain an enhancement in energy production in an available form such as ATP, since biosynthesis of stress related compounds are cost intensive (Kosová et al. 2011).

Resistance phases at molecular level are characterized by fine tuning of cellular metabolism to altered surrounding as well as by efficient levels of proteins capable of checking dangerous effects of stress on vital cellular functions and structures (Kosová et al. 2011). A rapid increase in ROS is caused by more of its generation and less of its detoxification which is a common manifestation of salinity stress. Salinity also leads to over-reduction of ETC and ROS are also formed as a product of various detoxification reactions, which include superoxide radicals (O_2.$^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (·OH). These ROS have the potential to disturb the cellular homeostasis and negatively affect many cellular components, their structure, and thus functions (Cruz de Carvalho 2008). Redox homeostasis is mainly maintained by proteins like L-ascorbate, peroxidase, MDHAR, glutathione peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) precursor, oxygen evolving enhancer protein 1, peroxiredoxin, thioredoxin peroxidase, glutathione transferase, ferredoxin-NADP reductase, and NADH-ubiquinone oxidoreductase 24 kDa subunit (UOR). To alleviate salinity induced oxidative stress, ROS scavenging machinery is activated in the foliage. There is differential expression of organelle based proteins under salinity (Table 11.4).

11.8 Salinity and Metabolism

Several metabolic pathways including secondary metabolism are significantly affected by salinity. Lignified and mechanically well developed xylem vessels are needed for ion exclusion through transpiration. Enhanced lignifications of xylem are possible by increased activity of SAM synthase, which plays important role in methylation and plays crucial role in lignin polymerization (Wang et al. 2009).

Table 11.4 Effects of salinity on proteins of different cellular parts

S. No.	Cellular parts	Functions	Reference
1.	Cell wall	Cell wall related genes, such as *ZmEXPB2* and *ZmXET1* has been associated with increased H3K9 acetylation in the promoter and coding region.	Li et al. (2012)
2.	Plasma membrane	Alteration in Na^+ levels induce signaling associated with an active Na^+ efflux via SOS1/SOS2/SOS3 complex	
		H^+-ATPase isoforms involved in providing energy for ion efflux	
3.	Endoplasmic reticulum	Enhanced fatty acid biosynthesis	
		Starvation associated message 22	
		Glycolysis and fermentation	Pang et al. (2010)
		Programmed cell death	
4.	Mitochondria	Enhanced level of Mn-SOD	Wang et al. (2009)
		Krebs cycle enzymes	Pang et al. (2010)
		Components of F_0-F_1 ATP synthase	Munns and Tester (2008)
		S-nitrosylation of PrxII F	
		Voltage dependent anion channel	Wang et al. (2009)
5.	Chloroplast	Enhanced abundance of stress and defense related proteins	Pang et al. (2010)
		Carbohydrate catabolism	Yu et al. (2011)
		OEC, Cyt b_6f, CF1-CF0 ATP synthase, RuBisCo, and carbonic anhydrase	Zőrb et al. (2010)
		Plastidial glycolysis	
		Alterations in thylakoid membrane composition	Zőrb et al. (2010)
6.	Nucleus	Nucleus represents the major organelle involved in plant phenotype remodeling in response to salinity since it is involved in stress signal transformation into changes in gene expression.	Ahmed et al. (2010)
		Histone modifications such as H3K4me3, H3K9ac, H3K9me2, H3K23ac, H3K27ac, H3K27me3, and H49c along with DNA methylation can be correlated with gene expression in response to high salinity	Kim et al. (2008)

Several proteins like xylose isomerase, peroxidases, etc. which are involved in lignification have also been found to have increased in salt-treated barley roots (Witzel et al. 2009).

Elevated levels of dissolved salt ions reduce the osmotic potential of water, which poses a threat of an osmotic stress on plant cells, to that of root cells especially which are in direct contact with soil water. Plant response to a decreased soil osmotic potential lies in a further decrease of intracellular osmotic potential which is possible

because of osmotic adjustments. Osmotic adjustment is the key mechanism which prevents plant cell dehydration, turgidity loss, and plasmolysis. Basically, osmotic adjustment of plant cell is made possible by accumulation of inorganic salt ions which has a low energy-cost but may cost more due to inhibitions caused to many intracellular enzymes by high salt concentrations. The other way for osmotic adjustment is by accumulation of organic hydrophilic compounds, commonly called as osmolytes. In salt-treated plants, alterations in metabolism of many osmolytes like proline and glycinebetaine have been observed. Proline biosynthetic enzymes like glutamine synthase (GS) and Δ-pyrrolin-5-carboxylase (P5CS) showed enhanced level, although, proline dehydrogenase (PDH), which is an enzyme involved in proline hydrolysis showed lower level (Pang et al. 2010; Kant et al. 2006; Taji et al. 2004). Similarly, an increased abundance of enzymes involved in GB biosynthesis like SAM synthase (SAMS), choline monooxygenase (CMO), and betaine aldehyde dehydrogenase (ALDH) has been observed in salt-treated Suaeda aegyptiaca and foxtail millet.

11.9 Conclusion

The ability of tolerant plants to sustain against high salt is determined by coordination between different physiological processes, metabolic pathways and protein or gene networks and on soil microbial content. Generally the halo-tolerant microbes in soil help in sustaining the plants through array of phenomenon and the solubilization of the nutrients is the key one. The proteins involved belong to highly diverse functional classes like photosynthesis, redox homeostasis, stress/defense, carbohydrate and energy metabolism, signal transduction, and membrane transport. Efforts have been made to understand the role of different proteins including the transcription factors in regulating the gene expression, so that the adverse effects of sodium can be minimized. To achieve a higher degree of understanding, regulation of gene expression using transcription factors is also discussed. The understanding will be helpful in modifying the crops using the genetic engineering.

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Part III

Water Treatment Technology

Prime Techniques for Pre- and Post-Treatments of Anaerobic Effluents and Solids

12

Suman Bajracharya, Nabin Aryal, Jayesh M. Sonawane, Suman Kharel, Shalik Ram Sharma, and Deepak Pant

Abstract

Several pre-treatment approaches have been explored to enhance the anaerobic fermentation kinetics and efficiency, which include thermal-alkaline treatment, free ammonia, sequential ultrasound techniques as well as grinding, and sieving. Additionally, valorization of mineralized compounds and production of reusable water can also be achieved via post-treatments. The post-treatment concept allows preserving or recovery of value-added byproducts in the form of manures, soil conditioners, and renewable energy. In this chapter, we explain the recent advancement in the pre-treatment and post-treatment of anaerobic digestate to enhance the anaerobic process and for the removal of undesirable compounds, recovery of energy, nutrients, and waste stabilization before disposal.

S. Bajracharya
Biochemical Process Engineering, Department of Civil, Environmental and Natural Resources Engineering, Luleå University of Technology, Luleå, Sweden

N. Aryal
Biological and Chemical Engineering, Aarhus University, Aarhus N, Denmark

J. M. Sonawane
Department of Chemical Engineering and Applied Chemistry, Centre for Global Engineering, University of Toronto, Toronto, ON, Canada

S. Kharel
Environmental Chemistry and Toxicology, Aarhus University, Roskilde, Denmark

S. R. Sharma
Section for Sustainable Biotechnology, Department of Chemistry and Bioscience, Aalborg University, Copenhagen, Denmark

D. Pant (✉)
Separation and Conversion Technology, Flemish Institute for Technological Research (VITO), Mol, Belgium
e-mail: deepak.pant@vito.be

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12.1 Introduction

Anaerobic digestion (AD) is a biochemical process that transforms organic matter into energy-rich methane and other value-added products such as bio-oils, under anaerobic condition (Amani et al. 2010; Adekunle and Okolie 2015). The process involves four bacterial groups such as hydrolyzers, acidogens, acetogens, and methanogens (Liu et al. 2002; Wang et al. 2013; Niu et al. 2014). Change in one of any aforementioned bacterial classes may result in the change of the anaerobic process and eventually affect the reactor performance. At the same time, the diverse microbial community in AD could provide reactor stability and higher performance (Nguyen et al. 2019). Besides the substrate material in the fermentation process, the processing time, organic loading, and bacterial consortium also define the final products from the AD (Zielińska et al. 2013).

The process of AD results in the production of anaerobic digestate (bio-effluent or bio-slurry/sludge) in addition to biogas [a mixture of methane (CH_4), carbon dioxide (CO_2), water vapor, hydrogen sulfide, and ammonia] (Dugba and Zhang 1999; USEPA 2002). During the AD process, carbon is converted to methane gas and released or separated from the digestate, whereas nitrogen (N), phosphorus (P), and potassium (K) are not removed or separated but are transformed from organic forms to inorganic forms. Approximately half of the organic N is transformed into inorganic ammonium (NH_4^+-N) form, which is directly available for absorption by plants (Gurung 1997; Möller and Müller 2012). This is the reason why digestate can be directly used as agricultural fertilizers by land spreading. High ammonium and organic matter content in anaerobic digestate create it as a suitable feedstock for agriculture or the chemical industry (Makádi et al. 2012). However, the harmful compounds must be removed prior to the reuse of the digestate. The treatment of digestate from anaerobic digestion is carried out to meet the guidelines of effluent disposal with the complete elimination of pathogens and the maximum removal or recovery of COD, suspended solids (SS), nutrients (nitrogen and phosphorus), and reduced inorganic compound (sulfur, ferrous, etc.). A billion gallons of digestate/sludge are produced every day globally, and therefore digestate/sludge treatment is an environmental concern globally (Cassie et al. 2010).

12.2 Digestate Pre-treatment and Phosphorus Recovery

Several pre-treatment approaches have been explored to enhance the anaerobic digestion efficiencies. Also, the addition of chemicals such as fermentation liquid with calcium proved to be an efficient way to recover short-chain fatty acids (SCFAs) (Ariunbaatar et al. 2014; Krishna and Kalamdhad 2014; Rodriguez et al. 2015; Sheng et al. 2019).

For higher methane production and volatile solids (VS) destruction, the free nitrous acid (FNA) pre-treatment has been proved (Zhang et al. 2019). This treatment allows a reduction in the hydraulic retention time (HRT) with similar performance. It was reported that with the shortened HRTs (7.5 and 12 days), the AD

reactor achieved VS destruction at almost 36%. Also, the methane production increased per unit of VS by a similar 31–35% (Zhang et al. 2019). Pretreated raw sludge and separated liquid slurry were explored in continuous up-flow anaerobic sludge blanket reactor separately to generate VFAs (Zhang et al. 2018b). It was observed that the highest VFA productivity of the system with organic loading rate (OLR) of 10 kg COD/m^3/d was fivefold higher than batch and semi-continuous fermentation with pretreated slurry. The system can be further combined with online nitrogen and phosphorus recovery, resulting in a further 20% VFA production with struvite recovery of 1.98 ± 0.28 g/g PO_4^{3-}. The processes show promising nutrients recovery with low consumption of energy for VFA production from wastewater activated sludge (WAS) (Zhang et al. 2018b). A thermal-alkaline pre-treatment and alkaline fermentation of sewage sludge increase VFAs production (Liu et al. 2018). In the wastewater treatment plant, VFAs are used as an external carbon source for biological nitrogen and phosphorus removal. The long-term process shows that anaerobic fermentation for VFAs production is a cheaper technique for carbon recovery. Sewage sludge generated in the improved biological phosphorus removal procedure generally contains ~5% of its dry weight as phosphorus.

Sequential ultrasound–thermal (55 °C) pre-treatment in an anaerobic breakdown of sewage sludge treatment increased methane yield without disturbing the process steadiness. However, this process results in a decline of up to 4.2% water recovery from the digestate (Neumann et al. 2018). Iron dosing is generally practiced in wastewater treatment. Sometimes it can be supplemented for odor and rust control, phosphate removal, or stoppage of emission of H_2S gas. The robust affinity among and phosphate and iron could decrease recovery efficacies via struvite precipitation. Also, it was observed that phosphate recovery might be advanced if a Fe-P composite like vivianite could be harvested from sewage sludge (Korving et al. 2018) The enhancement of phosphorus release from WAS via anaerobic fermentation can be achieved with the high-voltage pulsed discharge (HVPD) pre-treatment, 26.7% soluble ortho-P [$SOP_{(L)}$] was released as compared to the one without pre-treatment (Hu et al. 2018). The low retrieval of phosphorus from WAS is typically at little levels due to low phosphorus release (Xu et al. 2018).

The short-time anaerobic digestion (STAD) route is the most promising method for the stabilization of WAS and STAD process efficiency solely depends on the pH of the WAS (Zhou et al. 2019). Under anaerobic conditions, the biodegradation of nonylphenol (NP) is usually sluggish. The improvement of NP biodegradation and SCFAs accretion in activated sludge can be achieved by maintaining pH 10 with the addition of sodium lauryl sulfate. Degradation of 55% within eight days was achieved (Duan et al. 2019). Free ammonia (FA) to pretreat WAS enhances the phosphorus discharge; FA attributes the extra polymeric substance and cell envelope of bacterial sludge. Magnesium ammonium phosphate (MAP) was recovered in the form of phosphorus (Xu et al. 2018).

Mixing is another prime parameter and must be well augmented in anaerobic digestion system to accomplish exceptional biomaterials in sludge management. The mixing of 90 and 120 rpm can enhance the hydrolysis and acidification efficacy of

sludge (Ma et al. 2019). Generally, sewage composition does not affect the microbial richness (Granatto et al. 2019). The influence of the pre-treatment, including sieving and grinding and on AD of municipal solid waste, has been studied with the co-digestion of the liquid portion from hydrothermal carbonization of dewatered SS. The production of methane after pre-treatment was double than untreated one (De la Rubia et al. 2018). A recent study demonstrates that the carbon in the organic waste can be fully converted to CO_2 while keeping nutrients in the liquid effluent. Prolonging the residence time does not improve the effectiveness of carbon oxidation; however, adequate oxygen supply can result in high carbon transformation efficacies (>85%) and an odorless, clear effluent (Zhang et al. 2018a).

Bioconversion of the organic wastes in WAS and liquor wastewater (LW) to value-added products such as SCFAs via anaerobic fermentation is an ideal approach to treat organic waste (Luo et al. 2018). Generally, SCFAs concentration is found to be 5.4 g COD/L with an estimated 80% acetic and propionic acid under augmented conditions. A large amount of waste is generated from the coffee processing plant and is one of the threats to the environment. A series of the treatment used to process coffee wastewater such as thermophilic high solids co-digestion and WAS was proposed by an anaerobic membrane bioreactor (AnMBR). The COD removal efficiency of 92% with an average methane production of 0.28 L CH_4/gCOD was achieved at 50 g/L solids content in the AnMBR (Chen et al. 2019). The anaerobic dynamic membrane reactor (AnDMBR) was proposed for enhanced VASs production from SS. It was found that the membrane separation and organic loading rate (OLR) increased the productivity in AnDMBR, but it deteriorates with excess OLR (Liu et al. 2019a). VFAs yield and concentration, as well as substrate conversion rate, were observed as 7.8 kg VFA−COD/m^3 d, 60 g/L and 0.38 kg VFA−COD/kg VS, respectively (Liu et al. 2019a).

Nutrients recovery such as nitrogen and phosphate using thermophilic fermentation from food waste as a carbon source has been recently studied (Tang et al. 2019). As of late, the synchronous polyphosphate and iron phosphate recovery from iron-rich sludge was explored utilizing anaerobic fermenter with sulfate reduction (AF-SR) (Hu et al. 2019). Wastewater treatment plant based on the AF-SR system was proven to recover multiple elements such as P, N, Fe, S, and C. Anaerobic digestate of piggery wastewater comprises a high concentration of phosphorus and ammonia in unstable molar ratio. It was found that ammonia remains at a high concentration even phosphorous removed through struvite formation route. After the addition of leachate sewage sludge ash in 1:1.29 ratio ammonia and phosphorus removal efficiencies were 92% and 99.65%, respectively (Kwon et al. 2018). Phosphorous removal in a membrane bioreactor (MBR) was studied by administering ferric iron in acidogenic co-fermentation of the municipal wastewater (Li et al. 2014). Fe induced precipitation and effective P removal in MBR with a flat plate ceramic membrane. The study shows that the addition of $FeCl_3$ at 20 mg Fe/L could recover up to 95.6% of total P when fine-tuning the solution pH to 8, the P and Fe (II) form vivianite for the phosphorus recovery (Li et al. 2014). The pre-treatments discussed above can enhance the productivity of the fermenter and

recovery of nutrients; thus, the techniques can be potentially applied as the moderate release of manure in the agricultural field.

12.3 Identification of Recalcitrant Compounds After Anaerobic Digestion

The objectives of effluent treatment are to remove organic material, thus reducing the mass of degradable material, which prevents subsequent odors and possible pathogens. AD offers a wide range of advantages compared to aerobic digestion, which includes energy-saving, low sludge production, removal efficiency, construction, simple operation, fewer space requirements as the reactor can operate at high loading rates, and lower energy footprint, and moderate performance, especially for stabilization (Carlsson et al. 2012; Aryal et al. 2018a). Nevertheless, anaerobic treatment has some serious drawbacks in particular low nutrient removal and less efficient for the removal of the pathogen, longer start-up times, which requires careful handling to avoid bad odors; thus, it is essential for post-treatment of anaerobic efficient (Mai et al. 2018). Furthermore, since intrinsic characteristics of effluent AD alone are not sufficient to clean the additional compounds that might be causing the eutrophication of freshwater. Post-treatments are therefore necessary to remove some of the critical constituents in particular suspended solids, particulate ammonia nitrogen, phosphorus, sulfides, and pathogens.

12.3.1 Organic Compounds and Solids

Effluent after the anaerobic digestion has a significant amount of organic compounds, including non-biodegradable pollutants, intermediate products of biodegradation processes, and soluble microbial products (SMPs) that have been released during the metabolism process. It has been reported that COD contribution is significantly higher when AD operated at stress conditions in particular during nutrient deficiency, low HRT, relatively small and relatively high pHs such as 5 and 11. The VFAs increased thereby contributing up to 89% of the effluent COD (Kunacheva and Stuckey 2014; Kunacheva et al. 2017a, b). Recent research reported a significant amount of SMPs such as humic substances, polysaccharides, proteins, nucleic acids, lipids, carbohydrates, and small molecules in the effluent (Kunacheva and Stuckey 2014). More precisely, long-chain alkenes which contain C12–C24 alkanes esters and aromatic compounds were also reported (Zhou et al. 2009; Wu et al. 2019).

12.3.2 Micropollutants

The chemicals present in the effluents mostly relies on the source of the waste treatment. Researchers reported that wastewater contains different micropollutants

in particular pharmaceuticals chemicals, personal care products, steroid hormones, surfactants, industrial hazardous chemicals, and pesticides in the concentration range of between 0.1 and 10 mg/L or even higher which might create the operational problem during the anaerobic digestion process (Luo et al. 2014). The recent state of the art reported that anaerobic treatment might remove between 13% and 100% of micropollutants leaving behind the concentration range between 0.001 and 1 mg/L in the effluent; however, it depends on the property of the chemical (Luo et al. 2014). For example, alkylphenols, hormones, and pharmaceuticals (bisoprolol) were remained high concentration due to having lower degradation rates under anaerobic compared with aerobic (Gu et al. 2018).

Microbial pathogen removal is one of the major bottlenecks of AD for the safe operation of the process. Anaerobic digestion technology is not designed to remove the pathogens; thus, post-treatment is necessary for the removal of pathogens. The fecal coliform removal efficiency is around 108 to 107 magnitude, whereas helminth egg removal efficiency was 60–90% (Mai et al. 2018).

12.3.3 Nutrients and Soluble Methane

The discharge of nutrients in particular nitrogen and phosphorous as effluent may cause the eutrophication process in the surface water body. It has been reported that 1.0 kg of phosphorus amount can result in 111 kg growth of biomass, which corresponds to approximately 138 kg of COD (Mai et al. 2018). Nutrients such as nitrogen and phosphorus are hardly removed from the treatment process (Pant and Adholeya 2009). The recent state of the art reported that the concentration of nitrogen and phosphorous from 30 to 50 and 10 to 17 mg/L, respectively, is present in anaerobic effluents from municipal wastewater treatment (Foresti et al. 2006). During the AD process, organic nitrogen and phosphorus are hydrolyzed to ammonia and phosphate even enhancing the concentration in the effluent (Foresti et al. 2006; Moawad et al. 2009). AD is widely applied to produce the biogas; however, methane leakage from AD is one of the critical steps to control (Aryal et al. 2017a, 2018a; Aryal and Kvist 2018). Anaerobically treated effluent from municipal wastewater has reported 20 and 25 mg/L soluble CH_4 at 20–25 °C (Moawad et al. 2009; Mai et al. 2018). Additionally, the loss of dissolved methane in effluents was reported from 36% to 41% of the total methane produced from AD (Silva-teira et al. 2017).

12.4 Treatment of Anaerobic Digestate for Agronomic Uses

The digestate formed after anaerobic digestion comprises a solid fraction and a liquid fraction. The liquid fraction contains a high level of nutrients (N, P, and K), most of which are easily assimilated by plants. Although a lot of organic N and P are present in the solid fraction, predominately, it contains high organic matter including lignocellulose and helps in balancing the humic equilibrium of the soil (Bonten

et al. 2014). The high NH_4^+ concentration in digestate can lead to the volatilization of NH_3 during the storage and handling of digestate (Bonten et al. 2014).

Anaerobic digestate is used in agronomic purposes because the digestate is a valuable source of organic matter and nutrients (N, P, and K); it provides a good supply of iron, upgrades soil structure, and does not cause groundwater contamination as compared to chemical nitrogen fertilizers. Organic nitrogen in digestate is less susceptibility to leaching so it does not pollute groundwater (Vasileski 2007).

Agronomic assessments of anaerobic digestates from animal slurries show contradictory finding; the result mainly depends on the methodologies of digestate treatment and application on crops. The agronomic use of anaerobic digestate as fertilizers/soil conditioners was practiced broadly under agricultural land application for human crops production and forage/fodder production in grasslands and pastures. Significantly positive effects of digestates were obtained on grasslands (Rubæk et al. 1996) and in pot experiments (Dahlberg et al. 1988; Morris and Lathwell 2004). However, the results were not encouraging for the application of digestates in the settings of field trials (Möller and Müller 2012; Möller 2015). Application of digestate immediately mixing with a limited amount of soil during the pot experiments has been linked with positive agronomic results. When applied in this manner, $NH4^+$-N content and a portion of organic N fractions are delivered by digestate efficiently, compared to surface application in field condition. Besides the anaerobic digestates of animal wastes, the digestates of crop residues and cover crops have also shown positive results on soil N availability, N use efficiency, and crop yield (Emmerling and Barton 2007; Stinner et al. 2008).

Regarding the type of crops that have been assessed for the application of anaerobic digestates, agronomic significance has been demonstrated in the cultivation of organic vegetables (Liu et al. 2009; Furukawa and Hasegawa 2006; Kelderer et al. 2008). The N release of digestates was found to be comparable or even higher than that of commercial organic fertilizers for vegetable crops grown in the cold season (8 °C/16 °C) (Kelderer et al. 2008), and digestate:water ratio of 1:4 to 1:8 was found to be optimum (Liu et al. 2009).

The quality of anaerobic digestate depends on many factors for the use of agronomic purposes. It includes the type of solid waste or feedstock used for anaerobic digestion; quality of water used in mixing and feeding rate and storage, treatment and application of the digestate. The fertilizing potential of digestate also depends on possible nutrient losses during storage, handling, and/or application, especially due to volatilization of ammonia and leaching of nitrogen and potassium.

Digestate can be applied on the farm directly as liquid slurry or as solid application through recycling treatments such as solid–liquid separation, filtration, drying, or compositing (Möller and Müller 2012). It is often difficult to transport and apply the anaerobic digestate directly in its liquid form, so bio-slurry is often treated to get bio-solids before field application as a solid organic fertilizer. Anaerobic effluents are further treated for the production of concentrates and solid portion is further treated chiefly by means of biological processes e.g., composting, vermicomposting, etc. (summarized in Table 12.1).

Table 12.1 Treatments of digestate before using it in agriculture (Adapted and reproduced with permission from Monfet et al. 2018)

	Processes	Application	Main characteristics	References
Solid–liquid separation	Thickening: • Gravity settling • Filtration • Air flotation • Centrifugation	To separate solid and liquid fractions	Suspended solid fraction is concentrated up to 5–10%	Pell Frischmann Consultants Ltd. (2012)
	Dewatering – Filtration (Belt filter press, Chamber filter press, Vacuum filtration) – Centrifugation	Concentrates suspended solids in solid fraction	Solid concentration is from 15% to 35%	Chen et al. (2002)
Treatment of anaerobic effluents	Membrane filtration • Microfiltration • Ultrafiltration • Nanofiltration • Reverse osmosis • Forward osmosis	Removes suspended solids, microbes, macromolecules, and organic molecules and ions	Simple physical process that does not require chemicals. Operation and maintenance costs are lower. Permeates are rich in N and K and can be used as green fertilizer Membrane filtration is limited by fouling	Cath et al. (2006), Masse et al. (2007), Waeger et al. (2010), Vaneeckhaute et al. (2012)
	Struvite precipitation – Magnesium ammonium phosphate ppt	Recovery of N and P from the liquid fraction	Struvite is used as fertilizer	Uysal et al. (2010)
	Ammonia stripping – Air stripping – Stream stripping	Ammonia removal from the liquid fraction	Need to increase pH to 10–11; recovery of stripped NH_3 as ammonium salt for use in agriculture or chemical industries	Tao and Ukwuani (2015)
	Ammonia oxidation – Ozonation –Photocatalytic oxidation – Electrochemical – Bioelectrochemical	Removes ammonia	Oxidize ammonia to nitrate and N_2 gas	Battimelli et al. (2003), Aguilar et al. (2003), Rodríguez Arredondo et al. (2015), He et al. (2015)

(continued)

Table 12.1 (continued)

	Processes	Application	Main characteristics	References
	pH adjustment Acid treatment Alkali treatment	Stabilization of solids	Acidification avoids ammonia escaping and alkalinization increases the dewaterability of solids	Pell Frischmann Consultants Ltd. (2012)
Biological treatment of solids	– Composting – Vermicomposting	Degrade the remaining the organic matter with aerobic biodegradation, contributing to reducing potential phytotoxicity and eliminating pathogens	– For stabilization and biological valorization – For soil amendment	Chadwick (2005), Dinuccio et al. (2008)
	Biodrying: Air convection drying for 7–10 days	Moisture removal and conserves the highest carbon content in the dried solids	–Dry and stabilize organic waste faster without external energy input – Dried solids are biologically stable and odorless and have high calorific power The main drawback is the release of NH_3 during drying and its conversion to other gases (Avalos Ramirez et al. 2012)	Velis et al. (2009)

The technologies for post-treatment of digestates include initial mechanical processes for solid–liquid separation that segregate liquid effluent part and solid digestate portion. This separation then allows the treatment or valorization of each fraction by applying mechanical, physicochemical, or biological processes depending on the intended use of digestate. The solid fraction is rich in phosphorus,

whereas nitrogen and potassium (N–K) are found in both the liquid and solid fractions.

