Environmental Chemistry for a Sustainable World 44

K. M. Gothandam
Shivendu Ranjan
Nandita Dasgupta
Eric Lichtfouse *Editors*

Environmental Biotechnology Vol. 1

Springer

Environmental Chemistry for a Sustainable World

Volume 44

Other Publications by the Editors

Books
Environmental Chemistry
http://www.springer.com/978-3-540-22860-8

Organic Contaminants in Riverine and Groundwater Systems
http://www.springer.com/978-3-540-31169-0

Sustainable Agriculture
Volume 1: http://www.springer.com/978-90-481-2665-1
Volume 2: http://www.springer.com/978-94-007-0393-3

Book series
Environmental Chemistry for a Sustainable World
http://www.springer.com/series/11480

Sustainable Agriculture Reviews
http://www.springer.com/series/8380

Journals
Environmental Chemistry Letters
http://www.springer.com/10311

More information about this series at http://www.springer.com/series/11480

K. M. Gothandam • Shivendu Ranjan
Nandita Dasgupta • Eric Lichtfouse
Editors

Environmental Biotechnology Vol. 1

Springer

Editors
K. M. Gothandam
School of Bio Sciences and Technology
Vellore Institute of Technology
Vellore, Tamil Nadu, India

Nandita Dasgupta
Department of Biotechnology
Institute of Engineering and Technology
Lucknow, Uttar Pradesh, India

Shivendu Ranjan
Faculty of Engineering and the Built Environment
University of Johannesburg
Johannesburg, South Africa

Eric Lichtfouse
Aix-Marseille University, CNRS, IRD, INRA, Coll France, CEREGE
Aix-en-Provence, France

ISSN 2213-7114 ISSN 2213-7122 (electronic)
Environmental Chemistry for a Sustainable World
ISBN 978-3-030-38191-2 ISBN 978-3-030-38192-9 (eBook)
https://doi.org/10.1007/978-3-030-38192-9

© Springer Nature Switzerland AG 2020
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Switzerland AG.
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

‘Dedicated to all real sufferers for the lack of a clean environment’

Gothandam, Shivendu, Nandita and Eric

Preface

Biotechnology is constantly explored to develop methods for wastewater treatment for clean environment. In other words, new types of pollutants are also added to the waterbodies like engineered nanoparticles, microplastics, etc. Current wastewater treatment technologies are not sufficient to meet the ever-growing demands due to rapid industrialization and population growth, and they