The solid–liquid separation step includes thickening processes, namely gravity settling, filtration, air flotation, and centrifugation, after which the digestate contains up to 5–10% solids. Polyelectrolytes are additionally used to concentrate the digestate. The thickened slurry can be further dewatered by mechanical techniques such as filtration (belt filter press, chamber filter press, vacuum filtration) and centrifugation process (Chen et al. 2002; Chernicharo 2006), or using physicochemical and electrochemical techniques such as chemical coagulation and electrocoagulation (Buzzini et al. 2007) to obtain a solid cake that is 15–35% concentrated. The solid and liquid fraction so formed after the solid–liquid separation step should be stored and treated or valorized to reduce the nutrient loss and be able to use as agronomic purposes. The solid portion contains high dry matters and P contents but relatively low N and K contents, whereas the liquid fraction contains low dry matters and P contents but high N and K contents (Möller and Müller 2012).

Since the liquid digestate fraction is relatively more abundant in total N and NH_4^+ content, this can serve as potential N–K fertilizers, which is comparable to mineral N–K fertilizers or animal urine. Several methods are applied to extract and valorize the NH_4^+-N from the liquid fraction that include membrane filtration, struvite precipitation, ammonia stripping, and other advanced methods (ozonation and chemical oxidation, electrochemical oxidation, microwave). Membrane filtration method does not require chemicals addition and results in separation of retentate or concentrate from anaerobic digestate that is rich in solids and nutrients. Among the membrane filtration processes, microfiltration (MF) and ultrafiltration (UF) effectively remove suspended solids, macromolecules, and microorganisms, whereas nanofiltration (NF), reverse osmosis (RO), forward osmosis (FO), and electrodialysis (ED) remove small organic molecules and ions, such as ammonia (Masse et al. 2007; Waeger et al. 2010). Biological methods such as composting, vermicomposting, and biodrying can be carried out to the solid fraction of digestate for stabilization and biological valorization for soil amendment or biorefining. However, after the solid–liquid separation of digestates, the solid fraction should be treated and applied to the fields straightaway since significant N can be lost as NH_3 as it is volatilized in the first weeks of storage (Chadwick 2005; Dinuccio et al. 2008), specifically during the warmer season. Moreover, since composting is associated with high loss of N, it is considered to reduce the fertilizer value of digestates since the nutrient gets lost and releases greenhouse gases, for instance, N_2O (Avalos Ramirez et al. 2012). Nonetheless, the biological methods for the treatment of solid fraction help in the humic equilibrium of the soil.

12.5 Anaerobic Post-treatment of the Digestate (Effluent) from the Anaerobic Digestion of Organic Fraction of Municipal Solid Waste

The effluent from anaerobic digestion generally contains high organics and nutrients; thus, the effluent quality does not meet discharge limits. The effluent BOD concentration of 60–120 mg/L was reported in the effluent from UASB reactor treating municipal sewage systems (Chernicharo 2006). The effluents from anaerobic digesters usually require a post-treatment before final discharge. The main role of the post-treatment is to complete the removal of organic matter, as well as to remove constituents such as nutrients (N and P), sulfides, and pathogenic organisms (viruses, bacteria, protozoans, and helminths).

The whole organization of processes including mechanical, physicochemical, or biological post-treatments of digestate is shown in Fig. 12.1. Appropriate post-treatment processes should be selected according to the final use of the digestate. The reuse or recovery of resources from waste can be realized via AD, followed by the natural biological mineralization based post-treatments. Biological post-treatment processes stabilize the digestate, reduce organic loads, and also produce novel products such as biofuels. The biological treatments embrace the essential steps of the natural biological mineralization for the conversion of organic matter and nutrients in digestate. Several physicochemical and anaerobic/aerobic biological processes are reported in the literature for the post-treatment of effluents after anaerobic digestion which are summarized in Table 12.2.

Most of the effluents after AD of municipal wastes are post-treated in the polishing ponds, submerged aerated filters, or stabilization ponds. The common options of digestate effluent post-treatments are the trickling filter (TF), aerated bio-filter (ABF), rotating biological contactor (RBC), wetlands, chemically enhanced primary treatment (CEPT) and zeolite column, dissolved air flotation (DAF) aeration systems (Table 12.2). Settling processes such as polishing ponds or maturation ponds and the overland flow systems are usually employed at the full-scale level to remove the stabilized suspended matters from the digestate effluents. High rate aerobic post-treatment attains the effluent with low residual COD, lower than 50 mg/L. Anaerobic processes are also incorporated in the post-treatments in settling ponds, anaerobic filters but the methane produced in the post-treatment processes is not collected effectively due to improper collection system so the methane is burned in flares normally.

The effluent from the AD of organic fraction of municipal solid waste may not have agronomic uses due to the presence of other contaminants; thus, the more effective effluent treatment and removal of nitrogen are imperative for such a case. Among the biological post-treatments of effluents, the anaerobic processes such as anaerobic ammonium oxidation (anammox) for N_2 removal and bioelectrochemical approaches (Sevda et al. 2018) including microbial fuel cell (MFC) (Colombo et al. 2017a) and electro-fermentations (Roy et al. 2016) are more efficient, less energy-demanding, and economic as compared to other techniques. Moreover, the anaerobic processes could generate valuable products such as bioelectricity, biohydrogen,

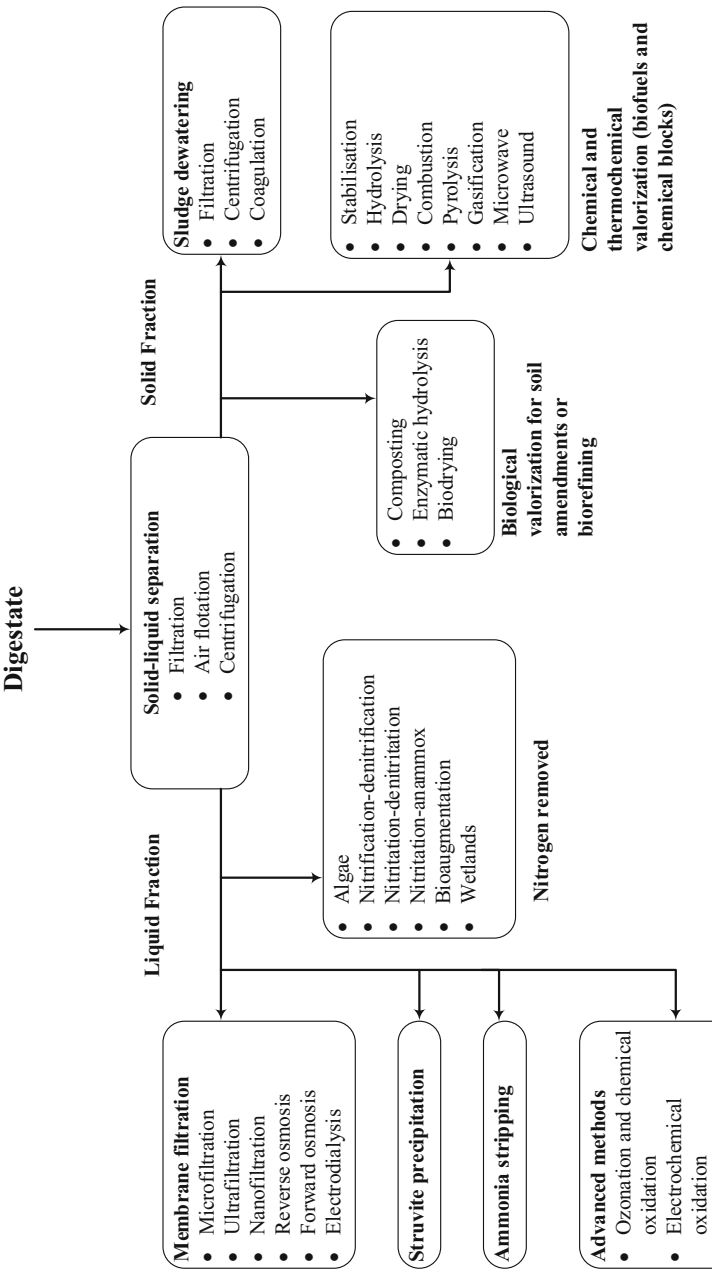

Fig. 12.1 Post-treatments processes for digestate (Reproduced with permission from Monfet et al. 2018)

Table 12.2 Overview of methods used for post-treatment of digestate effluent from AD and their performance (Reproduced with permission from Khan et al. 2011)

Post-treatment system	Effluent concentrations (removal efficiency %)							References
	BOD (mg/L)	COD (mg/L)	TSS (mg/L)	NH_4–N (mg/L)	Total N (mg/L)	Total P (mg/L)	Fecal coliform (most probable number/100 mL)	
Chemically enhanced primary treatment + zeolite column	32 (85)	45 (91)	24 (88)	0.3 (99)	0.5 (99)	0.5 (94)	1.0×10^5 (99)	Aiyuk et al. (2004)
Dissolved air flotation aeration systems	–	17 (98)	4 (98.4)	–	–	0.6 (98)	–	Penetra et al. (1999)
Coagulation-flocculation	>20 (91)	>50 (87)	>30 (82)				4.3×10^3 (99.9)	Jaya Prakash et al. (2007)
Slow sand filter	12 (92.6)	27 (91)	20 (91)				1.0×10^3 (99.995)	Tyagi et al. (2009)
Polishing ponds	24 (92)	108 (79)	18 (96)	20 (50)	25 (55)		5.8×10^2 (99.999)	Cavalcanti et al. (2001)
Constructed wetlands		52 (82)	174 (65)	14 (70)	17.5 (70)	0.74 (89)	1.0×10^3 (99.99)	de Sousa et al. (2001)
Duckweed ponds	14 (96)	49 (93)	32 (91)	0.41 (98)	4.4 (85)	1.1 (78)	4.0×10^3 (99.998)	El-Shafai et al. (2007)
Rotating biological contactor		43		2.2 (92)			9.8×10^2 (99.9)	Tawfik et al. (2002)
Aerated fixed bed reactor	11 (93)	54 (83)	10 (94)		30 (21)	3 (40)		Sumino et al. (2007)
Aerated bio-filter	26 (86)	78 (84)	23 (86)				4.1×10^5 (99)	Keller et al. (2004)
Trickling filter	17–57 (80–94)	60–120 (74–88)	<30 (73–89)					Chernicharo and Nascimento (2001)
Anaerobic filters	<40 (85–95)	60–90 (85–95)	<25 (77–94)					Chernicharo and Machado (1998)

(continued)

Table 12.2 (continued)

Post-treatment system	Effluent concentrations (removal efficiency %)							References
	BOD (mg/L)	COD (mg/L)	TSS (mg/L)	NH_4–N (mg/L)	Total N (mg/L)	Total P (mg/L)	Fecal coliform (most probable number/100 mL)	
Overland flow system	48–62 (53–83)	98–119 (77–83)	17–57	14–18			8.4×10^4–2.4×10^5 (99–99.9)	Chernicharo et al. (2001)
Activated sludge process		50 (85–93)	13–18 (82)					von Sperling et al. (2001)

biofuels. This section describes the anaerobic post-processing technologies to treat the digestate before disposal or final use.

12.5.1 Anaerobic Ammonium Oxidation (Anammox) for Nitrogen Removal

Nitrogen removal from the digestates can be performed either by the conventional nitrification–denitrification method or by the alternative anaerobic ammonium oxidation (anammox) method. The conventional method comprises ammonium oxidation to nitrite (nitritation), then to nitrate (nitration), and further followed by denitrification. This strategy uses ammonia-oxidizing bacteria (AOB) for nitritation and nitrite-oxidizing bacteria (NOB) for nitration, respectively. Denitrification comprises the biological process that sequentially reduces nitrate to nitric oxide, nitrous oxide, and finally, nitrogen gas.

Alternatively, advanced biological nitrogen removal comprises partial nitritation–anammox and nitrification–denitrification processes. The advanced biological nitrogen removal processes are comparatively cost-effective than conventional nitrogen removal. Since digestate effluent contains more than 70% of the NH_4^+-N from the whole digestate and low organics (100–200 mg/L; Foresti et al. 2006), the chemical oxygen demand (COD)/N ratio is low which is favorable for the effective advanced biological nitrogen removal process effective (Fuchs and Drosg 2013). Advance biological nitrogen removal is economically feasible since it requires less oxygen and less organic carbon. The anammox process is an effective method of nitrogen removal from liquid effluents. This process oxidizes ammonia to nitrogen gas in anoxic conditions using nitrite as an electron acceptor. An anammox reaction involves the intermediary formation of nitric oxide (NO) and hydrazine (N_2H_4) (Strous et al. 2006). Around 90% of nitrogen removal efficiency can be achieved in this process. However, in order to apply anammox, partial nitritation is applied prior to the anammox process that uses AOB to oxidize approximately 50% ammonium influent to nitrite, without further conversion to nitrate (Jetten et al. 1997). Several strategies have been used to reach partial nitritation by AOB: (1) limiting the growth of NOB by increasing free ammonia concentration, (2) reducing the dissolved oxygen concentration, (3) operating at temperatures at above 25 °C and at short solid retention time since AOB grows faster than NOB at this temperature (Magrí et al. 2013).

Advanced nitritation–anammox processes have been proven effective for nitrogen removal from liquid effluents in the full-scale application. A scheme to incorporate nitritation–anammox process in the wastewater treatment plant is provided in Fig. 12.2. Some commercially available technologies performing advanced nitritation–anammox process include DEMON®, ANAMMOX®, CleargreenTM, and SHARON coupled with ANAMMOX® (Monfet et al. 2018).

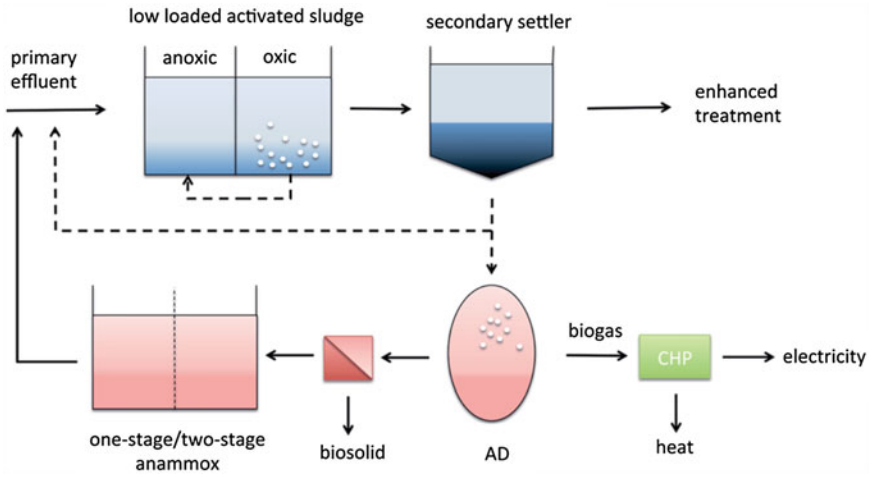

Fig. 12.2 Schematic overview of nitritation–anammox based wastewater treatment processes. *AD* anaerobic digestion, *CHP* combined heat and power (Adapted from Gao et al. 2014)

12.5.2 Microbial Fuel Cell/Bioelectrochemical Systems

Microbial fuel cell (MFC) is a system to produce bioelectricity from organic wastes using special microorganisms that can anaerobically oxidize the organic matters and generate electrons that are transferred to a solid electrode (anode) (Logan 2008). Bioelectricity is produced when the electrons generated at the anode from the microbial oxidation flow toward cathode through the external circuit to recombine with the proton and oxygen to produce water (Logan et al. 2006). MFC technology is governed by the electro-activity of bacteria which has an ability to transfer electrons to anode during the respiration process. A wide variety of substrates can be used in MFC including acetate (non-fermentable) (Bond and Lovley 2003), glucose (fermentable) (Feng et al. 2010), and also inorganic compounds such as sulfides (Rabaey et al. 2006). Recovery of energy as electricity from the waste streams along with the removal of waste is the main benefit of MFC technology which can transform the energy-intensive wastewater treatment process into energy-neutral or energy positive (Pasupuleti et al. 2016).

MFC could be incorporated in wastewater treatment by using it as digestate effluent post-treating system after the anaerobic digestion so that a final polishing step can be introduced to reduce the organic load further and recover the energy (Fig. 12.3). COD level has been lowered in the effluent to <1 g COD/L after AD which is a suitable organic loading for the operation of MFC. At the lab-scale with synthetic effluent, MFCs are reported to achieve a maximum COD removal rate of 3.99 kg COD/m^3 d (Peixoto 2012). However, a full-scale application of MFC has not been realized with real wastewater treatment except one or two test studies at pilot scales (Rossi et al. 2019).

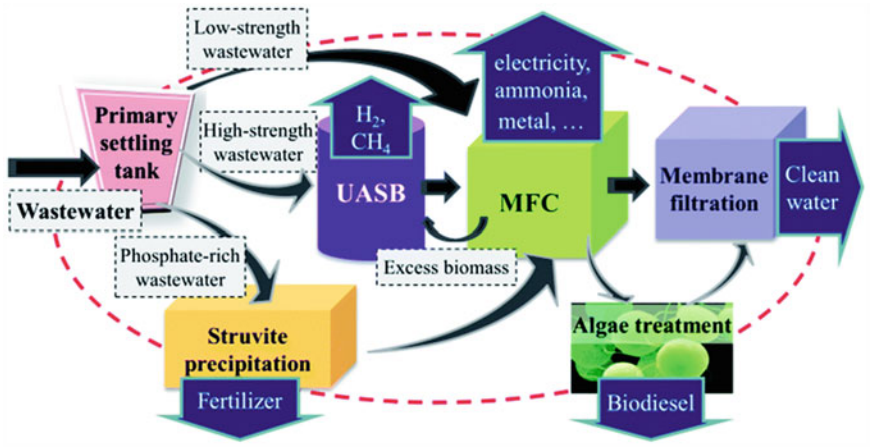

Fig. 12.3 Process flow for a conceptual MFC-centered hybrid process for wastewater refinery. The arrows indicate the water/sludge flow direction. (Reproduced from Li et al. 2014)

The power produced from MFC fed with real wastewater ranges up to only several tens of mW/m^2 (milliwatts per square meter of electrode surface) (Rodrigo et al. 2007), in contrast, about a few thousand mW/m^2 achievable with synthetic effluents. The power production has not upgraded significantly in MFC even though a number of studies applied advanced nanomaterials in the electrode and improved the reactor configurations (Bajracharya et al. 2016). Low conductivity and low buffer capacity of wastewater effluents treated in MFC are repeatedly referred to as one of the main issues responsible for reduced performances (Rozendal et al. 2008). However, a methane-producing MFC as a polishing post-treatment of the effluent from anaerobic digestion can be suggested for maximum energy recovery and removal of organics from the effluent (Gao et al. 2014).

Electricity production from organic waste in MFC may not have a substantial economic gain but the waste removal from effluent or possible conversion to value-added products in bioelectrochemical processes could be the more attractive aspect of applying MFC technology in waste treatment (Bajracharya et al. 2016). In this context, a recent concept of electro-fermentation and microbial electrosynthesis becomes realistic to use wastewaters as a source of energy and materials to recover value-added chemicals (Logan and Rabaey 2012; del Pilar Anzola Rojas 2018). In the AD of domestic wastes, a major hurdle is the accumulation of VFAs mainly from the anaerobic fermentation of food waste (Lee et al. 2015; Shi et al. 2017; Atasoy et al. 2018). A new approach to utilize fermentation products as electrical energy is shown by combining anaerobic fermentation and liquid catalytic fuel cells (AF-CF) (Liu et al. 2019b). Modern technology such as the electro-fermentation method could be a promising fermentation application in the future (Schievano et al. 2016; Civelek Yoruklu et al. 2019). Electro-fermentation is a process that involves electro-chemical control of the fermentative microbes and their associated metabolism with electrodes. Compared with conventional AD, electro-fermentation exhibits 34%

electrical efficiency for food waste utilization (Liu et al. 2019b). At the ambient temperature, the bioelectrochemical AD can save the heat input which is optional for organic waste such as sewage sludge in cold and moderate regions (Feng et al. 2018).

12.6 Post-treatment of Anaerobically Pretreated Landfill Leachate

Leachates are one of the most severe issues in environmental protection today. The disposal of highly complex and toxic leachate is an environmental threat due to the highly toxic nature and heavy pollutant load. The continuous evolution of applied methods and biotechnological innovations for the treatment of landfill have low efficiencies and thus need to be enhanced further. Methods currently being used are reasonably efficient over the traditional treatment methods, and advances are discussed in this section.

Reverse osmosis is the most effective option to treat the leachate. However, its fabrication, operation, and maintenance cost hinder its worldwide use. Recently, magnetic methods to treat landfill leachate have been explored to treat leachate by using magnetic particles together with magnetic sorption (Augusto et al. 2019). Landfill leachate was reported as the most promising feed for electricity generation in microbial fuel cells (Sonawane et al. 2017). Electrooxidation/electrocoagulation/electroreduction (EO/EC/ER) method for tertiary landfill leachate treatment was contemplated by Ding et al. (2018b). The influence of physical and chemical characteristic of the leachate directly affects the efficiency of the treatment system. EO/EC/ER can have the capability to remove carbonaceous and nitrogenous pollutants under ideal conditions. The system can remove 50–60% organic matter and 100% ammonia at a charge of 1.5 Ah/L with a power intake of 15 kW h/m^3. The investigation shows the EO/EC/ER is an efficient process and alternative tertiary treatment to treat leachate (Ding et al. 2018b). The electrooxidation and electrocoagulation method was developed to treat residual ammonia, organic pollutants, and total phosphorus in landfill leachate. The electrochemical reactor consisted of Boron-doped diamond or Titanium based dimensionally stable anodes as anode, Iron net as bipolar electrode and graphite felt or Ti net as a cathode. The voltage 7–14 V was applied with the removal efficiency of 100%, 65%, and 91% for ammonia, organics, and phosphorus at 1.5 Ah L^{-1}. The integrated bipolar system shows an efficient leachate treatment for residual pollutants (Ding et al. 2018a).

Anammox has proven to be an ideal process for nitrogen removal in low C/N landfill leachate. A post anammox can efficiently remove nitrogen from leachate. Fractional denitrification is accomplished by providing a feed for the continuous process by augmenting the electron donor lacking a state. The anammox process reached more than 95% efficiency (Wu et al. 2018). Photocatalytic UV-ZnO technique as a post-treatment consists of ZnO nanoparticles immobilized on stirring media to overcome the defect of the photocatalytic process. Maximum COD removal is achieved by 61–71%. Also, with this method, the biodegradability of

the leachate was enhanced from 0.15 to 0.55 and toxicity lowered by a further 79% (Ranjbari and Mokhtarani 2018).

In the combined approach, air stripping, enhanced coagulation, and Fenton oxidation have been studied. The air stripping of raw leachate removes 51.50% COD in 36 h. In Fenton oxidation, the highest removal of 67.70% of COD and enhanced coagulation could remove 56% of COD (De et al. 2019). Another approach of a synergetic effect of multi-stage organic oxidation, anammox for nitrogen removal and partial nitrification was explored to treat high ammonia, nitrogen, and COD in landfill leachate. This combination proved to an effective for landfill leachate treatment (Yuan et al. 2018). Ultrafiltration effluent from landfill leachate was further treated by electrooxidation process using variable anodes such as Ti/RuO_2-TiO_2, Ti/PtO_2-IrO_2, Ti/RuO_2-IrO_2, and Ti/IrO_2-Ta_2O_5, boron-doped diamond, Pt and stainless steel as a cathode. The COD removal from the anode and cathode configuration lies from 64.9% to 86.8%. Also, higher COD removal efficiencies attributed to chloride content in the leachate promoted indirect oxidation (Ukundimana et al. 2018). Pretreated landfill leachate has always been associated with toxicity even with low COD content. Toxicity reduction of such leachates was subjected to phycoremediation using ammonia tolerant *Chlamydomonas* sp. SW15aRL by adjusting N:P ratios in the leachate. With this process, it was seen that ammonia nitrogen was further reduced by 83% during microalgae treatment. The microalgae inhibition test shows increased growth after treatment. However, residual nutrients were also present (Paskuliakova et al. 2018).

12.7 Post-treatment Methods for Organic Solid Wastes

Organic solid waste, also known as organic-biodegradable-waste, has moisture content below 85–90% and can be of various origin such as domestic, municipal, industrial and commercial (Mata-Alvarez et al. 2000). These wastes can cause adverse effect like soil contamination through direct waste contact or leachate, contamination of surface and groundwater through leaching, air pollution via burning of wastes, odor in landfills, unrestrained release of methane by anaerobic digestion of waste, and dispersal of diseases by various vectors like bird, insects, and rodents (Ngoc and Schnitzer 2009). However, it can be a valuable resource when processed effectively via the application of innovative approaches and techniques. In order to reduce or eliminate the adverse impact on the environment and human health posed by it, various treatment methods are proposed and many of them are already in use. These techniques can reduce the volume and toxicity of solid waste.

12.7.1 Thermal Treatment

Thermal treatment comprises the application of heat to waste material. Few examples of such thermal treatment technique are described below.

12.7.1.1 Incineration

Incineration is the process of combusting solid digestate/waste in the presence of oxygen and converted into carbon dioxide, water vapor, and ash. Incineration is frequently practiced for the recovery of the heat energy from waste meanwhile reducing the volume of the waste by nearly 90% (Hjelmar 1996). However, incineration has its drawbacks such as the production of highly toxic substances like dioxin and furans, emission of metal oxides (McKay 2002).

12.7.1.2 Gasification and Pyrolysis

Gasification is fundamentally a thermochemical process which decomposes organic waste ingredients into gaseous products (Balat 2008; Rasmussen and Aryal 2019). Pyrolysis is an incomplete thermal degradation process performed in the absence of air unlike gasification, which uses a low amount of air (Demirbaş 2002). The high volume of gaseous products with less amount of char and ash are produced in the gasification process, but in pyrolysis, less volume of the gaseous products are formed with more amount of char and tar (Demirbaş 2002).

12.7.1.3 Open Burning

The unenclosed combustion of materials in an ambient environment is usually referred to as open burning (Lemieux et al. 2004). Among developing countries, it is considered the prevalent cause of involuntarily generated persistent organic pollutants (Fiedler 2007). However, this method is still in practice, as it bargains the low-cost solution to solid waste.

12.7.2 Biological Treatment Methods

Biological treatment methods use microorganisms to decompose biodegradable components in solid organic waste. They can be mainly classified into two types: aerobic, which needs oxygen for decomposing (e.g., composting), and another is anaerobic, which occurs without the presence of oxygen (e.g., anaerobic digestion).

12.7.2.1 Composting

One of the most appropriate alternative to treat and manage organic solid waste, from an environmental point of view is composting (Shen et al. 2012; Scoton et al. 2016). It is an anaerobic biological process in which microorganisms decompose organic materials to biologically stable products without any harmful effect on plants once amended as a soil supplement. It also ensures the reduction of waste volume, weight, and moisture content; minimizes potential odor; destroys pathogens; and increases potential nutrients for agricultural application (Wong et al. 2016). Composting contrasts considerably from the decay process occurring in nature because it is monitored and controlled plus aerobic conditions are maintained with a high-temperature phase (e.g., above 55 °C).

The most common composting techniques can be classified into three main categories, which are vermin-composting, windrows composting, and in-vessel composting (Wong et al. 2016).

12.7.2.2 Anaerobic Digestion

AD is one of the popular, ancient, and efficient biological processes for post-treatment of organic waste (Van Lier et al. 2001). AD occurs in the absence of oxygen where complex substrates are converted into digestate and biogas through the microbiological process. It occurs via mainly four steps, namely hydrolysis, fermentation, acidification, and finally, methane formation (Ariunbaatar et al. 2014). It has several advantages like the generation of biogas (methane) and fertilizer, reduction of greenhouse gas and solids, removal of pathogen, stable process, etc. The biogas upgrading technology can further enrich the methane for further utilization such as gas grid injection (Kvist and Aryal 2019). Nonetheless, a hybrid biological method that combines anaerobic digestion followed by aerobic post-treatment compensates the drawback of AD and helps to maintain better quality of effluent (Ghanimeh et al. 2018).

12.7.3 Landfills

12.7.3.1 Sanitary Landfills

It is a simple, low cost, and most often used method (Aziz et al. 2010). It involves controlled disposal of waste on land where it is allowed to decompose into biologically and chemically inert materials in a setting isolated from the environment until it is safe (Hossain et al. 2011). The practice of sanitary landfills reduces health and environmental risk.

12.7.3.2 Bioreactor Landfills

It is a complex heterogeneous system with various microorganisms that reside together enabling to speed up the waste decomposition process (Onay and Pohland 1998). Advantages of bioreactor landfills include a reduction in landfilling cost, operating cost, "post-closure care, maintenance, and risk," contaminating the life span of the landfill and environmental impact (Warith 2002).

12.7.4 Chemical Treatment

The use of chemical treatment combined with other technique is beneficial. For instance, the sludge degradation rate is very slow in anaerobic digestion, but the use of ozonation will break the cell membranes of biomass releasing substrate inside which will increase the degradation rate (Battimelli et al. 2003). Moreover, ozonation also aids in deodorization, better settlement, and a reduction in viscosity of sludge (Déléris et al. 2000). Similarly, other chemical oxidation processes like

alkaline-thermal treatment, chlorination also facilitate the breaking of cells (Rocher et al. 1999; Liu 2003).

The addition of either acid or alkali stabilizes the solid fraction. Loosing of ammonia is prevented by acid addition and an increase in dewaterability of solid fraction (to kill pathogens and neutralize odors) will ease by alkali addition (Pell Frischmann Consultants Ltd. 2012).

12.7.5 Membrane Filtration

It is a simple and cost-efficient physical process. A technique like microfiltration, ultrafiltration, nanofiltration, and reverse osmosis can retain solutes depending upon their sizes. Suspended solids, microorganisms, macromolecules, smaller organic molecules, and ions can be removed by the application of membranes (Waeger et al. 2010). Further permeates from membranes are rich in K and N which can be used as fertilizers (Vaneeckhaute et al. 2012). Membrane filtration is often limited by fouling of the membrane.

12.7.6 Ultrasound

The use of ultrasound is an eminent method for cell lysis (Harrison 1991). Numerous studies reported the beneficial aspect of using ultrasound for the treatment of sludge. For instance, one such previous study found that the application of ultrasonic disrupter improves biogas production of sludge (Onyeche et al. 2002). Other studies also found that it can increase the availability of organic matter and dewaterability of solid fraction leading to a decrease of excess sludge (Gonze et al. 2003; Na et al. 2007).

12.8 Resource Recovery from Anaerobic Effluents

Anaerobic digestion process often offers efficient removal of soluble organic carbon fraction and nitrogen and phosphorus; however, a complete removal is not possible. Recently numerous technologies have been tested for post-treatment of effluents in particular, ion separation via membrane, adsorption, and biological techniques are investigated (Hoang Nhat et al. 2018). Furthermore, some of the critical compounds and resources are also possible to remove from the effluent. Furthermore nutrient recovery such as nitrogen using electrooxidation, air stripping technique, precipitation, crystallization, and membrane filtration has been widely studied.

Among the tested processes biological technology such as anammox has shown promising result for nutrient recovery especially from low concentration of organic carbon contents and nitrogen in the effluent. For example, nitrogen uptake using the algal plant in the constructed wetland was widely applied. The nitrogen could utilize as a source for the growing photosynthetic algae. Nitrogen can undergo nitrification,

denitrification process, sedimentation, and ammonia volatilization with the biological process. In parallel, this biomass could be used as a source of energy in particular, feedstock for biogas plants, nutrient recycling (Hoang Nhat et al. 2018). However, the removal system requires longer retention time, ranging from one and a half days to 15 days for the stabilizing in settling tank. Phosphorus recovery from the effluent by microalgae is another successful example of the biological process for the post-treatment of effluent. Anaerobic membrane bioreactor equipped with microalgae has shown up to 98% phosphorus removal that results 0.1 mg PO_4^{-3}/L of phosphate concentration in effluent water (Hoang Nhat et al. 2018; Mai et al. 2018). Some of the resistance plants can be utilized to remove the hazardous compounds and metals from the effluent water (Gupta et al. 2013). The system has demonstrated the recovery of both nitrogen and phosphorus simultaneously, to produce the biomass, which could be utilized to produce biofuel. Methane slip from anaerobic digestion effluent should be prevented or required recovery system. A recent report has demonstrated the recovery of dissolved methane from effluent by applying methane trapping, stripping, membrane separation and biological recovery (Silva-teira et al. 2017). Furthermore, bioelectrochemical technologies, in particular, MFC, enzymatic fuel cell, and microbial electrosynthesis are under development and reported that anaerobic effluent treatment is possible for the recovery of nutrients, critical metals, and resources to valorize the waste product (Aryal et al. 2017b, c; Zeppilli et al. 2017). The CO_2 fraction from point sources such as biogas and industries could be utilized as carbon sources for further synthesis of chemicals and fuels in microbial electrochemical synthesis (MES) platform (Aryal et al. 2020; Bajracharya et al. 2017a, b, 2019; Aryal et al. 2018b, 2019).

Overwhelming use of fossils fuels, hiking oil prices, limited fuel reserves, environmental degradation, and concerns about massive climate change drive modern society to search for alternative renewable energy sources, for instance, organic wastes (Hallenbeck 2009). Toward utilization of renewable energy, hydrogen fuel is proposed as future energy sources that could be produced utilizing renewable energy and sustainable feedstock. Biological hydrogen production ($BioH_2$) has been thoroughly studied in recent years. Biological hydrogen production from waste biomass can be achieved by using photolytic microorganisms such as bacteria or algae or fermentative organisms (dark fermentation processes), the newly developed method is microbial electrolysis cell. The dark fermentation process is more attractive for high hydrogen production using organic wastes as compared to photo-fermentative processes. The dark fermentation process is considered as the most practically applicable owing to its potentiality to degrade organic wastes and a high H_2 production rate (Singh et al. 2015). As far, most investigations of biohydrogen production are still confined to use pure carbohydrates and carbohydrate-rich organic waste (Liu et al. 2010). Therefore, biohydrogen can be recovered from anaerobic digestion process.

12.9 Biohydrogen Production from VFA Obtained by Acidogenic Anaerobic Digestion of Organic Solid Waste

Overwhelming use of fossils fuels, hiking oil prices, limited fuel reserves, environmental degradation, and concerns about massive climate change drive the modern society to search for alternative renewable energy sources, for instance, organic wastes (Hallenbeck 2009). Wastes can easily be convertible to energy forms, for instance, biogas, biohydrogen, bioethanol, etc., via waste to energy technologies. The policies and investigations on alternative energy sources have recently become significant, particularly for global outlook constancy (Cheng and Hu 2010; Kothari et al. 2010).

Biological hydrogen production ($BioH_2$) has been thoroughly studied in recent years. Biological hydrogen production from waste biomass can be achieved by using photolytic microorganisms such as bacteria or algae or fermentative organisms (dark fermentation processes) and newly developed method is microbial electrolysis cell. Dark fermentation process is basically applicable owing to its degradation of organic wastes (Singh et al. 2015) or can use wastewater (Alzate-Gaviria et al. 2007; Li and Fang 2007) as substrates by mixed cultures to high H_2 production rate.

VFAs are the products of the first two stages of AD, i.e., hydrolysis and acidogenesis. Fermentative microorganisms (acid formers) convert the product of hydrolysis (sugars, amino acids, and short-chain fatty acids) into hydrogen, carbon dioxide, ketones (e.g., ethanol, methanol, glycerol, acetone), and a variety of VFAs (acetate, propionate, butyrate, lactate, etc.) and short alcohols (ethanol, methanol) (Ziemiński et al. 2014). In glucose fermentation, H_2 and CO_2 produce the highest of twelve moles of H_2 per mole of glucose degraded. When acetate and butyrate are the final products and associated with the highest theoretical yields of hydrogen during fermentation, the acetate and butyrate pathway yields, respectively, four and two moles of H_2 per mole of glucose. In general practice, maximum production of H_2 is associated with the combined fermentation products of acetate and butyrate. Less H_2 production is associated with propionate, and subsequently, decreased final products, for example, lactate and alcohols because the ultimate products of metabolic pathways avoid the most important H_2-producing reaction (Levin et al. 2004; Li and Fang 2007).

Therefore, biohydrogen is an alternative plan to convert organic wastes to hydrogen in dark fermentation and can be used as a substitute for methane (Kapdan and Kargi 2006).

12.10 Polyhydroxyalkanoates (PHA) Production from Organic Wastes Using Anaerobic Digestion

Polyhydroxyalkanoates (PHAs) from renewable resources have a broad range of functions and competencies and are supposed to be best for biodegradable plastics. PHAs can be produced by microbial fermentation of organic waste along with the VFAs and H_2 production. The effluent is acidic in nature due to the presence of VFA

and residual carbon content. PHAs can be synthesized under certain conditions of carbon excess and limited concentration of fundamentals growth nutrients, N or P (Salmiati et al. 2014).

PHAs have worldwide attention from industry and research because increasing concern toward climate change, decreasing petroleum resources, and increasing use of synthetic plastics (Chanprateep 2010). Production of PHA especially from renewable substrates such as organic waste is gaining considerable interest in recent years for decreasing the production costs. Complex organic materials are used as the substrates for PHA synthesis by PHA-producing microbes. Organic materials are complex in nature; therefore, initially hydrolysis is carried out to soluble intermediate followed by acidogenesis to produce VFAs and other monomer species (Możejko-Ciesielska and Kiewisz 2016).

Colombo et al. (2017b) reported bioplastics production utilizing VFA by using both and pure and mixed cultures (*Pseudomonas otitidis* and *Serratia ureilytica*) as biocatalyst. Synthetic acids (acetate, propionate, and butyrate), fermented food waste, and acidic effluents from the production of biohydrogen reactor were found by scientific study as substrates for PHA production.

12.11 Conclusion

The pre-treatment of digestate for AD has been applied to increase the efficiencies of microbial digestion process. The chemical, biological, and physical methods such as thermal-alkaline, microbial, fungal, free ammonia, sequential ultrasound, grinding, and sieving are widely applied techniques for pre-treatment. Furthermore, the effluent from anaerobic digestion generally contains high organics and nutrients; thus, the effluent quality does not meet discharge limits. The effluents from anaerobic digesters usually require a post-treatment before final discharge. The main role of the post-treatment is to complete the removal of organic matter, as well as to remove constituents such as nutrients (N and P), sulfides, and pathogenic organisms (viruses, bacteria, protozoans, and helminths). The value-added resources such as nutrients, biohydrogen, PHA, soil stabilizer, etc. could be recovered while doing post-treatment of digestate. Furthermore, digestate could further be utilized for the gasification process to synthesize the intermediate product of syngas.

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Nanoscale Materials and their Potential Application in Potable Water and Wastewater Treatment

13

Sumistha Das and Nitai Debnath

Abstract

Though three-fourth of the earth's surface is covered with water, those days are not far away when there will be a world war-like situation for pure drinking water demand. All our sweet water sources like rivers, lakes, ponds are becoming more and more polluted with contaminants from industries, sewage water, etc. We need to take some immediate action to combat this problem. Nanomaterial based technologies can be very useful in the management of water pollution as a whole, as these materials have several novel properties in comparison to their bulk counterpart. Different types of carbon, polymer, oxide nanomaterial based adsorbents, filtration membranes, etc. are gradually becoming very popular for the removal of toxic materials such as heavy metals, dyes, organic pollutants, etc. from water. Photocatalytic nanostructures like nano titanium dioxide can be used for not only wastewater management but also for purification of drinking water. Many nanomaterials which have antimicrobial property like nano silver or nano zinc oxide are now being used for decontaminating water from microbial pathogens. Moreover, nanosensors can be used for the detection of organic and inorganic pathogens in aquatic system. Though nanomaterial based water treatment technology is very promising, a lot more studies on toxicity of these nanomaterials need to be performed before the wide-scale application of this technology for water pollution management.

13.1 Introduction

While almost 70% of the earth is covered with water, a large population does not get pure drinking water. With the exponential demand of modern human civilization, we are heading towards a dead-end where all the natural potable water sources are going

S. Das · N. Debnath (✉)
Amity Institute of Biotechnology, Amity University Haryana, Gurgaon, India

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to be exploited and exhausted, while we are yet to find out any alternative resources. The situation is becoming graver day by day as our water bodies are gradually getting over burdened with pollution load. Environmentalists are working on several alternative strategies to preserve and restore water worldwide. However, the situation is still not very promising.

Huge amount of industrial wastes, excessive use of agrochemicals such as pesticides, fertilizers, herbicides in agricultural field, and domestic wastes are some of the key contributors to the growing problem of water pollution. Large number of pollutants from livestock, food waste, oil spill, etc. are major sources of organic water pollutants, whereas heavy metal leaching, industrial discharge, food processing waste, etc. are important reasons behind inorganic water pollution. Waste from pharmaceutical companies coming in contact with natural water resources is also increasing the pollution load day by day. The problem is more aggravated due to unauthorized and unplanned utilization of aquifer. Lack of appropriate sewage management is considered a key reason for water pollution. According to a report from the United Nations (UN), 80% of sewage water remains untreated and leads to further amplification of pollution to the water bodies. Moreover, deforestation for urbanization is not only destroying the natural ecosystem but also resulting in reduced rainfall which is ultimately leading to reduction of ground water table and growing pollution levels. Indiscriminate use of plastic, rubber, aluminum, etc. and their uncontrolled disposal in water bodies, and contamination of radioactive waste, are also becoming detrimental for overall water health with time.

Though several measures are taken by the government and private organizations globally and many environmental commissions are set to prioritize lessen water pollution, it is not enough to manage the colossal problem of water pollution. Here, we want to focus on application of nanomaterial in controlling water quality. Nanomaterials are interesting because of their novel properties which differ significantly from the bulk counterpart and have at least one dimension within the size range of 1–100 nm. These materials may be engineered or natural. In nano domain, due to the enhancement of surface area to volume ratio the materials become more reactive, whereas their optical properties get changed due to quantum size effect. Unique properties of nanoscale materials can be utilized to make miniaturized and more sensitive electrical, biomedical, and diagnostic devices. In the modern era, human amenities are flooded with different nanomaterials. Application of nanotechnology has the potential to revolutionize the sector of wastewater remediation, too (Haijiao et al. 2016).

This chapter will mostly focus on different nanomaterial based filtration techniques for maintenance of water quality. Study on different nanomaterial based adsorbents for removal of water contaminants will also be discussed. Detection of water-polluting objects is also of paramount importance. Nanomaterial based pollution markers are therefore, also discussed with cited examples. Similarly, nanoparticles based water treatment strategies are discussed with cited examples. Detection of microbial load is another parameter for water quality assessment. In this regard, various nanomaterials are emphasized for their role in waterborne pathogen detection.

13.2 Nanomaterials as Pollutant Adsorbents for Water Treatment

13.2.1 Carbon Nanomaterial Based Nano Adsorbents for Wastewater Treatment

World of nanomaterials are immensely vast and comprises of both natural and man-made nanostructures and nanocomposites. But carbon nanostructures are of particular interest due to their simple and inexpensive synthesis routes, huge availability, flexibility of size, variety of shapes and surface properties, as well as for their ultra-smart electronic properties. In general, three varieties of carbon nanomaterials are most popular as nano adsorbents in water treatment: Fullerenes (Sadegh and Ali 2019), carbon nanotubes (CNTs), and graphene (Machado et al. 2015).

13.2.1.1 Fullerene Based Nano Adsorbents

Fullerene is chemically C_{60} with 12 pentagons and 20 hexagons, with a carbon atom which has one π bond and two σ bonds at each corner of the shape to create a universal vertex. Structurally fullerene molecules are zero dimensional with sp^2 hybridization and have highly variable surface area ranging from 1.1 to 176 $m^2\ g^{-1}$. Various methods are utilized for the synthesis of fullerene clusters for example—laser ablation, hybrid plasma, thermal plasma, and combustion, etc. (Kroto et al. 1985; Wang et al. 2001). Surface area, charge, and other physicochemical properties may change depending on the synthesis method imparting unique features to these molecules. It is observed that fullerene clusters have very good adsorption properties because of its three attributes: large surface area available for interaction, groove space formed between nearby molecules, and the interstitial space (Machado et al. 2015).

Reports have shown that fullerenes have huge potential in adsorbing different organic pollutants such as organometallic compounds, naphthalene, aromatic hydrocarbons (polycyclic) 2,4-dinitrobenzene, etc. (Cheng et al. 2005; Yang et al. 2005; Ballesteros et al. 2000). It is found that the interaction between the pollutant and fullerene is physical in nature and much stronger than activated carbon in case of adsorbing organochlorine from aqueous solution. Berezkin et al. (2003) reported that fullerene molecules are more efficacious than activated carbon and soot materials for adsorption of organic compounds. It is reported that magnetic solid phase extraction based on fullerene and activated carbon adsorbent can be used for determination of azo dyes in water samples by capillary electrophoresis (Rodriguez et al. 2016).

13.2.1.2 Carbon Nanotube (CNT) Based Nano Adsorbents

CNTs are carbon allotropes where unique sp^2 carbon-carbon bonds impart superlative thermal, mechanical, and electrical properties and they are widely used in the field of optoelectronics, drug delivery, detection, therapy as well as wastewater management. Two different techniques are generally employed for the synthesis of CNTs. In case of high heat-based techniques, laser ablation is the popular method. Contrarily CVD (chemical vapor deposition) technique is utilized widely for

medium heat-based methods. These are considered as attractive nanoporous adsorptive materials because of having multiple pores. Moreover, during the formation of CNTs the folding of the carbon sheets into tubular form results in rehybridization of carbon orbital which ultimately incorporates newer surface charge and properties that inculcate better adsorption. Most of the kinds of non-covalent interaction like van der Waals forces, π-π stacking, electrostatic forces, hydrophobic interactions take part in CNT based adsorption of different substances from aqueous medium (Gupta et al. 2013). Further betterment can be achieved by surface engineering with different supportive chemical moieties to increase stability. Organic pollutants like 2-naphthol adsorption were successfully reported by core-shell CNT structures following the Freundlich model of adsorption isotherm (Xu et al. 2015). Another approach indicates incorporation of CNTs in diatom to remove organic dyes from contaminated water. Very recently Ihsanullah et al. (2015) reported removal of cadmium (Cd) pollutants from water with the help of acidified CNTs. Adsorption based removal of dyes from water can also be facilitated by different single-walled and multi-walled carbon nanotubes (SWCNTs and MWCNTs) depending on solution pH, concentration, and temperature. CNTs after modification with 8-hydroxyquinoline can be used to remove of Cu^{2+}, Pb^{2+}, and Zn^{2+} (Kosa et al. 2012).

13.2.1.3 Graphene Based Nano Adsorbents

Graphene family materials are also very popular adsorbents due to their unique characteristic features. Three dominant members of graphene family include graphene, graphene oxides, and reduced graphene oxide molecules. All these variants possess significant features and configuration making them suitable for adsorption. Graphene can also be used for detection of different contaminants because of its large surface area, which can accommodate different markers to identify the presence of several organic pollutants. Because of its distinctive chemical properties, the removal of environmental pollutants like dyes, phenolic compounds, and other organic contaminants from aquatic systems becomes easier (Sophia et al. 2016; Chowdhury and Balasubramanian 2014). For example, presence of large number of delocalized π electrons makes graphene attractable and suitable for further modification according to need (Liu et al. 2012).

Major limitation of large-scale graphene applications comes from the difficulties related to the preparation of individual isolated graphene sheet. To overcome this situation graphene oxide (GO) and reduced graphene oxide (rGO) molecules are new trends in the field. GO nanomaterials allow surface interactions due to π–π stacking favoring attachment of biological and non-biological markers for pollution identification (Xu and Zhu 2013). Similarly, rGO is having suitable surface area and porous structure making them good adsorptive material for environmental pollutants like organic dyes (Tiwari et al. 2013), thorium, (Pan et al. 2013) etc. Application of different forms of carbon based nanomaterials in adsorbing water pollutants is schematically shown in Fig. 13.1.

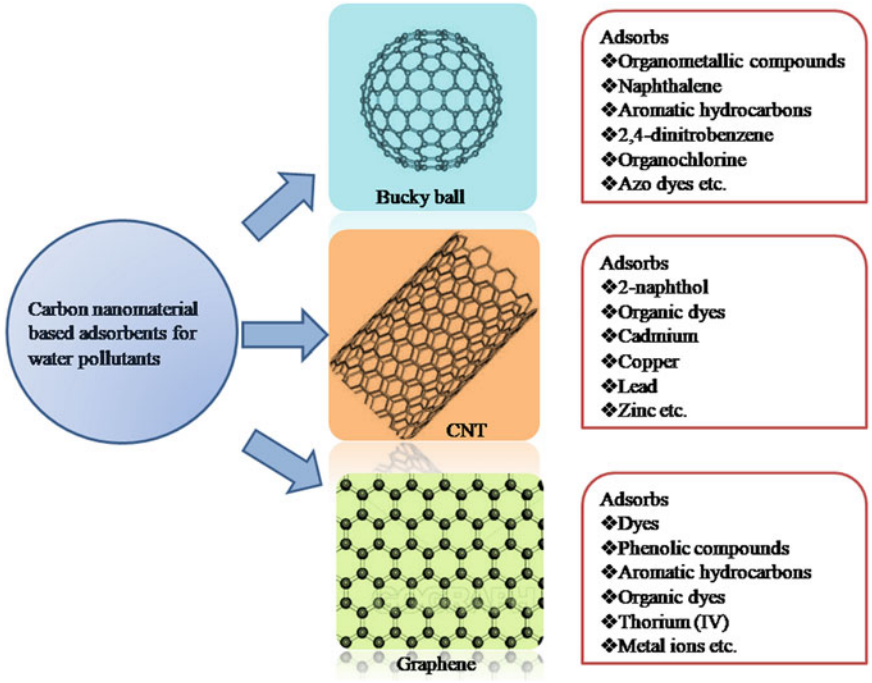

Fig. 13.1 Application of different carbon based nanoparticle as adsorbents

13.2.2 Metal and Metal Oxide Based Nano Adsorbents for Wastewater Treatment

Heavy metal based pollution of wastewater is a big threat today because of increased inflow of waste from industries like tanneries, batteries, and metal plating facilities and even from agricultural sector. Several popular methods like ion-exchange, precipitation, and membrane filtration have been exploited for heavy metal removal from water. The potential of adsorption with the help of metal and metal oxide nano adsorbents, is recently being explored for heavy metal removal from wastewater and they have the following advantages:

- Metal nano based adsorbents are chemically inert
- Non-toxic
- Pollutants can be removed from metal nano surface very easily
- Very small amounts of pollutants can be identified

Taman et al. (2015) showed successful removal of Cd^{2+} and Fe^{3+} ions from wastewater through copper oxide nano adsorbents. Similarly, arsenic toxicity can be controlled with the help of iron oxide nanoparticles. These iron oxide nanomaterials are not only good for reducing arsenic stress but also have a promotional effect on

overall growth of the plant (Praveen et al. 2018). Metal oxides based on titanium and zinc nano adsorbents are also widely used for remediation of water pollution. Titanium dioxide (TiO_2) nanomaterials have wide applications in water pollution control because of its photocatalytic property and cheap synthesis method (Bavykin et al. 2006). Zinc oxide (ZnO) nanostructures are also very promising for heavy metal removal from water. Study reported by Sheela et al. (2012), showed that ZnO nanoparticles (NPs) can be used as an adsorbent to remove Zn^{2+}, Cd^{2+}, and Hg^{2+} ions from aqueous solutions.

Mahmoud et al. (2015) reported efficacy of alumina adsorbents with immobilized 1-nitroso-2-naphthol for removal of pre-concentrators of Pb^{2+}, Cu^{2+}, Cr^{3+}, and Fe^{3+} from wastewaters. Silver NPs (AgNPs) are also potential adsorbent material for removal of pollutants from wastewater (Mohmaud et al. 2010; Fabrega et al. 2011).

13.2.3 Polymeric Nanomaterial Based Nano Adsorbents for Wastewater Treatment

Polymer-based nanomaterials are very unique in the field of pollution remediation because of their biocompatibility, easy synthesis, and unique redox properties. Amongst various approaches to remove heavy metals from water, adsorption promises to be the most effective because of its easy, economical, and simple technology. Polymeric nanostructures are now considered as good adsorbent material for heavy metal removal and other wastewater management strategies. For example, Mahmud et al. (2017) recently reported that polypyrrole (PPy) based polymeric nanopowder is very useful in removal of a number of heavy metal ions like arsenic, zinc, and cadmium from aqueous solution. Polymer-based hydrated manganese oxides (HMOs) can specifically attach heavy metals (Pb) and anionic legends like arsenate, phosphate, etc. (Tripathy and Kanungo 2005; Ren et al. 2011; Stroes-Gascoyne et al. 1987).

13.2.4 Nano Adsorbents for Treating Potable Water

Adsorption as discussed is by far the most useful technique to treat wastewater. However, these nano adsorbents can also be used for the treatment of potable water, which is becoming limited day by day. A large number of carbon based nano adsorbents are being used in potable water management. Non-carbon nano adsorbents are also equally popular. Several synthetic techniques like vapor deposition, laser ablation, arc discharge methods are employed for production of nanomaterial based adsorption systems.

Because of having high surface area and porosity carbon based nano adsorbents are highly suitable for treating a broad range of chemical pollutants including Cd^{2+}, Zn^{2+}, Pb^{2+}, benzene, etc. (Li et al. 2003) from drinking water. Both the varieties of carbon nanotubes MWCNTs and SWCNTs are very potent adsorptive material for water treatment (Madrakian et al. 2011; Yang et al. 2005). But the wide use of this

technology for potable water treatment has not become very much popular because of high cost (Yang et al. 2005). Among non-carbon based adsorptive materials AgNPs associated adsorbent fiber has an immense role in treating water because of their unique antimicrobial properties. WHO certified AgNP sheets as good adsorbents for water management (Dankovich and Gray 2011). Oxide NPs are also very efficient adsorptive materials. For example, zirconium oxide NPs embedded with amine-cross linked Shaddock Peel are very useful for removal of phosphate contaminants from water (Xing et al. 2017; Xu et al. 2015). Zero valent iron NPs are also promising adsorption material as iron nano adsorbents are very efficient in chromium removal from water (Wang et al. 2014).

13.3 Nanomaterial Incorporated Membranes for Water Treatment

According to WHO report (2019) worldwide 785 million people are facing the problem of unavailable drinking water, whereas more than 2 billion are suffering from the risk of scarcely accessible clean water. With gradual increase of human population as well as pollution level there is an urgent need to impose more stringent law related to wastewater treatment and newer strategies should be adopted to ensure availability of potable water to every inch of the globe. Conventionally, 'reverse osmosis' is the most popular method for water treatment. But it suffers from many limitations like high energy demand, reasonablly complicated procedure, and intolerable brine production. In this context, membrane distillation (MD) in amalgamation with nanotechnology can be a suitable alternative to the traditional water purification techniques (Wang et al. 2018; Gautam et al. 2019).

13.3.1 Nanofiber Membranes

Introduction of nanofiltration technology relied on introduction of a nanomaterial as a filtration material and is considered to be an intermediate between ultrafiltration and reverse osmosis. This technology has gained enormous popularity for its possible application to treat almost all kinds of water: brackish water, surface water, seawater, wastewater, groundwater, and even potable water. There are some important aspects to be considered while developing nanofiber based membrane distillation technology for wastewater treatment like membrane permeability, membrane stability in terms of chemical and mechanical stress as well as hydrophobicity. Nanofiber membranes are thin membranes for distillation purposes with suitable pore size, developed by electrospinning method that depends on generation of electrostatic field from a polymer solution where high hydrophobicity is also ensured.

Typically, electrospun nanofibers comprise of micron-sized pores appropriate for filtration mediated size exclusion of larger obnoxious particulate contaminants from

water. Gopal et al. (2006) developed heat treated electrospun nonwoven nanofiber membrane with bubble a limit of 4.6 μm which was suitable for successful exclusion of 7–10 μm particulate matter (Gopal et al. 2006, 2007). Nanofibers can be engineered to carry special functional groups to their surfaces for adsorption dependent microfiltration of heavy metal ions from water. Such nanofibers are referred to as "surface adsorption based electrospun membrane." Iron functionalized chitosan electrospun nano fiber (ICS-ENF) is such a membrane which can ultra-sensitively remove even trace amount of arsenic from wastewater (Min et al. 2016). This particular combination of iron-chitosan is far better than only chitosan electrospun fiber because of its potential to work with a 90% efficiency level for a wide range of pH (pH 4.3–7.3). The only disadvantage of this technology is its limitation to work in very high pH conditions where surface charge of electrospun membrane goes to a negative range, that in turn repels adsorption of negatively charged arsenic ions resulting in inefficiency of the technology to work at high alkalinity of contaminated water.

Purification of salty water to potable water is of very much need for geographical locations with limited availability of fresh water. In this scenario, nanofiber membrane distillation can be a suitable option where the membrane works as a separation unit of salty water and pure water. For example, Yalcinkaya et al. (2016) designed electrospun polyamide-6 nanofibers on polypropylene/polyethylene bicomponent spunbond nonwoven fabric as supporting substrate for piperazine or m-phenylenediamine (MPD) as separation layer to remove 97.4% $CaCl_2$ and 96.3% NaCl through thin film nano fiber composite (TFNC) of MPD-Triethylamine(TEA)-Synferol AH(Sy-AH). With this combination, salt rejection limit reached 98% level from sea water after consecutive three round treatment. Further progress in this technology was achieved from development of superhydrophobic electrospun PVDF nanofibrous membrane which was far better in maintaining water separation (Razmjou et al. 2012; Jiricek et al. 2016). The same group has also experimented with polyurethane nanofibrous membranes for sea water treatment with accuracy limit reaching 99% salt removal for all kinds of thickness of membranes tested (JilíIek et al. 2017; Jiricek et al. 2016).

13.3.2 Nanocomposite Membranes

Membrane based water treatments is well established technologies with significant limitations related to selectivity, permeability, etc. A new technology is very much in need to ensure water treatment. Nanomaterial and polymer moieties are combined to develop a new generation of membranes for wastewater treatment (Yin and Deng 2015). This membrane system has several valuable features added to the existing membrane based technology like hydrophilicity, porosity, charge density, and thermal and mechanical stability as well as novel functionalities and properties like antibacterial, photocatalytic, or adsorptive capabilities.

The concept of nanocomposite membrane originated for gas separation. However, this technology is now applied to an enormous extent starting from sensor applications, organic solvent nano separation to now wastewater management.

Nanocomposite membranes or nano enhanced membranes are generally designed based on the incorporation of nanomaterials as filler substance on a macroscopic platform or matrix. Thus, such combinations are also known as mixed matrix membranes (Ursino et al. 2018). A large number of different nano sized materials are being used in membrane technology to overcome several limitations like biofilm generation, fouling, porosity, etc. (Alpatova et al. 2013). CNTs are one of the most popular membrane materials so far. Due to their nano sized inner pores, selective permeability, pure water flux, lower fouling rate, and increased hydrophilicity, CNT based membranes are superior to traditional membranes. Moreover, surface modification of CNTs is a very simple addition of new features required for making it a more potent pollutant adsorbent membrane material (Fontananova et al. 2017).

TiO_2 nanomaterials are also widely used as nanofiber membrane, mostly in its rutile and anatase forms. This nanomaterial is also very popular because of its easy synthesis and cost-effectiveness. TiO_2 is a photocatalyst that yields a huge amount of reactive oxygen species like hydroxyl radicals, superoxide anions, and hydrogen peroxide, which makes them very effective as antimicrobial and virucidal material but at the same time they are very less toxic to human beings. Thus, TiO_2 NPs filled-membranes are very much effective for wastewater management with enhanced antifouling properties and a high flux recovery ratio (FRR %). Another advantage of using this material is its stability in terms of longevity and durability irrespective of water quality.

Antimicrobial application of AgNPs in the size range of 1–100 nm makes them very much suitable for nanocomposite membrane fabrication in amalgamation with polymers like chitosan, polyacrylonitrile, and polysulfone. Ag based nanocomposite membrane produces high biocidal activity, increases membrane permeate flux and hydrophilicity. Similarly, Cu compounds and Cu ion based nanocomposite membranes are also widely experimented in nanofiltration technology because of their antimicrobial efficacy (Xu et al. 2012). Incorporation of nano Cu in nanocomposite membrane ensures the change in pore size and surface properties that ultimately results in better hydrophilicity as well as a higher density of electrostatic charges along with biocidal functionalities (Ben-Sasson et al. 2016).

Apart from these many other nanomaterials like ZnO, GO, clay, Al_2O_3, SiO_2, Co NPs are also in use in nanocomposite based membrane fabrication.

13.3.3 Thin Film Nanocomposite Membranes

Thin film nanocomposite (TFN) membranes are a recent advancement in the field of membrane based wastewater treatment where ultrathin nanofilms are fabricated with different organic and inorganic materials for water flux and permeability properties. Yin et al. (2012) developed a porous MCM41 based thin film nanocomposite membrane with high retention capacity. This type of thin film has also shown improved hydrophilicity, roughness, higher zeta potential, and enhanced permeate water flux. Further improvisation can be incorporated with GO nanosheets as interlayer spacing agent, which can improve the water flux even better because

GO forms membrane channels that can augment permeability and separation ability. Freeze extraction technique for TFN construction is also found to be very promising. Zhang et al. (2016) proposed use of cellulose nanofibers with sub 10 nm pores. This filtration module can be applied for fast removal of NPs and substrates with diameters larger than 10 nm from water. Three-dimensional interconnected CNT sponge in ultrathin membrane can be utilized for removal of very minute amounts of methyl orange and rhodamine B molecules from water along with very tiny nanoparticulate matters like Au, TiO_2, Cd, etc. (Li et al. 2010).

Although a large number of researches are going on in nano and micro fabrication for wastewater management, still this technology suffers from many limitations. Fouling is one such issue which is a major challenge in making cost effective nano or ultra-thin membranes. Use of zwitterionic membranes are next generation materials to be considered to address the fouling issue with better grafting principles. Moreover, selectivity of desalination membrane also needs to be addressed. Recently, researchers in the field of water pollution management suggested the use of thin, detect free, and microporous support to overcome these limitations. Another limitation of the membrane based technology comes from the interference of different oxidants present in water like chlorine and ozone with membrane material like polyamide, etc. In most cases, this drastically reduces membrane durability. We are looking forward to the fabrication of multifunctional membrane with high flux, improved permeability, better separation, and equipped with antifouling, antimicrobial, and antiviral properties (Srivastava et al. 2004; Mahmoud et al. 2015).

13.4 Potential Application of Nanomaterial Based Photocatalysis for Water Treatment

In 1972, a breakthrough observation by Fujishima and Honda of photon mediated catalysis of water in the presence of TiO_2 molecules opened a new avenue for TiO_2 mediated photocatalytic degradations. The principle behind photocatalytic degradation in wastewater management relies on the light mediated breakdown of water contaminants into simpler forms of CO_2, H_2O, and other anions in presence of photoreactive materials. In nano domain, many materials possess such photoreactive properties. Because of the presence of a large band gap and less reactivity, nano TiO_2 is considered to be the most powerful nano photocatalyst. Almost all kinds of water contaminants (chemical and biological) can be treated with nano TiO_2. It is evident that under UV irradiation, nano TiO_2 is capable of degrading 14 different pharmaceutical wastes and this is why nano TiO_2 is now incorporated in wastewater treatment plants (Teixeir et al. 2016). Similarly, neodymium-doped TiO_2 hybrid NPs can efficiently remove contaminants from municipal waste (Shahmoradi et al. 2010). Apart from these other contaminants like pesticides, phenol, arsenic, and other toxic dye materials are also degraded and can be removed from wastewater under photocatalytic irradiation in presence of nano TiO_2 molecules (Nguyen et al. 2016; Alalm et al. 2015; Moon et al. 2014; Kim et al. 2016).

Another group of nanomaterials that is also used as photocatalyst are magnetic NPs. Gallic acid coated magnetic NPs can very specifically destroy pharmaceutical waste with high accuracy (Nadim et al. 2015). A core-shell gallic acid magnetic NP can effectively eradicate meloxicam, a drug from a water sample. Nanoconjugate of zinc oxide-polyvinylpyrrolidone NP is also proven to be very effective in the degradation of toxic dye from wastewater (Sidik et al. 2018). ZnO nanomaterial is also a very potential photocatalyst and is advantageous over many other semiconductor materials because of their large band gap. Further surface modification of nano ZnO can improve its photocatalytic activity (Uddin et al. 2012; Pant et al. 2012). This is why nano ZnO is incorporated within new age membrane based photocatalytic systems for removal of industrial dye (Hairom et al. 2014). Another advantage of using nano ZnO is their potent antimicrobial and antifungal activity making them useful in removal of microorganism based contaminants from water. Co-sputtering of Ag and ZnO NPs on glass substrate using the radio frequency technique enables the system to be useful for wastewater treatment. Depending on the chemical nature of pollutant in water, silica coated nano ZnO composites can economically provide better degradation of pollutants in comparison with nano TiO_2 composite based filtration system (Maučec et al. 2018). For removal of organic pollutants, Thomas et al. (2018) developed Sm^{3+}-doped graphitic carbon nitride nanosheets. Using thermal condensation approach, carbon and oxygen rich graphitic carbon nitride can be synthesized which has improved photocatalytic properties (Chan and Yu 2018).

13.5 Disinfection of Water with Antimicrobial Nanoparticles

Availability of potable water with ever increasing population is one of the major concerns globally. As per the WHO directive, chlorination is the most common method for removal of pathogenic microorganisms from water. This chemical treatment of water has major disadvantages as it changes taste, smell, and other qualities of drinking water.

In this context, the application of biosafe nanomaterials with antimicrobial properties can be used for treatment of potable water. These types of nanomaterials must have the following attributes:

- Easy synthesis
- Biocompatible
- Chemically inert
- High surface area to provide maximum biointeraction
- Economical
- Broad spectrum antimicrobial and antifungal properties

Several nanomaterials are available for combating microbial pathogens. Their antimicrobial action is mostly based on

- Intervention with microbial cell wall structure
- Oxidative stress generation
- Replication inhibition of microbial genetic machinery

ZnO NPs are well known for their antibacterial and antifungal efficacy. The generation of reactive oxygen species (ROS) is the major mechanism of nano ZnO based antimicrobial activity. The advantage of ZnO based nanostructure is their ability to interact with both bacterial membrane and the genetic core structure. Normally ZnO NPs are considered to be safe for the mammalian system, for which these nanomaterials are very popular in food industry and environmental applications. ZnO NPs are proved to highly effective against a wide spectrum of microbes like pathogenic *Staphylococcus aureus* and *Escherichia coli*. Different studies have reported that ZnO nanostructures have a potentially detrimental effect on both gram-positive and gram-negative strains relevant to water quality because of ROS and Zn^{2+} generation (Zhang et al. 2016). It is also observed that nano ZnO in the size range of 18–21 nm is highly effective against *S. epidermis*. Although the relationship between size and antimicrobial activity of ZnO NPs is yet to be established, the common observation is that there is a positive correlation between scaling down the size of nano ZnO and its antimicrobial efficacy (Nair et al. 2009).

AgNPs are also widely used for wastewater treatment nowadays. These are most commonly used for the removal of coliform microbes from wastewater (Jain and Pradeep 2005). Silver NPs of different sizes, shape, and surface areas are tested and are found to have high antimicrobial activity (Moran et al. 2005). Silver NPs are used in different antimicrobial filtration setups as coating material for their unique broad spectrum antifungal and antibacterial efficacies (Tiwari et al. 2008).

Mg and MgO NPs are also having biocidal efficacy against gram-positive and gram-negative bacteria like *E. coli* and *Bacillus megaterium,* and it can even control bacterial spores of *B. subtilis* (Stoimenov et al. 2002). Polyethylenimine NPs are also having bactericidal effect against *Streptococcus* mutants (Park 1998). All these antimicrobial NPs discussed here are now being utilized in the municipal waste management system (Dimapilis et al. 2018).

13.6 Detection of Waterborne Pathogen with the Help of Nanoparticles

Nanoparticles play a major role in cutting edge disease detection and therapy and can be used for detection of water borne pathogens as well (Vikesland 2018). Major reasons for which nanomaterials are preferred for detection are

- Small sample volume
- Chance of false positive signaling is less
- Surface modification can ensure desirable features of specific biosensors
- Various optically active nanostructures are available for detection
- Less requirement of high throughput instrumentation facilities for detection
- Less labor intensive

13.6.1 Optical Nanosensors for Detection of Waterborne Pathogens

Many of the metal and metal oxide NPs are optically active. Surface functionalization of such nanomaterials makes them perfect for the detection of waterborne pathogens with simple colorimetric assays. For example, the gold nanomaterial is surface plasmon active and responds to irradiation at a specific wavelength and makes it a useful detection tool for various medical conditions. Silver nanostructures are not only used in removal of microbial pathogens from water but also can be used for biosensing (Moustafa 2017). Zhou et al. (2014) reported the use of SERS active silver nanomaterial for the detection of water borne infections in living conditions.

13.6.2 Magnetic Nanosensors for Detection of Waterborne Pathogens

Magnetic nanomaterials are very popular in sensing devices because of their specialized features responsive in magnetic field. Josephson et al. (2001) reported the detection of oligonucleotide sequences using supermagnetic nanosensors. Magnetic nanosensors can also detect the molecular interactions occuring at very low levels using magnetic resonance imaging technique (Perez et al. 2004). Identification of infectious *E. coli* strain became easier with the ultrasensitive technique by an amalgamation of multiparametric magneto-fluorescent nanosensors (Tuhina et al. 2016; Banerjee et al. 2016).

13.6.3 Eletrochemical Nanosensors

Electrochemical nanosensors are composed of highly sensitive nano electrodes that are ultrasensitive and can detect minute changes in potential. These sensors can be used for water quality assessment as well. For example, *E. coli* strain O157:H7 identification was reported by Nguyen et al. (2018) using the immune-detection technique. Similarly, aptamer immobilized electrochemical nanosensors based on amine functionalized metal-organic framework can be utilized for *E. coli* detection in wastewater (Shahrokhian and Ranjbar 2018). Polyaniline grafted gold nanomaterial based voltammetric sensors are also reported for oligonucleotide based pathogen detection (Shoaie et al. 2018). Beeman et al. (2018) reported ultraviolet treatment and detection of water borne *E. coli*.

13.7 Conclusion

Water is one of the major constituents of life and its quality needs to be maintained at any cost for our survival. Nanomaterials are nowadays taking a key role in water pollution detection and management. This chapter focused on the utilization of

nanomaterial based techniques to determine potable water quality and manage water pollution as a whole. A large number of nanomaterials are involved in removal of heavy metals like As, Pb, Cr, etc. Many nanomaterial based filtration devices have become popular to control water pollutants. Nanomaterial based adsorptive platforms are also in use for their selectivity and controllable pore size. Due to the inherent property of surface reactivity of nanosized materials, these can rapidly interact with microorganisms and this is why these are now broadly used for the removal of microbial contaminants. Nanomaterials are very easy to engineer according to the need for management of water pollution. It is very common to use nanomaterials for the identification of undesirable biofilms in water body or water storage facilities.

As the size range of these structures is between 1 and 100 nm, these can conveniently enter the systemic level and there the impact of overexposure to nanomaterials needs to be evaluated thoroughly. Although many studies are going on at different level for nanotoxicity assessment, safe exposure limit to different nanomaterials is yet to be established. Biocompatibility and biodegradability are also major parameters that we need to consider for nanostructure based environmental pollutants removal. It is not recommended to apply any non-biodegradable substance even for sequestration of water pollutants. Although application of nanoscience in wastewater management is a promising science, we always need to be very much convinced about the technology before we commercially use them for water pollution management.

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Efficiency of Graphene-Based Forward Osmosis Membranes

14

Hanaa M. Hegab, Ranwen Qu, Christopher P. Saint, Linda Zou, Deepak Pant, Milena Ginic-Markovic, and Ahmed ElMekawy

Abstract

Although forward osmosis (FO) has escalating prospective applications, several key challenges are still facing this technology, such as membrane fouling, concentration polarization and reverse solute flux. This necessitates the development of novel membranes by integrating nanomaterials that possess extraordinary features such as the evolutionary Graphene (Gr) family nanomaterials. Gr-based nanomaterials have promising ability to be integrated with FO membranes, to enhance water flux and overcome the challenges of FO technology, i.e. reverse solute flux and fouling. In this chapter, the fabricated Gr-based membranes for FO application were evaluated, analysed and compared, particularly the performance of different fabrication strategies including surface modification, free standing and bulk fabrication. Accordingly, here we critically review all the studies

H. M. Hegab · R. Qu
Department of Chemical Engineering, Monash University, Clayton, VIC, Australia

C. P. Saint · M. Ginic-Markovic
Future Industries Institute, University of South Australia, Adelaide, SA, Australia

L. Zou
Natural and Built Environments Research Centre, University of South Australia, Adelaide, SA, Australia

Department of Chemical and Environmental Engineering, Masdar Institute of Science and Technology, Abu Dhabi, United Arab Emirates

D. Pant
Separation and Conversion Technologies, VITO-Flemish Institute for Technological Research, Mol, Belgium

A. ElMekawy (✉)
Genetic Engineering and Biotechnology Research Institute, University of Sadat City (USC), Sadat City, Egypt
e-mail: ahmed.elmekawy@gebri.usc.edu.eg

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performed to integrate Gr materials with FO membranes, focusing on membrane efficiency including water flux and reverse solute flux, in addition to antifouling ability. Moreover, the different strategies for Gr materials integration are assessed.

14.1 Forward Osmosis (FO) Basic Concepts

The problems of potable water scarcity and escalating water needs have become a severe global issue owing to economic development and fast population growth. To overcome this challenge, the desalination of seawater was suggested as one of the most promising strategies to provide substitutable water resources (Shannon et al. 2008; Chung et al. 2012a). Even though several techniques have been developed for water desalination, e.g. distillation, pressure driven membrane processes (PDMPs) and solvent extraction, they consume high energy which limits any further improvement. This is why the osmotically driven membrane process (ODMP), which employs the driving force of the osmotic pressure variance through a permeable membrane, has attracted increasing attention in the last few years (Shen et al. 2016). ODMP has the ability to solve the problems of global water shortage by integration into water treatment processes (Shaffer et al. 2015). This has encouraged the performance of extensive research into the utilization of forward osmosis (FO) technology in environmental and industrial wastewater treatment. FO membrane technology has several applications in different fields such as wastewater treatment (Cath et al. 2005a; Holloway et al. 2007), desalination (Chung et al. 2012b), osmotic power generation (Achilli and Childress 2010; Yip et al. 2011; She et al. 2012), pharmaceutical industry (Yang et al. 2009) and food processing (Garcia-Castello and McCutcheon 2011). Modified FO membranes could be applied in different types of bioreactors to overcome biofouling challenge. A traditional membrane bioreactor (MBR) can employ osmotic membrane bioreactor instead of using micro/ultrafiltration membranes (Adnan et al. 2019). The FO-based MBRs are novel biotechnological systems which require low tendency biofouling FO membrane. Moreover, FO membrane was integrated into microbial fuel cells (MFCs) to enhance their wastewater treatment potential, which are known as osmotic MFCs (Yang et al. 2016).

The concept of FO technology depends on separating two solutions with dissimilar concentrations using a semipermeable membrane (Fig. 14.1). One of these solutions is a concentrated draw solution (DS) and the other is a more diluted feed solution (FS) (Shaffer et al. 2015), in which the water in the FS permeates through the membrane to the DS (water flux (J_w)) (She et al. 2015), and sometimes the draw solutes in the DS diffuse to the FS in a reverse direction (reverse solute flux (J_s)) to the water permeation (Tang et al. 2010; Xiao et al. 2011). The extents of J_w and J_s are influenced by the internal concentration polarization (ICP) which occurs inside the membrane porous support layer, in addition to external concentration polarization (ECP) close to the membrane surface. Both of ICP and ECP can result in the decline of operative driving force in terms of the osmotic pressure (concentration) difference

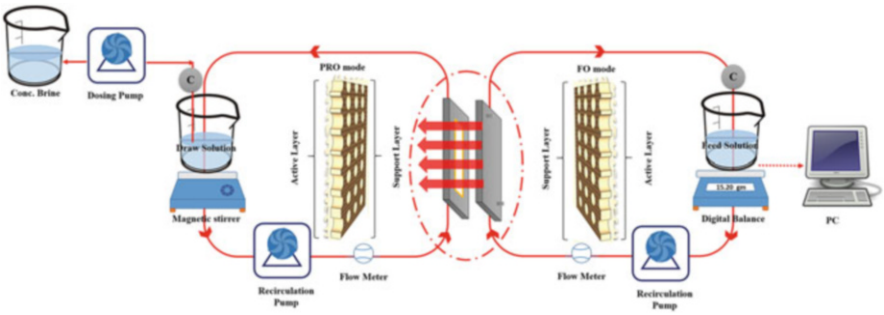

Fig. 14.1 Schematic drawing of FO and PRO setups

through the membrane (Mehta and Loeb 1978a; Tang et al. 2010). The specific J_s has been proposed to measure the membrane selectivity, and is calculated from the ratio of the J_s to the forward J_w, in which a greater ratio indicates a decline in membrane selectivity and a lower FO productivity (Zhao et al. 2012). Accordingly, the FO performance can be evaluated by these parameters; membrane selectivity and permeate flux.

By applying the osmotic pressure difference to energize the transportation of water through the membrane, FO can overcome numerous problems of hydraulic PDMPs (Shaffer et al. 2015). As there is lower or no hydraulic pressure needed for FO technology compared to PDMPs (e.g. RO, nanofiltration (NF), microfiltration (MF) and ultrafiltration (UF)), it shows several potential benefits including reduced energy consumption (generally used for pumping the FS and DS solutions) (McGinnis and Elimelech 2007), low fouling tendency (Achilli et al. 2009; Mi and Elimelech 2010b) and enhanced water recovery, compared to RO membranes (Martinetti et al. 2009).

Efforts were conducted for the development of thin-film composite (TFC) FO membranes as they display increased J_w and decreased J_s, particularly in contrast to the commercial cellulose triacetate (CTA) FO membranes produced by Hydration Technology Innovations Inc. (HTI™ Co., Albany, OR) (Wei et al. 2011; Puguan et al. 2014). A TFC-FO membrane normally comprises a thin active layer (i.e. polyamide (PA)), in control of high salt rejection, backed by a porous mechanical support layer, for reliable water permeation (Bui et al. 2011; Wei et al. 2011; Han et al. 2012a; Bui and McCutcheon 2013; Widjojo et al. 2013; Emadzadeh et al. 2014a; Wang et al. 2015). Several FO membranes, in both flat sheet and hollow fibre modules, have been fabricated in the last half century in which by ICP and the thickness of membranes were reduced (Shi et al. 2011; Widjojo et al. 2011). The most common one was that commercialized by HTI where the membrane is prepared using CTA reinforced by an implanted polyester screen mesh (Cath et al. 2005b). Thin-film porous hollow fibre supported with an extremely thin RO-like surface layer was prepared by Wang et al. (2010), while ester-based FO membrane

supported with dual selective surface layers was fabricated (Zhang et al. 2010). However, the characteristic physicochemical surface features of the PA TFC membranes accelerate biofouling to be a major problem (Mi and Elimelech 2010b; Ang et al. 2011) and their performance, including J_w and J_s, also needs to be optimized compared to that of PA TFC membranes applied in RO (Greenlee et al. 2009).

14.2 Challenges of FO Technology

Although FO technology has achieved a number of advancements, it faces some key challenges, which highpoints the need for the enhancement of membrane design to decrease membrane fouling and diminish the loss of draw solutes (McGinnis and Elimelech 2007; Achilli et al. 2009; Mi and Elimelech 2010b). These limitations are related to the concentration polarization (CP), membrane fouling and J_s, which are illustrated in Fig. 14.2.

14.2.1 Concentration Polarization

CP is the result of the concentration variation between the FS and the DS in ODMPs across an asymmetric FO membrane. Two types of CP can be distinguished in FO systems; ECP and ICP. In general, ECP takes place at the surface of the membrane's dense active layer, while ICP takes place inside the membrane's porous support layer. The negative effect of ICP on the J_w of FO membrane is greater compared to

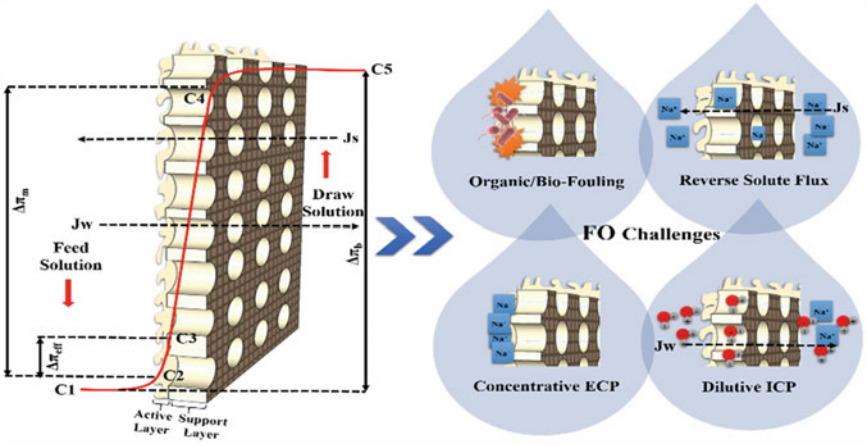

Fig. 14.2 Schematic illustration of FO technology challenges including ICP, ECP, fouling and J_s

that of ECP (Cornelissen et al. 2008; Hancock and Cath 2009). Two types of ECP can occur depending on the membrane orientation; the first is the concentrative type which takes place upon the facing of the membrane support layer to the DS, while the other is the dilutive type which takes place as the membrane support layer faces the FS. ECP results in reducing the osmotic pressure by the side of the membrane active layer surface on the DS part. When the FS concentration is quite low in the FO mode, the concentrative ECP coefficient can be demonstrated according to Eq. (14.1).

$$\frac{\pi_{m-\text{feed}}}{\pi_{b-\text{feed}}} = \exp\left(\frac{J_w}{k_{\text{feed}}}\right) \tag{14.1}$$

where $\pi_{\text{b-feed}}$ is the osmotic pressure of the FS in the membrane bulk, while $\pi_{\text{m-feed}}$ is the osmotic pressure of the FS at the membrane surface. k_{feed} is the mass transfer modulus on the feed part. Likewise, the dilutive ECP coefficient in FO mode can be indicated by Eq. (14.2).

$$\frac{\pi_{m-\text{draw}}}{\pi_{b-\text{draw}}} = \exp\left(-\frac{J_w}{k_{\text{draw}}}\right) \tag{14.2}$$

where $\pi_{\text{b-draw}}$ is the osmotic pressure of the DS in the membrane bulk, while $\pi_{\text{m-draw}}$ is the osmotic pressure of the DS at the membrane surface. k_{draw} is the mass transfer modulus on the draw part. The water flux in FO mode can be demonstrated by Eq. (14.3) (Cath et al. 2006).

$$J_w = A\,(\sigma\Delta\pi - \Delta P) \tag{14.3}$$

where A is the water permeability factor of the membrane, σ is the reflection modulus, $\Delta\pi$ is osmotic pressure change through the membrane and P is the employed hydraulic pressure. The difference ($\sigma\Delta\pi - \Delta P$) embodies the operative driving force. In FO mode, the driving force is due to the osmotic pressure change between the DS and the FS, and therefore, the J_w can be indicated by Eq. (14.4).

$$J_w = A\,(\pi_{\text{draw}} - \pi_{\text{feed}}) \tag{14.4}$$

where π_{draw} is the osmotic pressure of the DSs, while π_{feed} is the osmotic pressure of the FSs.

ICP is one of the key phenomenon in ODMPs, where the J_w decline in FO mode is mainly attributed to ICP (Mehta and Loeb 1978a, b; Gray et al. 2006; McCutcheon and Elimelech 2006), leading to more than 80% reduction in J_w (Mehta and Loeb 1978a, b). Both types of ICP (dilutive and concentrative ICPs) can take place inside the membrane support layer according to the membrane alignment. Dilutive ICP will develop when the DS is employed opposed to the membrane support layer, where the water infiltrates across the membrane from the FS side to the DS side. In the opposite membrane alignment, where the FS is in front of the membrane support layer, the FS solutes accumulate inside the support layer of the membrane leading to the development of concentrative ICP. In FO mode, where the DS is facing the

membrane support layer, the dilutive ICP takes over the J_w, which can be indicated by Eq. (14.5) (Loeb 1997).

$$J_w = \frac{1}{K} \ln \frac{A\,\pi_{\text{draw}} + B}{A\;\pi_{\text{feed}} + B + J_w} \tag{14.5}$$

where K is termed as the solute resistivity, and it measures the salt passage to the membrane support layer, while B is termed as the solute permeability modulus of the membrane.

To mitigate ICP and increase water flux, the draw solute diffusion passing the support layer should be controlled. The characteristics of the FO membrane support layer are very vital and have a huge impact on ICP. The perfect support layer is expected to have low structural parameters including high porosity with low tortuosity and very thin layer, which will reduce the diffusion path of draw solute. Membrane hydrophilicity can also minimize ICP. The hydrophobicity of the support layer, like polysulfone (PSf) and polyester (PE), reducing the operative porosity of the support layer (McCutcheon and Elimelech 2008). Furthermore, the membrane characteristics can be defined through the structural parameter S (Eq. (14.6)) (Gerstandt et al. 2008; Yip et al. 2010).

$$S = KD = \frac{t_s\,\tau}{\varepsilon_{\text{eff}}} \tag{14.6}$$

where D represents the solute diffusion coefficient, t_s is the thickness of the support layer, τ is the tortuosity of the support layer and ε_{eff} is the actual porosity of the support layer. The scientific research focused on FO membrane has been quickly applied to commercially available products such as OasysWater Inc., Boston, MA; HTI TFC-FO membrane modules, which have low structural parameters around 500 μm (Phillip et al. 2010; Yip et al. 2010; Cath et al. 2013; Tiraferri et al. 2013). The development of new commercial FO membrane and its effect on water flux are illustrated through A, salt permeability, B, and S values, using 1M sodium chloride draw solution and deionized water feed solution.

14.2.2 Membrane Fouling

Membrane fouling is the main and expected problem in all membrane-based processes (Lee et al. 2010; Tang et al. 2011), hence lesser membrane fouling entails high water productivity, less need for cleaning and extended membrane lifetime, thus decreasing capital and operational expenditure. Nevertheless, membrane fouling in ODMPs is not the same as that in PDMPs owing to the absence of hydraulic pressure being applied in the first type of process. It was observed that intermolecular adhesion and organic fouling in FO mode are closely interrelated, showing the main function of this interaction in developing the organic fouling and the subsequent cleaning process, where the membrane material plays a significant role (Mi and Elimelech 2008, 2010a, b). The FO membrane fouling is also controlled by

the dual effect of chemical and hydrodynamic interactions. The organic and inorganic FO membrane fouling are almost entirely reversible by water washing, due to the less compressed fouling layer where there is no hydraulic pressure employed. However, the J_s from the DS can cause the accelerated formation of FO fouling cake layer based on osmotic pressure. Upon the facing of the DS to the membrane support layer, the accumulation of draw solutes can occur at the surface of the membrane active layer via reverse diffusion, improving the concentration polarization and lowering the net osmotic energetic force (Lee et al. 2010).

14.2.3 Reverse Solute Flux

The solute reverse diffusion from the DS across the membrane to the FS in ODMPs is also expected due to the concentration variances and is strongly related to the membrane fouling. Several multivalent ions, such as Ca^{2+} and Mg^{2+}, can interact with the reverse diffusion foulants in the FS, which is likely to augment the membrane fouling (Zou et al. 2011). Moreover, the solutions of multivalent ions can also lead to the development of more severe ICP due to their lower solution diffusion coefficients and greater ion sizes (Zhao and Zou 2011). In general, TFC-FO membranes possess temperate selectivity as compared to the innovative seawater TFC-RO membranes (Fritzmann et al. 2007; Greenlee et al. 2009), because of the high frequency of J_s in FO membrane processes, which also speeds up the onset of FO membrane fouling (Zou et al. 2011).

14.3 Water Treatment with Graphene (Gr)

The integration of hydrophilic nanomaterials, e.g. modified carbon nanotubes (Wang et al. 2013), titanium dioxide (TiO_2) (Emadzadeh et al. 2014a, b) and porous zeolite nanoparticles (Ma et al. 2013), within the support layer of the TFC-FO membranes has improved the membrane performance. The structural and physical integrations of these nanomaterials with the membrane support layer escalate its hydrophilicity and/or porosity, leading to a remarkable enhancement in the J_w of the modified TFC-FO membranes and simultaneously lower the phenomenon of ICP (Han et al. 2012b; Ong et al. 2015).

Driven by their attraction to develop the advantages of incorporating nanomaterials, researchers were interested in integrating Gr derivatives into different water filtration membranes. As the flux through a membrane is inversely proportional to its thickness, the water permeability of extremely thin membranes should greatly improve. Gr is two dimensional (2D) one atomic layer thickness of sp2-hybridized carbon (Kranbuehl et al. 2011; Georgakilas et al. 2012; Polschikov et al. 2013). This advantage makes it a very promising material for FO membranes. Moreover, due to its outstanding strength (1 TPa) (Lee et al. 2008; Singh et al. 2011), porous Gr can be employed as a standalone material for FO membrane without the

need for a support layer, and therefore, displays zero ICP when external hydraulic pressure is not applied.

One derivative of Gr is the Graphene oxide (GO) which is a promising material for the modification of the support layer of TFC-FO membranes because of its exceptional properties, as it profusely encloses oxygenous functional groups, e.g. epoxy, carboxyl and hydroxyl groups (Hu and Mi 2013). GO nanosheet is a 2D single-layer one atomic thick, which ranges from 1 to 2 nm (Stankovich et al. 2006; Hu and Mi 2013). GO nanosheets can develop superior interaction with the polymer matrix as compared to other types of materials due to their tremendously high surface area-to-volume ratios (Stankovich et al. 2006). In contrast to conventional membranes, which work by separating the solutes from FS as water permeates across the pores of the membrane active layer, a GO membrane depends on the nanointerlayer gaps to filter water as it meanders within the deposited GO layer on a support substrate. The long path length, as a result of the capillary effect, triggers the water to move almost with no friction (Kannam et al. 2012; Nair et al. 2012), which is considered as a favourite material feature for producing high flux membranes.

These exceptional physical and chemical features of GO nanosheets are promising for producing novel composite materials which possess distinctive structure, strong hydrophilicity, great chemical stability and improved antifouling ability (Stankovich et al. 2006; Hu and Mi 2013; Lee et al. 2013; Mi 2014; Perreault et al. 2014). However, the antibacterial was completely negated when GO was covered up inside the bulk polymer matrix as compared to that being deposited onto the membrane surface (Liu et al. 2011; Tu et al. 2013). Earlier studies have combined the GO with the selective layer of the membrane via layer-by-layer self-assembling (LbL-SA) method (Choi et al. 2013; Kim et al. 2013) or chemical crosslinking (Perreault et al. 2014), and obtained enhanced separation and water permeability, antifouling ability and chlorine resistance. Nevertheless, these modified membranes may experience the decline of water permeability over time as the GO layer can interfere with water infiltration (Chae et al. 2015).

The nanochannels created in 2D GO or reduced graphene oxide (rGO) nanosheets membranes allow the permeation of ions and gases with tinier sizes than those of the formed channels, while obstructing all other larger varieties (Mi 2014). For instance, even though GO membrane in dry state (void spacing = 0.3 nm) is not penetrable to most gases, water vapour can permeate easily, rendering it a superlative membrane for the selective removal of water (Nair et al. 2012). On the other hand, the exposure of GO-based membranes to aqueous solution caused the hydration effect to increase the spacing between GO nanosheets (0.9 nm), which in addition to the destabilization of the membrane, reduced its selectivity, where tiny hydrated ions (e.g. Mg^{2+}, K^{+} and AsO_4^{3-}) can penetrate the expanded nanochannels (Joshi et al. 2014). Alternatively, rGO membranes displayed superior water stability as they can keep the dense interlayer spacing owing to the considerable low amount of hydrated functional groups (Mi 2014). Yet, the main problem of rGO-based membranes for water separation is the elevated transport resistance caused by the narrow interlayer spacing.

14.4 Gr-Based FO Membranes

FO membrane is the core of effective and productive FO processes with improved separation performance. The applicable FO membrane should possess several improved features in terms of solute rejection, stability, water permeability and antifouling ability (Shen et al. 2016). Although Gr-based materials were successfully applied in the enhancement of FO membranes' properties, most of these studies still suffer from the absence of a practical technique for anchoring the Gr nanosheets to the membrane surface. In the following sections, we will review and analyse the trials of integrating Gr derivatives with FO membranes elucidating how those modified membranes were fabricated and compare their performances.

14.4.1 Fabrication and Modification

Researchers have fabricated different types of FO membranes, comprising TFC (Sukitpaneenit and Chung 2012; Liu et al. 2015a; Wang and Xu 2015; Wang et al. 2015), cellulosic (Su et al. 2010; Zhang et al. 2011; Ong and Chung 2012) and LbL-SA membranes (Saren et al. 2011; Qi et al. 2012). TFC membranes were developed by the creation of a PA layer on a substrate by interfacial polymerization (IP) method, and since then, have attracted much attention for different applications of water treatment due to their outstanding separation process under a wide range of operational conditions, e.g. pH and temperature. Nevertheless, traditional TFC membranes suffer from several problems regarding low J_w and fouling tendency attributed to their comparative hydrophobic surfaces due to the presence of trimethyl chloride (TMC) and m-phenylenediamine (MPD) monomers (Rana and Matsuura 2010). Several studies were conducted to overcome these problems by using suitable modification techniques, e.g. submerging the emerging PA membrane into active solvent (Li et al. 2013; Zhang et al. 2013), applying surfactants or additives in the monomer solutions (Cui et al. 2014) and grafting zwitterions (Azari and Zou 2012; Yu et al. 2014; Mi et al. 2015), the hydrophilic polymer chains (Bernstein et al. 2011; Lu et al. 2013) or nanoparticles (Tiraferri et al. 2012) on the membrane surface of the PA layer.

GO nanosheets are one of the nanomaterials that have been integrated with FO membranes through several techniques ranging from surface modification to bulk fabrication. GO was successfully used to modify the membrane surface via three different techniques. The first approach depends on the development of a covalent bond between the GO nanosheets and the PA TFC membrane by chemical modification (Perreault et al. 2014), while the second technique depends on formation of non-covalent attachment of GO nanosheets to the membrane surface via LbL-SA method (Hu and Mi 2014; Kang et al. 2019). The third approach uses an adhesive polymer to attach GO nanosheets by one pot reaction (Hegab et al. 2015a, b). These techniques of surface modification comprise different types of materials, e.g. photocatalytic nanoparticles such as TiO_2 (Dreyer et al. 2010; Koinuma et al. 2012) and carbon-based nanomaterials (Perreault et al. 2014; Hegab et al. 2015a),

macromolecules (Kim et al. 2003; Mo et al. 2007; Greenlee et al. 2009; Tiraferri et al. 2011) and biocidal nanoparticles such as silver nanoparticle (AgNPs), either combined with the support layer (Hu and Mi 2013; Hegab et al. 2015b) or modifying the TFC membrane surface.

GO has also been mixed with the polymeric matrix to fabricate different bulk nanocomposite membranes, such as polyvinylidene fluoride (PVDF) microfiltration/ultrafiltration (Wang et al. 2012; Xu et al. 2014; Zhao et al. 2014), polyether sulfone (PES) nanofiltration membranes (Zinadini et al. 2014) and polysulfone (PSf) (Lee et al. 2013).

One of the main drawbacks of the blending technique is the absence of GO direct exposure to foulants, which restricts the interaction between foulants and GO. Accordingly, the superior GO features, in terms of antifouling, will not be entirely exploited. The direct deposition of GO nanosheets on the membrane surface is a more effective technique for fouling control, hence the GO/foulants interaction can be maximized. The exceptional structure of GO confers a considerably greater contact area for the convenient interaction between GO and the foulants. Therefore, GO could be grafted on the surface of a PA membrane (Perreault et al. 2014) or stacked along with the amine-functionalized GO by electrostatic interactions (Choi et al. 2013) to improve the membrane antifouling property against bovine serum albumin (BSA) and bacterial cells.

Polymers were used in several studies to stabilize the GO on the membrane surface. GO nanosheets were applied to modify the PA selective layer of the TFC-FO membranes using a poly L-Lysine (PLL) through two different approaches; LbL or hybrid grafting techniques (Hegab et al. 2015a). Moreover, a novel technique for attaching GO nanosheets on the TFC-FO membrane surface was studied employing the polydopamine (pDA) bioadhesive polymer, where the pDA was deposited via self-assembly in addition to oxidative polymerization, which result in the reduction of GO nanosheets and their immobilization on the membrane surface (Hegab et al. 2016a). GO nanosheets were also immobilized onto the surface of FO TFC membranes via a single-step surface modification, including the self-assembly and oxidative crosslinking of tannic acid (TA) and polyethylene imine (pEI) (Hegab et al. 2016b).

Aside from the polymer-based surface modification of FO membranes, silver nanoparticles (AgNPs), as an effective biocidal material, was applied as a FO surface modifier (Soroush et al. 2015). The complications of using biocidal nanoparticles include their propensity to agglomerate and their release into the surrounding aqueous environment as they detach from the surface. The best strategy to overcome these problems is to apply carbon nanocomposites, instead of employing one type of nanoparticle (Cristiani and Perboni 2014). In one trial, AgNPs-GO nanosheets were attached to the PA surface, via covalent bonding, to improve the membrane hydrophilicity and biocidal features. AgNPs were formed in situ by using the wet chemical reduction method to reduce silver nitrate on the GO nanosheets surface (Yin et al. 2013), which was then attached to the surface of TFC-FO membranes by covalent bonding. Similarly, in another study, the AgNPs were synthesized by the same method including the utilization of GO nanosheets, and then was attached to the

surface of TFC-FO membrane to improve the antibacterial properties of the membranes (Soroush et al. 2016). GO nanosheets are suitable for attachment with Ag^+ ions in order to control the shape, size and distribution of the synthesized AgNPs, due to the presence of oxygenous functional groups in GO. The employment of GO leads to the production of small, uniform, stable and distributed AgNPs in addition to improved silver loading and ion release control.

Several applicable and effective techniques for the fabrication of freestanding ultrathin rGO membranes were reported. One of these techniques was exemplified by Liu et al. (2015b), in which a reinforced GO membrane was shaped on a mixed cellulose ester (MCE) filter through the filtration of diluted GO dispersion. The formed GO/MCE membrane was then positioned over a hydriodic acid solution, where the GO membrane rapidly changed to a black colour, confirming the reduction of GO to rGO by the acid vapour. Another strategy was followed to achieve freestanding membrane including stacking GO nanosheets with acryl binder and utilizing the (UiO-66) metal-organic framework intercalated into the GO nanosheets, tuning the d-spacing by adding potassium cation to co-polymerized GO (Balapanuru et al. 2019; Pang et al. 2019; Talar et al. 2019). Furthermore, a new water filtration membrane was fabricated by LbL as it is an effective method to generate dense layers on a membrane support (Kang et al. 2014). This method was successfully used to integrate the negatively charged GO nanosheets with a porous poly(acrylonitrile) (PAN) support through the positively charged poly (allylamine hydrochloride) (PAH) by electrostatic interaction (Hu and Mi 2014). Similar strategy was applied to fabricate five bilayers of GO and oxidized carbon nanotubes (CNT) on a polyethersulfone membrane to achieve GO-OCNTs membrane (Kang et al. 2019).

Additionally, GO-based FO membranes were fabricated in several studies. The first trial was conducted by Park et al. (2015), in which GO nanosheets were combined with the PSf to produce a novel membrane with a PSf/GO composite support layer. The PA active layer was then employed on the PSf/GO via IP to obtain the TFC-FO membranes. Moreover, GO nanosheets were produced and combined with the PA selective layer to obtain a new TFNC FO membrane by applying the IP method, using an aqueous blend of MPD-GO solution and through the incorporation of polyoxometalate based quaternary GO open frameworks (POM- QGO OFs), GO/Fe_3O_4 nanohybrids, graphene quantum dots (GQDs), pDA/GO within the PA active layer (Shen et al. 2016; Rastgar et al. 2018; Seyedpour et al. 2018; Choi et al. 2019; Shakeri et al. 2019; Xu et al. 2019). The TFC-PA FO membrane was also prepared using different techniques in which rGO modified with graphitic carbon nitride was produced and applied for porous PES substrate modification (Wang et al. 2015) and through the intra-crosslinking of GO nanosheets using m-xylylenediamine (MXDA), followed by inter-crosslinking by trimethyl chloride (TMC) (Jin et al. 2018).

14.4.2 Performance

The performance of Gr-based FO membranes was simulated and proved to be superior to their pristine equivalents through several studies. One of these studies used fluorinated porous Gr with improved salt rejection (Gai et al. 2014). The J_w was 28.1 L/cm^2 h, which was nearly four times that of the conventional CTA FO membrane. Positive simulation results were also obtained when GO membrane was applied in the FO process (Gai and Gong 2014). The ICP dropped to zero and the J_w of the GO membrane reached 3.8 L/cm^2 h, which was approximately 1.7 $\times$ 10^3 order of magnitude higher than that of the classic CTA FO membrane. Moreover, reverse solute passage across the membrane was not detected when the pore-diameter was less than 9.4 Å.

These progressive simulation results for Gr-based FO membranes encouraged several investigators to experimentally evaluate the performance of such modified membranes. Polymeric materials, e.g. pDA, PLL and poly tannic acid (pTA), were used along with GO to modify the surface of TFC-FO membranes in three separate studies (Hegab et al. 2015a, 2016b, c). The J_w increased by 21.5%, 7.3% and 20% for pDA, PLL and pTA modified membranes, respectively, while J_s decreased by 80%, 51.4% and 80%, compared to the pristine one. In addition, AgNPs were also applied with GO for the surface modification of TFC-FO membranes in two studies. Inconsistent results were obtained in both studies, where the first one claimed an increase in both J_w (3%) and J_s (3.5%) (Soroush et al. 2016), while the second one observed a decrease in J_w (1.8%) and increase in J_s (16.7%) (Soroush et al. 2015). These results might be ascribed to the development of an extra layer on the membrane surface, that could behave as a barrier and reduce the J_w (Soroush et al. 2015).

Freestanding GO FO membranes were fabricated through different strategies and their performances were evaluated. The first trial was performed by Hu and Mi (2014), in which they fabricated a GO-PAH FO membrane with increased J_w (82.1%) and J_s (98.2%) compared to the HTI™ membrane. A similar fabrication trial, by using GO-PAH, was performed in a different study (Hu et al. 2016). The same results were obtained, but with less magnitude, in which J_w increased by 44.9%, while J_s decreased by 83.3%, compared to PA membrane. Both membranes; the GO and PA, have identical solute permeability, however, the GO membrane possesses a much greater water permeability, showing a lesser extent of water permeation resistance. The cellulose ester was used with rGO by Liu et al. (2015a) to fabricate a freestanding rGO membrane with 100 nm thickness (Liu et al. 2015b). The obtained J_w of the rGO membrane was 57 L/m^2 h, applying deionized water (DI) water as FS and sodium chloride (NaCl; 2 mol/L) as DS, which was about five times (78.9%) the J_w of the commercial CTA. This indicated that the ICP was nearly removed by this distinctive freestanding rGO membrane. Moreover, a low J_s (1.3 g/m^2 h) was obtained, which is nine times lower compared to that of CTA.

Furthermore, bulk fabrication strategy was applied in several studies as one of the most convenient and stable techniques for integrating GO with FO membranes. GO nanosheets were integrated with the PSf to produce a PSf/GO composite membrane

support layer, which was subsequently combined with the PA active layer, resulting in a modified TFC-FO membrane (Park et al. 2015). The introduction of GO increased the J_w by 68.4%, while negatively increase the J_s by 48.6%. This was attributed to the development of dilutive ICP that reduced the osmotic pressure gradient inside the support layer. Similarly, rGO was integrated with the PES layer of TFC-PA FO membrane (Wang et al. 2015). The J_w of the modified membrane was 50 L/m^2 h (16% greater than the control); whereas the J_s increased by 38.6%. Escalating the DS concentration resulted in increasing the J_w due to higher osmotic pressure variance through the membrane. Simultaneously, J_s leakage from DS to FS increased, because of the greater salt concentration gradient across the TFC membrane's active layer. Alternatively, GO was integrated into the PA active layer in order to fabricate a novel TFC membrane for FO application (Shen et al. 2016). The J_w of all GO-based TFC membranes were greater than that of the control TFC membranes, in which J_w increased with the mounting GO loading. This improvement was ascribed to the collective effects of the thinner selective layer, improved hydrophilicity and extra passages formed. The J_s in all the GO-based TFC membranes were less than that of the control TFC membranes.

Although several trials have been performed to integrate Gr nanomaterials with the FO membrane, three different approaches have been tried; surface modification, freestanding and bulk modification, and several initial facts could be established. It was clear from all of these studies that the highest J_w achieved for the Gr-modified FO membrane was 79 L/m^2 h, upon peeling off the freestanding POFG-Acryl from Polypropylene substrate. However, the average J_w variation of the freestanding membranes (81%), compared to the pristine, was superior to that of the other methods (Table 14.1). On the other hand, by calculating the average deviation in J_s from the control for each method, it was clear that there is no significant difference in J_s except for bulk modification. According to these two parameters; J_w and J_s, freestanding is still the best technique to integrate Gr into FO membranes, only if the J_s of the modified membranes are to be optimized.

14.4.3 Fouling Mitigation

Although Gr is well known for its antibacterial ability, the propensity of the modified FO membranes towards biofouling was investigated in very few studies. The biofouling formation on surface modified polymeric GO-based FO membranes was assessed using mixed aquatic culture in three studies (Hegab et al. 2015b, 2016b, c).

The modified membranes GO-pDA, GO-PLL and pTA considerably postponed the biofouling onset and the declines in normalized flux (J_w/J_{w0}) were 22%, 47% and 53%, respectively. Furthermore, the biocidal activity of these modified membranes surpassed 98%. The integration of the GO nanosheets with polymers led to superior features and morphology, resulting in better biofouling resistance ability, along with improvement in the biocidal activity of modified membranes. This could be ascribed to the amphoteric nature of polymeric materials with the possible existence of

Table 14.1 The features and performance of Gr-based FO membranes fabricated by different

TFNC material[a]	Fabrication method	Feed/draw solutions	J_w[b] (L/m^2 h)	J_w variation (%)[c]	J_s[d]	J_s variation (%)[e]	Contact angle (°)	Antibacterial (%)	Tested bacterial cells	Antifouling[f]	Reference
Gr[g]-membrane preparation strategy—surface modification											
TFC[h]-PA[i]/GO[j]-pDA[k]	Self-assembly and oxidative polymerization	DI[l]/NaCl[m] (2M)	13	21.5 ↑	0.68 mg/min	80 ↓	9.5	98.5	Mixed aquatic culture	22	Hegab et al. (2016a)
TFC-PA/GO-pLL[n]	Hybrid grafting	DI/NaCl (2M)	11	7.3 ↑	1.7 mg/min	51.4 ↓	29.8	99	Mixed aquatic culture	47	Hegab et al. (2015a)
TFC-PA/GO-pTA[o]	Self-assembly and oxidative crosslinking	DI/NaCl (2M)	16.9	20 ↑	0.96 mg/min	80 ↓	19	99.9	Mixed aquatic culture	53	Hegab et al. (2016a)
TFC-PA/AgNPs[p]/GO	In situ formation	DI/NaCl (1M)	N/A	3 ↑	N/A	3.5 ↑	19	98	*E. coli*[q]	N/A	Soroush et al. (2016)
TFC-PA/AgNPs/GO	In situ wet chemical reduction	DI/NaCl (1M)	5.4	1.8 ↓	0.6 mol/m^2 h	16.7 ↑	25	96	*E. coli*	N/A	Soroush et al. (2015)
Gr[a]-membrane preparation strategy—freestanding											
MCE[r]/rGO[s]	Vacuum filtration	DI/NaCl (2M)	57	78.9 ↑	1.3 g/m^2 h	89.6 ↓	78	N/A	N/A	N/A	Liu et al. (2015a)
GO–PAH[t]/PAN[u]	Layer-by-layer assembly	DI/sucrose (1M)	14	82.1 ↑	0.11 mol/m^2 h	98.2 ↑	N/A	N/A	N/A	N/A	Hu and Mi (2014)
GO–PAH/PAN	Layer-by-layer assembly	DI/sucrose (0.9M)	7.8	44.9 ↑	0.05 mol/m^2 h	83.3 ↓	17	N/A	N/A	0.8	Hu et al. (2016)

GO-OCNTs	Layer-by-layer assembly	DI/Na_2SO_4 (1M)	8.7	33 ↑	0.25 g/m^2 h	4 ↑	42.5	N/A	N/A	N/A	Kang et al. (2019)
POFG- Acryl/ polypropylene	Peeling off	DI/NaCl (2M)	79	81 ↑	3.4 g/m^2 h	99 ↓	N/A	N/A	N/A	N/A	Balapanuru et al. (2019)
UiO-66-GO/ nylon	Vacuum filtration	DI/NaCl (2M)	29.2	N/A	12.9 g/m^2 h	N/A	26	90%	*E. coli*	N/A	Pang et al. (2019)
GO/KCl/ nylon	Spin Coating	DI/NaCl (1M)	56	N/A	6.8	N/A	11	N/A	N/A	N/A	Talar et al. (2019)
Gr[a]-membrane preparation strategy—bulk fabrication											
TFC-PSf[v]-GO/PA	Interfacial polymerization	DI/NaCl (0.5M)	19.77	68.4 ↑	3.5 g/m^2 h	48.6 ↑	62	N/A	N/A	N/A	Park et al. (2015)
TFC-PA-GO	Interfacial polymerization	DI/NaCl (2M)	33	33.3 ↑	7 g/m^2 h	1.4 ↓	57	N/A	N/A	10	Shen et al. (2016)
TFC-PES[w]-rGO/PA	Interfacial polymerization	DI/NaCl (3M)	50	16 ↑	0.22 mol/m^2 h	38.6 ↑	53	N/A	N/A	N/A	Wang et al. (2015)
TFC-POM-QGO-OFs/PA	Interfacial polymerization	DI/NaCl (1M)	34.7	48 ↑	11.9 g/m^2 h	45 ↑	50	N/A	N/A	N/A	Shakeri et al. (2019)
TFC-MMGO-Fe3O4/PA	Interfacial polymerization	DI/NaCl (1M)	56	67.8 ↑	9.8 g/m^2 h	29.5 ↑	48.7	N/A	N/A	82	Rastgar et al. (2018)
TFC-GQDs/ PA	Interfacial polymerization	DI/NaCl (2M)	53	26 ↑	110 mmol/m^2 h	35 ↓	59	90–95%	*E. coli*	60	Seyedpour et al. (2018)
TFC-PEI-GQDs/PAN	Interfacial polymerization	DI/$MgCl_2$ (0.5M)	12.9	10.8 ↑	1.41 g/m^2 h	32.8 ↓	37.5	N/A	N/A	N/A	Xu et al. (2019)
TFC-PDA-GO/PA	Interfacial polymerization	DI/NaCl (1M)	24.3	36.6 ↑	3.8 g/m^2 h	11.6 ↓	44.3	N/A	N/A	N/A	Choi et al. (2019)

(continued)

Table 14.1 (continued)

TFNC material[a]	Fabrication method	Feed/ draw solutions	J_w[b] (L/m^2 h)	J_w variation (%)[c]	J_s[d]	J_s variation (%)[e]	Contact angle (°)	Antibacterial (%)	Tested bacterial cells	Antifouling[f]	Reference
TFC-GO-MXDA/PA	Interfacial polymerization	DI/ trisodium citrate (0.25M)	13.2	N/A	0.1 mol/ m^2 h	N/A	67	N/A	N/A	N/A	Jin et al. (2018)

[a]TFNC: thin-film nanocomposite
[b]J_w: Water flux
[c]Variation % is calculated according to the control J_w
[d]J_s: reverse solute flux
[e]Variation % is calculated according to the control J_s
[f]Reduction % in normalized flux (J_w/J_{w0})
[g]Gr: graphene
[h]TFC: thin-film composite
[i]PA: polyamide
[j]GO: graphene oxide
[k]pDA: polydopamine
[l]DI: deionized water
[m]NaCl: sodium chloride
[n]pLL: poly L-Lysine
[o]pTA: poly tannic acid
[p]AgNPs: silver nanoparticles
[q]E.: *Escherichia*
[r]MCE: mixed cellulose ester
[s]rGO: reduced graphene oxide
[t]PAH: poly (allylamine hydrochloride)
[u]PAN: poly (acrylonitrile)
[v]PSf: polysulfone
[w]PES: polyethersulfone

positive primary/secondary amino groups on the membrane surface leading to partial antibacterial activity upon their interaction with the negatively charged bacterial membrane surfaces (Kochkodan et al. 2008). Additionally, GO has its own biocidal activity (Kang et al. 2007; Wang et al. 2008; Soroush et al. 2015), which is attributed to the existence of defective edges, providing active sites for the production of reactive oxygen species (Sanchez et al. 2012; Hegab et al. 2016c), causing stress and leading to the destruction of the bacterial cell membrane (Akhavan et al. 2012). This antibacterial activity is augmented by the physical contact with the GO sharp edges, resulting in bacterial cell membrane damage (Akhavan and Ghaderi 2010; Hu et al. 2010). Thus, the direct interaction between the bacteria and the membrane surface can result in cellular lysis and consequently the hindrance of biofilm development leading to a lessening in biofouling formation and its related problems.

Additionally, organic fouling was also investigated in few studies against alginate and BSA (Hu et al. 2016; Shen et al. 2016). It was observed that a cross-linked alginate gel layer could be developed on the surface of the membrane in the presence of Ca^{2+} ions, in the aqueous solution of synthetic wastewater, acting as linkers between alginate molecules (Mi and Elimelech 2008; Tiraferri et al. 2012). This gel layer will result in amplifying the transport resistance, and considerably reduces the J_w. The first study applied a fabricated GO FO membrane and observed that the J_w/J_{w0} of GO-based TFC membranes slowly declined with time compared to the control TFC membrane, demonstrating the lower fouling tendency (Shen et al. 2016). The other study evaluated the organic fouling of a freestanding GO-PAH membrane and observed that the GO membrane displayed antifouling ability comparable with that of typical PA membrane (Hu et al. 2016). Alginate fouling resulted in more flux drop compared to BSA fouling, particularly for the classic PA membrane, where its J_w decreased to about 80% and 60% of the original flux in the absence and presence of Ca^{2+} ions, respectively (Hu et al. 2016). The foulants, with hydrophobic nature, could easily be adsorbed on the hydrophobic surfaces and diminish the interfacial energy, without any important thermodynamic gain for the extreme hydrophilic surface. Moreover, the strongly fixed water molecules can bind to the hydrophilic surface by a hydrogen bond and develop a skinny water border layer which acts as a hurdle to limit the adsorption of hydrophobic foulants. Additionally, some negatively charged foulants are electrostatically repulsed with the membrane surface of the GO-based TFC membranes, which also carry the same charge due to several functional groups, e.g. hydroxyl, carboxyl and epoxide (Shen et al. 2016). The GO-based TFC membranes, which possess smooth surfaces, have the ability to circumvent the accumulation of foulant particles on the PA as a result of the limited adhesion spots on the membrane surface, which contributes towards the improvement of antifouling ability. The adsorption of organic foulants primarily occurs on the GO nanosheets basal plane, in which water mainly passes the GO membrane in proximity to the GO's oxidized edges, resulting in foulant adsorption not creating too much interference to water flux (Hu et al. 2016).

Furthermore, the dual biocidal effect of GO-Ag modified membranes was observed in several studies (Soroush et al. 2015, 2016). Membranes modified with GO-AgNPs displayed 80% bacterial inactivation compared to the PA pristine

membrane (50%) (Soroush et al. 2016). This combined biocidal effect is owing to the antibacterial mechanisms of both the GO nanosheets and AgNPs. GO nanosheets are physical biocidal materials and can rupture bacterial cell membranes with their sharp edges (Perreault et al. 2014; Romero-Vargas Castrillón et al. 2015) or control lipid peroxidation initiated by the oxidative character of GO (Hegab et al. 2016b). Producing the reactive oxygen species or the direct oxidation of cellular elements also contributes towards the bactericidal activity (Mejías Carpio et al. 2012; Perreault et al. 2015; Hegab et al. 2016c).

14.5 Conclusion

The Gr material, with its 2D structure, potentially provides several significant advantages, in terms of its facile fabrication method and the enhancement of the physicochemical features of FO membranes, compared with conventional membrane materials, e.g. PA. This unique GO structure lends it to be an outstanding candidate for developing a novel type of membranes by integrating GO nanosheets through different strategies, e.g. surface modification, free standing and bulk fabrication. These techniques are relatively eco-friendly and cost-effective as the entire fabrication process can be performed in aqueous solution, whereas the typical membrane fabrication techniques, e.g. IP, regularly involve complicated chemical reactions and apply organic solvents. Only a few number of research studies have investigated the biofouling of FO membrane, limited attention has been presented to resolve the drawback of FO membrane J_s and develop reliable techniques to impede the bacterial proliferation on the membrane surface by means of a facile practical coating. Moreover, the fouling development on the Gr-based FO membranes was investigated in very few studies, which highlights the urgent need for detailed research about the role of Gr as an antifouling material in modified membranes and how to maximize this role by applying the suitable technique of Gr integration with the FO membranes.

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Constructed Wetland: A Green Technology for Wastewater Treatment

15

Ashutosh Kumar Choudhary and Parveen Kumar

Abstract

Water pollution is an emerging global environmental issue which causes several thousands of death daily. This chapter describes the suitability of constructed wetlands (CWs) for wastewater treatment, types of CWs, operating parameters, mechanism of contaminants removal, role of plants and microbes in treatment, and the treatment performance for different wastewaters. Wastewater generated by various industries contains high concentrations of recalcitrant pollutants and color, which have serious impacts on wastewater receiving water bodies. In the view to protect surface water bodies and to meet out standard discharge norms, it is the demand of time to reduce the pollution load of wastewaters by adopting sustainable wastewater treatment technologies. The conventional treatment systems are primarily less efficient to remove recalcitrant compounds and color from wastewater. To overcome this problem, CWs could be one option for domestic and industrial wastewater treatment. These systems are simple and have proved to be sustainable and green technology to improve wastewater quality.

A. K. Choudhary
Himalayan School of Science and Technology, Swami Rama Himalayan University, Dehradun, Uttarakhand, India

P. Kumar (✉)
School of Basic and Applied Sciences, G. D. Goenka University, Gurugram, Haryana, India
e-mail: praveen.kumar@gdgu.org

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15.1 Introduction

Water pollution is an emerging global environmental issue which is leading to several thousands of death daily. Inadequate availability of clean potable water has become one of the most persistent problems which cause serious human health issues. Many cities in developing world generally have poor implementation and management of sewage treatment facilities due to lowest priority among the various infrastructure advancements (Konnerup et al. 2011). Therefore, the discharge of large volumes of untreated wastewater into aquatic bodies is a general practice. On the other hand, wastewater generated by various industries contains high concentrations of recalcitrant organic compounds, color, inorganic nutrients, and metals which cause serious impacts upon discharge to receiving aquatic bodies. Generally, small scale industries cannot fully afford the advanced wastewater treatment facilities. Moreover, conventional treatment facilities are not sufficient to meet wastewater discharge norms directly into aquatic bodies. The conventional treatment methods generally adopted by Indian industries include conventional primary and secondary treatment processes which are not so efficient for the degradation of the recalcitrant compounds and color from wastewaters. It has been practically observed that the conventionally treated wastewater still contains high pollution load that imparts toxicity to the wastewater. In order to protect surface water bodies and to meet increasingly stringent wastewater discharge limits, it is essential to reduce the pollution load of industrial wastewaters by adopting sustainable wastewater treatment technologies.

Constructed wetlands (CWs) have been effectively used to treat industrial and municipal wastewaters since 1950s by Dr. Käthe Seidel in Germany (Vymazal 2011) and have emerged as an eco-friendly wastewater treatment process in both developed and developing countries. CW systems have emerged rapidly over the last 30 years and have been implemented worldwide as a choice over conventional wastewater treatment systems. These systems are eco-friendly, require minimal energy input, and are easy to maintain, which makes them appropriate for industrial wastewater treatment.

15.2 Constructed Wetlands (CWs)

Constructed wetlands (CWs) are specially designed engineered establishments that are utilized to control water pollution from small to large scale. These systems use natural processes utilizing shallow beds or channels, typically less than 1 m in depth, specified plants, substrate (soil, coarse sand, and/or gravel), and a diversity of microorganisms for the removal of contaminants from wastewaters (EPA 2004; Choudhary et al. 2011). It receives primary treated wastewater and treats it to secondary level standards or even better. CWs are accomplished by reducing all types of contaminants including inorganic pollutants, organic matter, toxic compounds, color, heavy metals, and pathogenic microbes from wastewaters. Generally, CWs have low maintenance and operational cost but in some countries the

prices are comparable with traditional wastewater treatment systems. Moreover these systems have esthetic values over traditional wastewater treatment facilities (Kadlec et al. 2000). In the last several decades, these systems have been utilized to treat the wastewaters originated from different sources i.e. municipal wastewater, agricultural runoff, acid mine drainage, landfill leachate, urban storm water, and industrial wastewater including petrochemical, chemical, textile, paper and pulp, food processing, and tannery industries (Kadlec et al. 2000; Choudhary et al. 2013, 2015; Kumar and Dutta 2019; Ghimire et al. 2019; Kumar and Singh 2020).

15.2.1 Types of CW

Constructed wetlands are broadly classified into three categories i.e. surface flow (SF) constructed wetlands, subsurface flow (SSF) constructed wetlands, and hybrid systems that utilize both surface and subsurface flow wetlands, based on the flow of wastewater in root zone.

15.2.1.1 Surface Flow (SF)

Surface flow constructed wetlands (SF-CWs) are frequently used in North America and Australia. These facilities have been generally used to control agricultural runoff and acid mine drainage pollution (Reed et al. 1995). Generally, SF-CWs have been efficiently utilized in Europe to remove nitrogen from wastewater (REED 1993). A SF wetland consists of a low depth bed (<1 m), soil, or other medium to support the roots of plants and water control structures that maintain a low depth of water usually 0.2–0.4 m. In these systems, flowing wastewater surface is always above the bed surface (Fig. 15.1). The upper water surface layer is aerobic due to diffusion of atmospheric oxygen while in deeper root zone environment is usually anaerobic. Their cost of construction and operation is low (EPA 2000) but generally have a low treatment efficiency in compression to SSF-CWs (Lee et al. 2009). A wide range of submerged and floating plants are available which can be used in SF-CWs like *Typha*, *Scirpus*, *Digitaria*, *Cyperus* spp., etc. While treating wastewater, SF wetlands look like natural wetlands and provide habitat for wildlife with esthetic benefits. It works as a buffer zone between tertiary wastewater treatment facilities

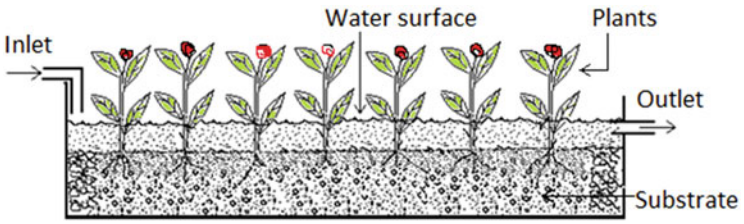

Fig. 15.1 Surface flow constructed wetland (adapted from Choudhary et al. 2011)

and natural waterways (Vymazal 2007). Therefore, SF-CWs may be a feasible choice for the environmental restoration of surface aquatic bodies. The major drawback of SF systems is that it usually requires larger land than other treatment facilities (EPA 1995). Based on the dominant plant species i.e. free-floating aquatic plants, emergent aquatic plants, or submerged aquatic plants dominated systems, SF-CWs can further be classified (Vymazal 2007). The main contaminants removal processes are sedimentation, filtration, adsorption, oxidation in upper water surface, and reduction in deeper water surface and substrate. The heavier particles predominantly settle down near inlet open water zone whereas lighter particles might only settle down after passing through wetland channel.

15.2.1.2 Subsurface Flow (SSF)

A subsurface flow (SSF) CW system has a sealed bed or channel with a high porosity substrate like small rocks, gravel, and soil or mixture of all of these. The wastewater level is designed to stay below the top layer of the substrate (Fig. 15.2). The wastewater flows horizontally or vertically into the substrate under the influence of gravity, depending on the type of the wetland system. These systems commonly have been used to reduce biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, toxic metals, and nutrients from domestic and industrial wastewaters (EPA 2000). Mostly, these systems have been used in Europe and South Africa (Lee et al. 2009). SSF-CWs are more efficient on area basis as compared to SF systems (Kadlec 2009). The plant species generally used in SSF-CWs includes lily (*Canna indica*), cattail (*Typha* spp.), common reed (*Phragmitis australis*), bulrush (*Schoenoplectus*), and *Pseudacorus* spps. Because of the higher hydraulic restrictions offered by the substrate, SSF-CWs are suitable to treat wastewaters having relatively low suspended solids particles. The treatment efficiency of SSF-CWs systems is comparatively higher than that of SF-CWs due to the porous substrate which provides greater surface area and higher hydraulic retention time for wastewater. The only disadvantage of SSF-CWs is that it is costlier to build on a unit area basis than SF-CWs (EPA 1995, 2000). It is further sub-classified into two categories: first is horizontal flow (HF) CWs and second is vertical flow (VF) CWs, based on the flow of wastewater in root zone. A horizontal subsurface flow (HSSF) system consists of a sealed bed with high porosity substrate

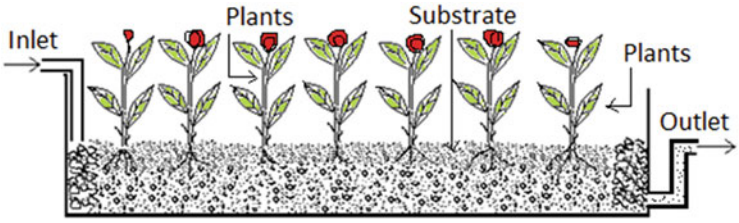

Fig. 15.2 Horizontal subsurface flow constructed wetland

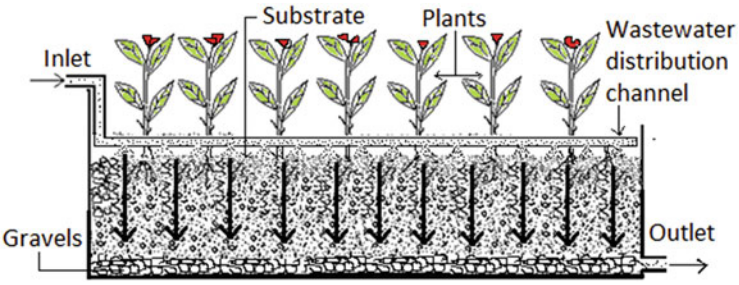

Fig. 15.3 Vertical subsurface flow constructed wetland

for the growth of plant species (Fig. 15.2). The wastewater level is designed to stay just below the upper most layer of the bed. The effective bed depth for SSF-CWs is normally <0.6 m for efficient contaminants removal (Kadlec and Knight 1996). The pre-treated wastewater should be fed through inlet zone for the proper and long-term functioning of HSSF-CWs, which further passes slowly through the bed/channel under the upper surface horizontally subjected to reach the outlet zone. The vertical flow constructed wetlands (VF-CWs) system has a bed packed with substrate to support plants growth (Fig. 15.3) with vertically downward flow of untreated wastewater through the bed. Here, the treated wastewater is collected by an installed collection system at the base. These systems are more efficient than HSSF-CWs when there is a limitation of space availability.

15.2.1.3 Hybrid

The hybrid systems act more efficiently for the removal of contaminants from wastewaters. There has been a growing interest in these systems, even though it is comparatively more expensive to develop and complex to operate than other CWs. Hybrid CWs have been used in different parts of the world mainly for the efficient removal of total nitrogen (TN) because different types of CW configurations provide different redox conditions, which are appropriate for nitrification and denitrification processes (Vymazal 2011). However, single-stage CWs cannot efficiently remove total nitrogen (Vymazal 2007) due to lack of aerobic and anaerobic conditions simultaneously. To overcome this problem, different types of CW can be configured to get the benefits of individual treatment systems. Hybrid systems were developed from original hybrid systems designed at the Max Planck Institute in Krefeld, Germany (Seidel 1965). This system has two stages of many parallel VF channels followed by two or more HF channels in series. To configure hybrid systems different types of combinations are possible such as HF–VF CWs, VF–HF CWs, HF–SF CWs, and SF–HF CWs. There has been a growing demand in achieving fully nitrified effluents but HF systems alone cannot do this because of their inadequate oxygen transfer potential. On the other side, VF systems are very efficient for nitrification due to the presence of oxygen rich environment (Vymazal 2005).

15.2.2 Components of CW

A CW is having a well-designed bed/channel that includes substrate, liner, wastewater, wastewater flow control structures, water loving plants, and naturally developed microbial communities. The beds usually consist of pits with depth of 0.30–0.50 m, lined with a liner to avoid water losses and are partially filled with 0.20–0.30 m of soil in SF-CWs and 0.20–0.50 m with sand and gravel in SSF-CWs. These beds should slope about 1–3% upstream (EPA 1995, 2000). The bed length should be three times greater than the width to make sure that the wastewater is not passing too fast through the wetland channel. The U.S. Department of Agriculture (USDA) (1991) guidelines suggest an overall width to length ratio of 1–4. CWs must be sealed by using liners to avoid the possibility of ground water contamination and also to prevent ground water from infiltration into the bed. For this purpose, different types of synthetic liners can be used such as asphalt, butyl rubber, and plastic membranes. The liner must be covered uniformly with 8–10 cm of soil/sand to avoid the penetration of plants roots in the liner. The substrate supports the wetland plants, offer sites for biochemical transformations, and accumulation of contaminants. Substrates include soil, coarse sand, gravel, calcite, slag, marble, zeolite, fly ash, dolomite, limestone, organic materials, shell, and activated carbon. The coarse sand used in SSF-CWs make possible an efficient treatment by supporting filtration and adsorption processes and by providing a surface for microbial growth. Gravel bed systems are widely used in Asia, Australia, North Africa, New Zealand, and South Africa. The selection of substrates determines the hydraulic conductivity and the capacity of absorbing contaminants. The poor hydraulic parameters would result in channel clogging and low adsorption of pollutants by substrates which further affect the long-term performance of CWs. Soil properties play an important role in CWs. Therefore, parameters like pH, cation exchange capacity, electrical conductivity, texture, and organic content should be considered in selecting the substrate medium. The pH of the soil regulates the transformation and removal of contaminants. As per the guidelines of EPA, pH should be in a range from 6.5 and 8.5 (EPA 1995). The electrical conductivity of substrate in root zone affects the ability of microbes and plants to remove contaminants from wastewater. It is suggested that soils having electrical conductivity less than 4 mmho/cm are best for the growth of plants. Sandy and gravel soils have high porosity due to this wastewater moves speedily through the wetland channel. The texture of soil also affects growth of roots and the adsorption/absorption of contaminants. Loamy and medium textured soils are generally recommended, as these soils have high retention capacity for contaminants and no restriction on plants growth. While, the high density soils (e.g. clays and shales) should not be used as substrate due to poor root growth and low hydraulic conductivities (SCS (Soil Conservation Service) 1991). The substrate must have adequate organic content to support plants and microbial growth, primarily in the initial phase of the wetland setup. Organic matter like spent mushroom compost, sawdust, hay, or straw can be used conveniently. Organic material supports microbial growth by providing a source of carbon and

creates the anoxic conditions that are necessary for nitrate reduction and neutralization of acidic mine drainage.

One of the main components of CWs is water flow control structure. Wastewater level is regulated by flow control structures installed at inlet and outlet zones of the treatment channel. It allows flexibility so that wastewater flow can be regulated and adjusted to get desired treatment results. Flow structures are installed in a way to handle maximum design flows and to minimize short-circuiting. Inlets at SF-CWs are generally simple with an open-end pipe, channel, or gated pipe which releases wastewater into the bed. In SSF-CWs systems, inlet structures include manifolds (surface and subsurface), open trenches perpendicular to the direction of flow, and weir boxes with single point. The inlet zone of bed should have coarse rocks of dimension 8–15 cm to ensure rapid infiltration. A flow splitter should also be needed for parallel beds. At SF-CWs, the wastewater level is regulated by outlet flow structures, which might be a riser pipe with adjustable settings, weir, or spillway. A variable-height weir with detachable stop logs make possible easy adjustment of the water levels. On the other side, spillways are not adjustable but simple to build. The installation of an adjustable outlet is recommended to maintain a satisfactory water level in the wetland channel (EPA 1995).

The most significant component of CWs is vascular (the higher plants) and non-vascular plants (algae). The role of plants in CWs is principally to make growth and provide aerobic conditions to the treatment bed. With this, vegetative growth provides attachment sites for the growth of microorganisms and also generates detritus matter which further supports microbial metabolism by providing organic carbon. Additionally, vegetation stabilizes the root zone environment by increasing the bed permeability. It has been generally observed that algae develop in the system very easily and helps in increasing the dissolved oxygen concentration in water by performing photosynthesis which further affects the rate of degradation of contaminants. Small plants or parts of plants such as rhizomes, tubers, rootstocks, or cuttings can be easily used to grow vegetation in CWs. The collection of vegetation should be done in early spring season and plant them on the wetland bed to reduce the mortality. The vegetative propagules are usually spaced at 30–90 cm intervals. Before starting the wastewater treatment in CW, vegetation should be allowed to grow because it requires an acclimatization phase to overcome the planting stress. Satisfactory vegetation growth generally takes few months in regular growing season. The plant species commonly used in CW systems include emergent plants, floating leaved plants, free-floating plants, and submerged plants. In last three decades about 150 water loving plant species have been effectively used in worldwide (Vymazal 2013). The most commonly used growing plant species are *Phragmites* (Poaceae), *Scirpus* (Cyperaceae), *Typha* (Typhaceae), *Iris* (Iridaceae), and *Juncus* (Juncaceae). The commonly preferred submerged species are *Potamogeton crispus*, *Myriophyllum verticillatum*, *Hydrilla verticillata*, *Vallisneria natans*, and *Ceratophyllum demersum*. The plants with floating leaves used for treatment purpose are generally *Marsilea quadrifolia*, *Nymphoides peltata*, *Trapa bispinosa*, and *Nymphaea tetragona*. The free-floating plants that have been used in different treatment studies are *Lemna minor*, *Eichhornia crassipes*, *Salvinia natans*,

Table 15.1 Plant species for SF-CWs

Scientific name	Region	Reference
Aquatica	China	Sheng et al. (2013)
Candocks	China	Sheng et al. (2013)
Carex	USA	Heyvaert et al. (2006)
Eichhornia crassipes	Argentina	Maine et al. (2006)
Ipomoea aquatica	China	Li et al. (2010)
Ipomoea aquatica forsk	China	Sheng et al. (2013)
Juncus	USA	Heyvaert et al. (2006)
Lemna	USA	Heyvaert et al. (2006)
Lolium perenne sp.	China	Li et al. (2012)
Lolium multiflorum sp.	China	Xian et al. (2010)
Panicum elephantipes	Argentina	Maine et al. (2006)
Scirpus	USA	Heyvaert et al. (2006)
Typha spp.	Canada	Bosak et al. (2016)
Typha domingensis	Argentina	Maine et al. (2006)

Table 15.2 Plant species for SSF-CWs

Scientific name	Wetland type	Region	Reference
Acorus calamus	VF	China	Gu et al. (2007)
Agave sisalana	VF	China	Gu et al. (2007)
Andropogoneae saccharum	HSSF	Ethiopia	Dires et al. (2018)
Canna indica	HSSF	India	Choudhary et al. (2015)
	VF	China	Gu et al. (2007)
Colocasia esculenta	HSSF	India	Choudhary et al. (2015)
Cyperus papyrus	HSSF	Ethiopia	Dires et al. (2018)
Eichhornia crassipes	VF	India	Kumar and Singh (2017)
Pennisetum pedicellatum	HSSF, VF	India	Thalla et al. (2019)
Phragmites australis	HSSF	India	Rai et al. (2015)
	VF	Belgium	Auvinen et al. (2017)
Phragmites karka	HSSF	Ethiopia	Alemu et al. 2017
Schoenoplectus tabernaemontani	VF	Canada	Rozema et al. (2016)
Scirpus atrovirens	HSSF	Ethiopia	Dires et al. (2018)
Typha domingensis	HSSF	Ethiopia	Dires et al. (2018)
Typha latifolia	HSSF	India	Rai et al. (2015)
	VF	China	Gu et al. (2007)

and *Hydrocharis dubia*. Among the above mentioned plants species, *Phragmites australis* has been utilized for different treatment studies in Europe and Asia, *Phragmites australis* and *Typha domingensis* in Central/South America, *Typha latifolia* in North America, *Cyperus papyrus* in Africa, and *Scirpus validus* in Oceania, used in SF-CWs (Table 15.1) (Vymazal 2013). Similarly, some common plant species used in SSF-CWs around the world are *Phragmites australis* and *Typha* spp. Likewise, ornamental species like *Canna indica* and *Iris pseudacorus* can also be used for treatment (Table 15.2).

A basic inherent part of CWs is that their treatment mechanism is principally regulated by native micro flora and fauna i.e. algae, bacteria, yeast, fungi, and protozoa. A number of chemical reactions mediated by microorganisms transform a variety of inorganic and organic substances into harmless elements/compounds. Microbes alters the redox conditions of the substrate, as a result improves the treatment efficiency of the system.

15.2.3 Design Parameters

The construction of efficient CW requires simple design and integration with the natural landscape of the selected site. The CW treatment systems are generally established as close to the wastewater source as possible and should be large enough to accommodate present requirements and possible future expansion. The area required for an HSSF treatment system is generally 5 m^2 per population equivalent (PE) while a VSSF system requires about 1–3 m^2 PE^{-1} for sewage treatment. For hybrid systems, the land area requirement is about 0.70–0.93 m^2 PE^{-1} (Vymazal 2011). The efficiency of any CW system depends upon the hydrology and other environmental factors. Precipitation, evapotranspiration (ET), hydraulic retention time (HRT), hydraulic loading rate (HLR), and effective water depth also play a significant role in the overall performance of the system. It is mandatory to prepare a hydrologic budget prior to designing a CW treatment system because any alteration in HRT or wastewater volume can significantly affect the treatment performance (Choudhary et al. 2013, 2015).

15.3 Treatment Mechanism

The treatment mechanism for the removal of different types of pollutants in CWs can be broadly classified into three different categories (Fig. 15.4) as physical processes (settling, filtration, diffusion, absorption, and volatilization), chemical processes (oxidation, reduction, photochemical oxidation, precipitation, adsorption, and ion exchange), and biological processes (microbial degradation, phytodegradation, phytovolatilization, evapotranspiration, plant uptake, and microbial uptake).

The primary mechanism operative in CWs is solid/liquid separations and structural transformations (EPA 2000). Separations usually include filtration, absorption, adsorption, sedimentation, and ion exchange. Contaminants transformation is mainly due to biochemical reactions occurring under aerobic and/or anaerobic environment regulated by root zone. The contaminants removal efficiency in CWs mainly depends on the rhizosphere interactions between soil, pollutants, plant roots, and diverse microorganisms. The soil is the main factor which promotes the growth of vegetation and microbes. It is reported that fine textured soil material facilitates greater growth of vegetation and thus increases the rate of pollutants removal (Garcia et al. 2005). Plants are directly involved in the uptake of inorganic and organic pollutants and indirectly in the degradation of pollutants by providing oxygen rich

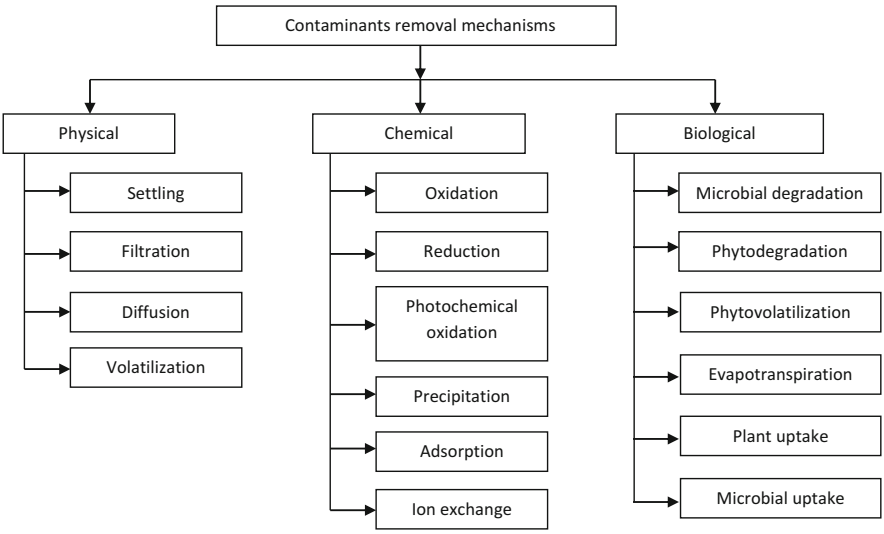

Fig. 15.4 Contaminants removal mechanisms in CWs (Adapted from Choudhary et al. 2011)

environment in root zone that also facilitates microbial activity for reduction/transformation of pollutants. The key factors that influence the uptake of pollutants by wetland plant species are concentration of pollutant in wastewater, physicochemical properties like octanol–water partition coefficient (log Kow), polarity, acidity constant (pKa), etc. (Stottmeister et al. 2003; Wenzel et al. 1999). According to Sandermann (1992), the metabolism of pollutants can be classified into three phases in plants i.e. transformation, conjugation, and compartmentation. Fermentation and respiration are the key metabolic pathways by which microorganisms break down organic matter into simple components like as carbon dioxide (CO_2), nitrogen (N_2), and water (H_2O) (Faulwetter et al. 2009). Some microbial transformations are aerobic while others are anaerobic. Microorganisms efficiently degrade a variety of the organic pollutants; however the rate kinetics of degradation varies significantly, depending on physiochemical properties of the compound and root zone environment under wetland bed.

15.3.1 Suspended Solids

Suspended solids in wastewater contains a variety of pollutants, like inorganic nutrients, organic matter/components, and toxic metals. The leading physical mechanisms for suspended solids reduction are filtration, flocculation, and sedimentation (Kadlec 2009). The surface forces accountable for the removal of suspended solids include electric forces which might be attractive or repulsive depending on the surface charges and Vander Waal's force of attraction (Metcalf 1991).

15.3.2 BOD and COD Removal

Both BOD and COD are considered as very important parameters for wastewater pollution control. In CWs, removal of BOD and COD is supposed to happen through sedimentation and filtration of suspended matter in the void spaces of substrate media (Reed 1993). Removal of BOD is primarily due to microbial degradation pathways under aerobic conditions and entrapment processes (Watson et al. 1989). The soluble organic matter is reduced by the microbial growth supported by media surface and roots of vegetations. According to DeBusk (1999), organic matter contains roughly 45–50% carbon, which is utilized by a micro flora and fauna as a source of energy for growth (DeBusk 1999). For this purpose oxygen is directly provided by the plants to convert organic carbon into carbon dioxide. Organic matter which is present in soluble form also gets removed by adsorption and absorption processes. The extent of adsorption or absorption depends on the physiochemical properties of both the soluble component and the interacting surface of wetland components like vegetation, substrate, and litter (EPA 2000).

The kinetics of degradation processes in the wetland decides degradation or accumulation of pollutant in treatment system and further regulates the mass balance of a specific pollutant in different components of the CW system. In a newly established wetland, sorption onto the surface of substrate is higher as a result of the high adsorption/absorption capacity of available unexposed substrate (Omari et al. 2003). If no free sorption site is available under substrate, then system amalgamate the pollutants and no sorption–desorption equilibrium condition is reached. This condition further increases the pollutants retention in treatment system and supports microbial degradation (Imfeld et al. 2009).

15.3.3 Nitrogen Removal

Nitrogen is one of the primary inorganic constituent in domestic and industrial wastewaters that cause a serious problem of eutrophication in receiving water bodies which have an effect on dissolved oxygen level of aquatic body. It might also cause toxicity to aquatic biodiversity. Conversely a variety of inorganic and organic nitrogen compounds are important for all living creatures. A wide range of inorganic nitrogenous species which are present in environment are ammonium (NH^{4+}), nitrite (NO_2^-), nitrate (NO_3^-), nitrous oxide (N_2O), and dissolved nitrogen gas (N_2). All these inorganic species are considerably removed by vegetation in CW through uptake at low hydraulic loading rates (HLR). While organic nitrogen is generally present as amino acids in proteins, uric acid produced by birds and insects, urea excreted by mammals, purine, and pyrimidines as building blocks of DNA (Kadlec and Knight 1996). In CWs, plants convert inorganic nitrogenous compounds into organic compounds. Aforesaid all the forms of nitrogen are important as depending on the available forms in the substrate, plant species only uptake desired forms of nitrogen available in the solution (Lambers et al. 1998). It has been observed several

times that key mechanism accountable for nitrogen removal is nitrification–denitrification complex mediated by the microorganisms in wetlands (Reddy et al. 1989).

Nitrification is primarily an oxidation of ammonium to nitrate facilitated by nitrifying bacteria. It requires aerobic conditions to carry forward and is carried out in two steps: first step is mediated by Nitrosomonas bacteria in which conversion of ammonium to nitrite takes place (Eq. (15.1)) and second step is conversion of nitrite to nitrate by Nitrobacter bacteria (Eq. (15.2)). In both the steps, nitrifying bacteria drive energy from the oxidation of ammonia and nitrite whereas CO_2 is utilized as carbon source for growth (Vymazal 2007). The summarized nitrification reactions are as follows:

$$NH_4^+ + 3/2O_2 \rightarrow NO_2^- + H_2O + 2H^+ \tag{15.1}$$

$$NO_2^- + 1/2O_2 \rightarrow NO_3^- \tag{15.2}$$

The process of nitrification is affected by pH of wastewater, alkalinity, reaction temperature, carbon source, microbial diversity, concentrations of ammonium-N and dissolved oxygen in wastewater (Lee et al. 2009). While in denitrification, organic matter is utilized by microorganisms like Pseudomonas, Bacillus, and Micrococcus, using nitrate instead of oxygen as an electron acceptor. In this process, first nitrate is reduced to nitrous oxide and further reduced to atmospheric nitrogen (Verhoeven and Meuleman 1999). It can be summarized by the following Eq. (15.3) (Hauck 1984):

$$2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow N_2O \rightarrow N_2 \tag{15.3}$$

This process contributes to about two-third fraction of total nitrogen removal in wetland bed (Reddy and D'Angelo 1997). The kinetics of denitrification is regulated by several factors like temperature and pH of wastewater, concentration of nitrate, microbial population, availability of organic carbon, HRT, growth of vegetation, concentration of dissolved oxygen, and type of substrate. Ammonification also plays an important role in removal of nitrogen in CWs. It is a complex process in which organic nitrogen is utilized and converted into ammonia by microorganisms. It is a fast process than nitrification and rate of reaction is affected by wastewater parameters, carbon to nitrogen (C/N) ratio, and microenvironment of the substrate (Reddy and Patrick Jr. 1984). With all this, a process of volatilization also contributes to the removal of ammonia nitrogen (Vymazal 2007). Anaerobic ammonium oxidation known as Anammox is a recently discovered nitrogen reduction process in which ammonium is directly oxidized to N_2 by nitrite mediated by a group of planctomycete bacteria in the presence of oxygen. As per the studies carried out by Saeed and Sun (2012), during Anammox reaction several intermediate products like hydrazine and hydroxylamine are also formed.

15.3.4 Phosphorus Removal

CWs provide suitable environment for the inter-conversion of all forms of phosphorus. In domestic and industrial wastewaters phosphorus occurs in different organic and inorganic forms but insoluble forms of phosphorus are usually not biologically available until transformed into soluble inorganic forms. Soluble phosphorus is easily uptake by vegetation and gets accumulated in tissues or utilized for metabolic activities. Phosphorus uptake by plants is generally very high in the initial phase of growing season (Vymazal 1995). Most of the phosphorous as PO_4^- is removed from wastewater in the course of sedimentation while its transformations in wetland are caused by microbial interactions, mineralization, adsorption/desorption, leaching, and precipitation (Vymazal 2007). In adsorption, movement of soluble inorganic phosphorus takes place from wastewater to soil mineral surfaces, where it gets accumulated without penetrating the substrate particle surface. Precipitation takes place by reaction of phosphate ions with metallic cations like Al, Mg, Ca, or Fe, forming weakly crystalline solids under appropriate environment. Some common phosphate minerals which precipitate in root zone are apatite, hydroxylapatite, wavellite, strengite, variscite, and vivianite (Reddy and D'Angelo 1994).

15.3.5 Metals Removal

A significant quantity of heavy metals is continuously discharged into aquatic bodies by industries. Some of the discharged metals are highly toxic and cause serious problems to natural ecosystems. In CWs, different complex processes are involved in the removal of metal from wastewater. These are filtration, sedimentation, precipitation, adsorption, and uptake by plants and microorganisms (DeBusk 1999). Out of all, sedimentation and filtration are the key processes involved in the removal of heavy metals. In sedimentation heavy metals aggregate into particles having large size to sink easily (Walker and Hurl 2002). For the metal precipitation several factors like pH of the wastewater, solubility product (Ksp) of the metal species, metal ions concentration, and other relevant anions play an important role. When the concentration of anions and cations is such that their product exceeds Ksp, precipitation takes place (Sheoran and Sheoran 2006). In this way, heavy metals get easily trapped in the wetland sediments and removed from the wastewater. Some studies also reported that metallic species get adsorbed to substrate or get involved in the chelate formation with organic content present in solution. In addition to this, mechanism for metal removal also includes oxide formation (Weider and Lang 1986). Wetland vegetation and microbial uptake also contributed significantly for metal removal but the rate of removal varies widely and depends on concentration of metals in wastewater, vegetation growth, and type of plant species (Sheoran and Sheoran 2006).

15.3.6 Pathogen Removal

A variety of pathogens are present in domestic sewage which deteriorate the quality of surface water bodies if discharged untreated. CWs have outstanding pathogen removal potential as reported in different research studies. CW systems work as filter where a combination of physiochemical and biological processes together contribute in the removal of pathogens (Brix 1993). Physical factors include exposure to ultra-violet rays, filtration, sedimentation, and aggregation with sediments. Chemical factors which cause pathogen removal are oxidation reactions and exposure to toxins produced by plants and microorganisms that already present in root zone (Gersberg et al. 1989). Several other biological processes that significantly remove pathogens are ingestion by nematodes, protozoans, lytic bacteria/bacteriophages attack, and natural death (Ottova et al. 1997).

15.4 Role of Plants in CW

The primary role of vegetation in CWs is to supply oxygen in root zone which further regulates the activity of microorganisms and the treatment of wastewater in CW systems. The root zone is the dynamic reaction zone under substrate where physicochemical and biological processes occur in between contaminants, microorganisms, plant roots, and bed sediments. Plants generally uptake those pollutants which are weak electrolytes and compounds having intermediate lipophilicity. For the compounds which are having strong polarity, it is very difficult to cross biomembranes, which results in limited uptake (Briggs et al. 1982). It is well reported that plants have the largest genome structure as compared to other life forms (Voet et al. 1998). This results in very complex secondary metabolism in plants. Till date more than 80,000 secondary metabolites have been reported in plants (Richter 1998). The reason behind this is that plant metabolism resembles more the reactions occurring in animal liver than the bacterial metabolism (Sandermann 1994). For instance, in plants phase I transformation reactions like hydroxylation, sulfoxidation, and N- and O-dealkylation are catalyzed by P-450 enzymes which play a key role in detoxification of herbicides (Pflugmacher and Schröder 1995; Barret 1995).

Research studies carried out by Choudhary et al. (2013) shows that plants directly uptake some of the chloro-organic compounds from pulp and paper mill wastewater in HSSF-CW and indirectly they maintain aerobic environment in root zone for the oxidative degradation of chloro-compounds specifically chlorophenols by microorganisms. In this study, pulp and paper mill wastewater, having 26 different chlorophenolic compounds, was fed to wetland system. Out of 26, four different categories (chlorophenols, chlorosyringaldehyde, chloroguaiacols, and chlorovanillin) of about 14 chlorophenolic compounds were detected in the harvested plant biomass of *Canna indica*. Among all detected compounds, 5,6-dichlorovanillin contributed to the highest concentration of 623.00 ± 14.62 μg kg^{-1} in plant biomass followed by 2,6-dichlorosyringaldehyde (222.00 ± 10.07 μg kg^{-1}) and 4-chloroguaiacol (154.00 ± 6.90 μg kg^{-1}). Other

chloro-compounds were detected in minor quantities. Category wise, accumulation of chlorovanillin in plants was highest with 53.4%, followed by 19.0%, 14.3%, and 13.4% for chlorosyringaldehyde, chloroguaiacols, and chlorophenols, respectively. In chlorophenols, monochlorophenols accumulated to 38.4% with highest amount in plant biomass. In this study one interesting observation was that only mono-, di-, and trichlorophenolic compounds were accumulated in plant biomass. This can be explained on the basis of log K_{OW} value of the compound. If log K_{OW} value for a compound is less than 4 it can be easily uptake by the plant species while if it is more than 4, uptake by plants is generally not possible. For mono-, di-, and trichlorophenolics the log $K_{OW} < 4$ and for tetra- and pentachlorophenolics the log $K_{OW} > 4$ (Shiu et al. 1995; Xie et al. 1984). Similarly, wetland plants uptake heavy metals also but the rate of removal depends on concentration of heavy metals in wastewater, type of plant species, and vegetation growth.

15.5 Microbial Transformation and Degradation of Pollutants

A diversity of microorganisms present in root zone of the vegetation performs a significant role in the reduction and removal of contaminants most preferably organic matter. Cellular respiration and fermentation are two main processes by which microorganisms convert organic components into carbon dioxide, ammonia, nitrogen, hydrogen sulfide, methane, acetic acid, and water (Dvorak et al. 1992).

15.5.1 Organic Contaminants

The microorganisms generally present in wetlands are autotrophic bacteria, heterotrophic bacteria, fungi (basidiomycetes and yeasts), and some species of protozoa (Kadlec and Wallace 2009). A significant factor which principally influences the degradation process by microbes is the chemical structure of compounds (Dua et al. 2002). The compounds having simple structure, possessing high water solubility, could be efficiently degraded by microorganisms. Organic compounds with complex structures resist degradation process mainly due to the lack of appropriate genes in microorganisms. The degradation rate is comparatively slower for complex aromatic compounds by non-specific enzymes through co-metabolism reactions which do not significantly support the growth of microorganisms (Seffernick and Wackett 2001).

Aerobic chemoheterotrophs degrade organic compounds with comparatively faster rate than chemoautotrophs. Oxidation of organic compounds by these bacteria utilize O_2 as a final electron acceptor (Garcia et al. 2010), and liberate CO_2, NH_3, and other simple compounds. The performance of these microorganisms is basically depends on the concentration of oxygen and organic content in wetland bed. The rate of microbial degradation is also closely associated with the biodegradability index i.e. BOD/COD ratio in wastewater. For untreated domestic wastewater, the range of BOD/COD ratio varies from 0.3 to 0.8. A BOD/COD ratio of 0.5 or more indicates that the organics present in the wastewater are easily degradable, whereas if this ratio

is less than 0.3, it indicates low or non-biodegradability of organic contaminants. Under anaerobic conditions, degradation follows two main steps i.e. fermentation followed by methanogenesis in which anaerobic heterotrophic bacteria utilize organic content for metabolic growth (Saeed and Sun 2012).

15.5.2 Chlorinated Compounds

Microorganisms are efficient enough to degrade a variety of organic contaminants but the rate of degradation depends on chemical and structural properties of target compound, and the physiochemical environment in wetland bed. Because of organic by nature, biodegradation and transformation are the two major mechanisms for removal of chlorinated organic compounds in CWs. The degree of degradation or transformation of specific organic compounds mainly depends on factors like concentration and water solubility (Imfeld et al. 2009). A diversity of microorganisms like *Mycobacterium* sp., *Pseudomonas* sp., *Sphingomonas chlorophenolica*, *Flavobacterium* sp., *Novosphingobium lentum*, *Rhodococcus chlorophenolicus*, *Desulfitobacterium hafniense*, etc., show strong affinity to interact with contaminants leading to complete degradation of the chlorinated compound or some structural changes (Czaplicka 2004; Field and Alvarez 2008). These compounds become water soluble after one or more initial reductive dechlorination steps, and consequently might be degraded by aerobic and anaerobic microbial interaction. These microbial interactions occur simultaneously in root zone of the treatment system. In anaerobic conditions, chlorophenols can undergo reductive dechlorination in the presence of suitable electron-donating components. While in aerobic micro environment, chlorophenols can serve as sole electron and carbon sources to support microbial growth (Field and Alvarez 2008). The rate of aerobic degradation is comparatively slower for higher chloro-compounds in comparison to lower ones (Amon et al. 2007). According to reported studies, biodegradation of pentachlorophenol is faster under anaerobic conditions and pentachlorophenol gets converted into a mixture of di-, tri-, and tetrachlorophenols (D'Angelo and Reddy 2000). Under aerobic root zone environment, higher chlorophenolics are resistant to microbial degradation as chlorine atoms hinder with the action of most of the oxygenase enzymes, which normally initiate the degradation of aromatic compounds (Copley 1997). Some aerobic bacteria which degrade chlorophenolic compounds are *Sphingomonas chlorophenolica*, *Arthrobacter* sp., and *Flavobacterium* sp. (Miethling and Karlson 1996; Yang et al. 2006). Higgblom et al. (1998) investigated the o-methylation of chloroguaiacols following p-dechlorination/hydroxylation by *Rhodococcus chlorophenolicus*.

A number of fungal species like *Auricularia auricular*, *Agrocybe semiorbicularis*, *Coriolus versicolor*, *Flammulina velutipes*, *Pleurotus ostreatus*, *Dichomitus squalens*, *Hypholoma fasciculare*, and *Stereum hirsutum* have been reported for the potential to degrade a range of pesticides like triazine, phenylamide, dicarboximide, phenylurea, organophosphorus, and chloro-organic compounds (Bending et al. 2002).

Many highly halogenated compounds like as polychlorinated biphenyls (PCBs) are extremely resistant to biodegradation, due to their physiochemical properties i.e. low water solubility and the lack of a compatible structural site for the attachment enzymes. As per evidence pentachlorophenol (PCP) breaks down under the alternating aerobic and anaerobic conditions prevalent in wetland substrate (DeBusk 1999). The degradation of haloaromatic compounds proceeds via formation of halocatechols as intermediates which are then cleaved by dioxygenases. Under anaerobic conditions, the breakdown of aromatic compounds occurs through reductive dehalogenation (Farhana and New 1997) which further leads to the formation of more biodegradable compounds (Pitter and Chudoba 1990). According to Tiedje et al. (1969) and Sharpee et al. (1973) reductive dechlorination of 2,4-DCP is followed by carboxylation, ring fission, acetogenesis, and methanogenesis which led to the complete mineralization of 2,4-DCP. Proposed pathway for biodegradation of 2,4-dichlorophenol under aerobic environment by bacteria is shown by Eq. (15.4) (Tiedje et al. 1969; Sharpee et al. 1973).

$$\text{2-chloro-4-carboxymethylenebut-2-enolide} \rightarrow \text{2-chloromaleylaceticacid} \rightarrow \text{Succinicacid} \tag{15.4}$$

In the similar way, anaerobic biodegradation of pentachlorophenol takes place through reductive dechlorination (Uotila et al. 1991). Aerobic degradation of chlorophenols has been reported in soil and freshwater lakes. The studies on phenols and chlorophenols under aerobic soil environment showed that phenol, o-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, and 2,6-dichlorophenol might be degraded fast as compared to pentachlorophenol, 3,4-dichlorophenol, 2,4,5-trichlorophenol, and m-chlorophenol, whereas 3,4,5-trichlorophenol and 2,3,4,5-tetrachlorophenol persist in for several months. Chlorine attachment at meta position increases compounds toxicity and unavailability to soil microorganisms due to their adsorption on sediments. These are several possible causes for low biodegradability of compounds (Balfanz and Rehm 1991). As per the studies a number of bacteria are there which can utilize chlorophenols as a carbon source in aerobic conditions. Chlorophenols are easily metabolized by bacteria under anaerobic substrate environment which support reductive dechlorination by a number of intermediate steps. The reductive dechlorination usually utilizes the availability of electron-donating substrates. Mikesell and Boyd (1986) reported the general encountered reductive dechlorination pathway during anaerobic pentachlorophenol biodegradation which is shown in Eq. (15.5) (Mikesell and Boyd 1986).

$$\begin{aligned}\text{Pentachlorophenol} &\rightarrow \text{2, 3, 4, 5-tetrachlorophenol} \\ &\rightarrow \text{3, 4, 5-trichlorophenol} \rightarrow \text{3, 5-dichlorophenol} \\ &\rightarrow \text{3-chlorophenol} \rightarrow \text{Phenol} \\ &\rightarrow \text{Carbondixode} + \text{Methane}\end{aligned} \tag{15.5}$$

A number of fungal species also shown affinity to mineralize pentachlorophenol (Mileski et al. 1988) and produce extracellular enzymes which polymerize humic

substances with chlorophenols (Bhandari et al. 1996). In soils the pathway for anaerobic transformation of pentachlorophenol is replacement of chlorine sequentially by hydrogen atom which leads to the formation of phenol, benzoate, acetate, CO_2, and CH_4 (Kuwatsuka and Igarashi 1975).

15.6 Treatment Performance of CWs

CWs have been effectively used for industrial wastewaters treatment (Table 15.3). Kumar and Singh (2017) studied municipal wastewater treatment using VF-CW and reported 15.61–83.6% removal of nitrogen, 78.94–80.45% removal of sulfate and phosphate. The integration of Iron Scraps barrier in wetlands improved contaminants removal. Rai et al. (2015) studied seasonal removal of trace elements and nutrients from urban sewage using HSSF-CW. The plants showed higher trace elements removal efficiency in summer (62.22–86%) than in winter (51.39–78.59) with higher accumulation in root than shoot. The average removal efficiency for physicochemical characteristics is found in the range of 55.3–91.61% and 64.8–94.1 during winter and summer. The enhanced removal during summer is due to seasonal variation in temperature and hydrology of wetland. Hence, HSSF-CW is reported to be a sustainable technology for sewage treatment in developing countries. Heyvaert et al. (2006) studied remediation of storm water runoff through SF-CW and reported significant reduction of nutrients (59–83%) and sediment. The sub-alpine CW showed consistent treatment efficiency during winter when the wetland is covered with snow. Pelissari et al. (2014) studied dairy cattle wastewater treatment for nitrogen transformation in HF-CW and VF-CW systems. Nitrification is not observed in HF-CW while 73% removal of NH4-N is achieved by nitrification in VF-CW. The plants uptake 5.2% and 0.88% influent nitrogen in HFCW and VFCW, respectively. Wojciechowska (2017) studied multistage HSSF CW for landfill leachate treatment. The availability of labile organic matter limited denitrification. The municipal wastewater and landfill leachate co-treatment improved treatment performance and biodegradability. Mojiri et al. (2016) worked on co-treatment of municipal wastewater and landfill leachate using HSSF-CW having two adsorbents layers (zeolite and ZELIAC). The trace elements are removed up to 86-87.1% while the removal of physiochemical characteristics ranged from 86.7 to 99.2%. The translocation factor is found >1 and Typha is reported as a hyperaccumulator plant. Rozema et al. (2016) observed the performance of VF-CW for treatment of winery and domestic wastewater in cold climate. The removal of physicochemical parameters ranged from 83 to 99%. The total phosphorus removal decreased as the wetland aged and the treatment efficiency is not affected by cold climate. Kim et al. (2014) reported significant COD and organic nitrogen removal (>94%) for treatment of domestic and winery wastewaters using VF-CWs. The denitrification mostly happened in partially saturated VF-CW without additional carbon addition. Alemu et al. (2017) studied tannery wastewater treatment through hybrid wetland and reported 82–99.9% removal efficiency. The treated wastewater meets discharge standard and can be reused for irrigation. Saeed et al. (2012) studied

Table 15.3 Treatment efficiency of CW for wastewater treatment

Wastewater source	CW configuration	Treatment efficiency (%)			Reference
		BOD_5 and COD	Nitrogen, phosphate, sulfate	Miscellaneous	
Municipal	VF CW, HRT: 48 h	–	NH_4^+: 83.6, TN: 82.4, NO_2^-: 15.6, NO_3^-: 48.9, SO_4^{2-}: 80.5, and PO_4^{3-}: 78.9	–	Kumar and Singh (2017)
Urban	HSSF CW, HRT: 36 h	BOD: 91.6	NH_4^+: 55.3, NO_3^-: 63.1, TP: 58.1	TSS: 82.7, Pb: 78.6–86, Cu: 72.5–84, Zn: 68.4–83.5, As: 63.2–82.2, Cr: 64.5–81.6, Co: 65.1–76.9, Ni: 51.4–68.1, Mn: 53.3–62.2 (summer)	Rai et al. (2015)
Storm water runoff	SF CW	–	NH_4^+: 40, NO_3^-: 83, TN: 49, TP: 66	TSS: 74	Heyvaert et al. (2006)
Dairy	HSSF CW (flow: 3.98 m^3/week) and VF CW (flow: 4.5 m^3/week)	COD: 68–74	NH_4^+: 58–80 TN: 23–59	–	Pelissari et al. 2014
Landfill leachate and municipal	Multistage HSSF CW, HRT: 7.1 day	BOD: 88–95, COD: 47.8–86.6	TN: 68.9–98.5	–	Wojciechowska (2017)
Landfill leachate and municipal	Adsorption and HSSF CW, contact time: 50.2 h	COD: 86.7	NH_4^+: 99.2	Color: 90.3, Ni: 86, Cd: 87.1	Mojiri et al. (2016)
Winery	VF CW, HLR: 22.3 mm/day, flow rate: 16,620 L/day	BOD: 99 COD: 99	NH_4^+: 85, TKN: 94, TP: 83	TSS: 98	Rozema et al. (2016)
Domestic and winery	Hybrid (trickling filter + VF CW), flow rate: 70 m^3/day	BOD: 97.9 COD: 94.9	TN: 70.9, TKN: 97.2, TP: 59.6	TSS: 98.8	Kim et al. (2014)
Tannery	Hybrid (hydrolysis-anaerobic-SBR-HSSF CW), flow rate: 23.63 m^3/day	BOD: 98.2 COD: 97.7	NH_4^+: 93.5, NO_3^-: 79.7, TN: 90.8, SO_4^{2-}: 82, S^{2-}: 99.9	Cr: 97.4	Alemu et al. 2017

(continued)

Table 15.3 (continued)

Wastewater source	CW configuration	Treatment efficiency (%)			Reference
		BOD_5 and COD	Nitrogen, phosphate, sulfate	Miscellaneous	
Tannery	Hybrid (VF-HF-VF) CW, HLR: 6 cm/day, HRT: 4.8 day VF-12.5 day HF-2.4 day VF	BOD: 98 COD: 98	NH_4^+: 86, NO_3^-: 50, PO_4^{3-}: 87	TS: 55	Saeed et al. (2012)
Pulp and paper	HSSF CW, HRT: 5.9 day	BOD: 90 COD: 84	–	Color: 94, AOX:87, chlorophenolics: 87, cRFA: 93	Choudhary et al. (2015)
Pulp and paper	HSSF CW, HRT: 5.9 day	–	–	AOX: 89.1, Chlorophenolics: 67–100	Choudhary et al. (2013)
Aquaculture ponds	VF CW, HRT: 0.58–1.44 day	BOD: 70.5	NH_4^+: 61.5, NO_3^-: 68, PO_4^{3-}: 20	TSS: 81.9, chlorophyll a: 91.9%	Gu et al. (2007)
River	Floating beds	COD: 80	NH_4^+: 85.2, sulfide: 90	TSS: 85.2	Sheng et al. (2013)
Lake	Combined floating-bed systems	–	TN: 52.7, NH_4^+: 33.7, TP: 54.5	TOC: 49.2, chlorophyll a: 80.2	Li et al. (2010)
Refinery	Perennial grass floating-bed system, HRT: 35 day	COD: 52.2–66	TN:59.1–69.5, TP: 55.7–72.3	–	Li et al. (2012)
Swine	Macrophyte floating-bed system, HRT: 35 day	COD: 80.7–85.4	TN: 79.6–84.0, TP: 88.3–90.4	Sulfonamides: 89–99	Xian et al. (2010)
Tool factory (metallurgic)	SF CW, HRT: 7–12 day	–	NO_3^-: 70 NO_2^-: 60	Cr: 86, Ni: 67	Maine et al. (2006)
Textile	Hybrid CW, HLR: 566–5660 mm/day, HRT: 0.2–1.8 day (VF CW), 0.7–7.1 day (HF CW)	BOD: 98 COD: 98	NH_4^+: 86, NO_3^-: 50, PO_4^{3-}: 87	–	Saeed and Sun (2013)
Potato farm	SF CW, HRT: 0.3 months	BOD: 79–96	TN: 62–86 TP: 54–90	TSS: 97–99	Bosak et al. (2016)

Municipal and hospital	VF CW (aerated), HRT: 0.5–2 day	–	–	Metformin: 99, valsartan: 99	Auvinen et al. (2017)
Hospital	HSSF CW, HRT: 4 day	–	–	Antibiotic resistant bacteria: 80.8–93.2	Dires et al. (2018)

TN total nitrogen, *TP* total phosphorus, *TS* total solids, *TSS* total suspended solids, *TKN* total Kjeldahl nitrogen, *AOX* adsorbable organic halides, *cRFA* chlorinated resin and fatty acids, *TOC* total organic carbon

treatment of tannery wastewater using hybrid wetland system and reported 50-98% removal efficiency. The coco-peat media with high porosity allowed oxygen to transfer in VF-CW for nitrification and organics removal. The SSF-CW showed higher removal of phosphorus with iron-rich cupola slag media. Choudhary et al. (2013, 2015) studied removal of chloro-organics from wastewater of pulp and paper mill using HSSF-CW planted with *Colocasia esculenta* and *Canna indica*. The removal efficiency varied from 67 to 100%. Chloro-organics are found to accumulate in wetland components i.e. in plant biomass and soil. Plant species showed significant accumulation for di-chlorophenol (DCP) and no affinity for chloro-resin and fatty acids. The major fraction of chloro-organics is degraded in the constructed wetland. Gu et al. (2007) assessed performance of group of CWs for treatment of re-circulating aquaculture wastewater and reported 20–91.9% removal efficiency. Sheng et al. (2013) studied remediation of heavily polluted river using integrated ecological floating beds and observed >70% average removal. The unpleasant odor stopped and farmers started using water for irrigation. Li et al. (2010) studied a novel integrated ecological floating bed (plants, filter-feeding bivalve, and bio-film carrier) for lake water purification. The better removal of nitrogen and phosphorus has been reported. Li et al. (2012) studied nutrients and pollutants removal from refinery wastewater using perennial grass (fodder crops) floating-bed system and reported 52.2–72.3% removal efficiency. *Lolium perenne Topone* showed best results with highest survival rate. The grass floating beds may significantly enhance the microbial activity in the wastewater. The plant residues can be beneficially reused as animal feed. Xian et al. (2010) studied swine wastewater treatment using floating-bed CW and reported 83.4–99.5% removal efficiency. Dryan demonstrated best pollutant removal efficiency. The root showed higher potential for assimilation of nutrients and antibiotics than shoot. Maine et al. (2006) investigated metal and nutrient removal from metallurgic plant wastewater using SF CW. The system is found efficient for metals removal (66–95%). Bosak et al. (2016) studied potato farm wastewater treatment with SF CW and reported 54–99% removal efficiency. The best treatment performance is reported during spring months. Auvinen et al. (2017) studied performance of aerated SSF CW for removal of pharmaceuticals from municipal and hospital wastewaters. The single stage of SSF CW was unsatisfactory in controlling discharge of pharmaceuticals. The pharmaceuticals removal efficiency improved significantly when continuous aeration is applied. Dires et al. (2018) investigated removal of antibiotic resistant bacteria from hospital wastewater using HSSF-CWs. Considerably high removal of antibiotic resistant bacteria is reported in wetlands having vegetation than the non-vegetated systems. Efficacy of CW filter containing different macrophytes (*Phragmites*/*Typha*) for mine wastewater treatment has been reported to successfully remove sulfate (up to 70%), lead (up to 65%), zinc (95%), BOD (80%), and ammonia nitrogen (95%) (Scholz et al. 2002; Aisling et al. 2004). Wanga et al. (2009) performed mesocosms experiment using a VF-CW for the treatment of liquid sludge in the Mediterranean region. In this study, three plant species, broadleaf cattail (*Typha latifolia L.*), common reed (*Phragmites australis.*), and yellow flag (*Iris pseudacorus L.*), were planted as monoculture and feed with a liquid sewage sludge from a food industry, with high COD

concentrations (COD > 8000 mg/L). Removal efficiency was more than 87% for total Kjeldahl nitrogen and about 98% for TSS and COD. Kadlec and Knight (1996) reported more than 90% removal of coliforms and more than 80% for *Streptococci* in wetland systems. Similarly, Neralla et al. (2000) reported up to 99% reduction in fecal coliform by CW.

15.7 Operation and Maintenance

The first most condition for the proper operation of treatment system is pre-treatment of wastewater prior to treatment by CW. Clogging is a main operational issue related to SSF wetlands and can ultimately limit the lifetime of the system. So it is highly recommended to remove the suspended solids from the wastewater prior to wetland treatment. Based on initial predictions, the longevity of HSSF-CW systems were about 50–100 years (Conley et al. 1991) but with time this estimation has been changed to 15 years (Cooper et al. 1996), 10 years (Wallace and Knight 2006), and 8 years (Griffin et al. 2008), respectively, as per different opinions. Clogging or chocking occurs due to build up of suspended particles present in wastewater and other system parameters. Removal of suspended solids is very significant to avoid or reduce the clogging of bed. In controlling the system operation hydrology plays a very crucial role and it should be regulated time to time to attain acceptable treatment results. The optimization of hydraulic loading rate (HLR) and hydraulic retention time (HRT) should be carried out before full scale operations of the wetland treatment facility. Here we can conclude that greater the HRT more would be the treatment efficiency of the system. For CWs, HRT is the average time that wastewater takes to pass from inlet to the outlet in the wetland bed. It is generally expressed as mean volume of the treatment bed divided by mean outflow rate. If, anyhow short-circuiting develops, effective HRT differs considerably from the calculated HRT as per the given below Eq. (15.6) (EPA 1995).

$$\mathrm{HRT} = nLWd/Q \tag{15.6}$$

where: n = porosity of substrate media (% as a decimal), L = length of bed (m), W = width of bed (m), d = depth of wastewater in bed (m), Q = flow rate (m^3/day)

A HRT of 5–6 days is sufficient for the efficient removal of contaminants from wastewater (Choudhary et al. 2013). Here porosity is equal to void volume/total volume by percent. For coarse sand, porosity is about 32% and for gravel it is 35–40% (Reed 1993). HLR is also an important parameter for treatment operation which is equal to the loading on a wastewater volume per unit area of CW bed (Eq. (15.7)) (EPA 1995).

$$\mathrm{HLR} = (\mathrm{Parameterconcentration})(\mathrm{Wastewatervolume/area}) \tag{15.7}$$

SF-CWs in general contain water depths <0.4 m, and HLR range varies 0.7–5.0 cm/day, which corresponds to a wetland area of 2–14 hectare/1000 m^3/

day of flow (Kadlec and Knight 1996) and the required surface area is about 3–10 m^2/PE depending on climatic and design parameters. In countries having warm climates relatively less area is required because of higher biological activity while in cold climates the minimum required area should not be less than 5 m^2/PE. The organic loading should not be more than 4–10 g BOD/m^2 day in cold climates (Morel and Diener 2006). To maintain efficient operation of VF-CWs, recommended surface area is typically 3–4 m^2/PE to treat domestic wastewater in cold regions and 1–2 m^2/PE in warm climatic conditions. The organic loading should be limited to 20 g COD/m^2 day and 60–70 g COD/m^2 day, in cold and warm climates, respectively. The hydraulic loading for VF-CWs in cold climates should not exceed 100–120 mm/day (Hoffmann et al. 2011). The hydraulic loading should be checked regularly and should not exceed the recommended input values.

Evapotranspiration (ET) is one of the unavoidable factors which affect the wetland water budget and consequently the HRT. ET is the total water loss through transpiration by plants and evaporation from the upper surface of wetland. If ET exceeds the total hydraulic loading in the system, additional water should be added to keep the system wet. The ET rate depends on the weather conditions i.e. time of the day, temperature, wind speed, humidity, and designing of the system. Higher ET rate results in significant water loss which consequently affects the HRT for treatment. Without considering the ET values, it is not possible to maintain HRT accurately. CWs must be properly managed if they are to perform well.

15.8 Conclusions

Constructed wetlands (CWs) are simple and have been proved to be a sustainable and green wastewater treatment technology that uses natural processes to improve wastewater quality. Wetland vegetation and microorganisms play an active role in whole treatment process. These systems mainly depend on renewable energy sources such as solar and gravitational energy for treatment operation. It is one of the best choice for treatment of domestic and industrial wastewater because of low operational cost than traditional wastewater treatment facilities and simplicity of operation with high treatment efficiency. It also enhances the esthetic value of the local environment and acts as a habitat for flora and fauna. Furthermore, it facilitates water reuse and recycling which is also an important factor.

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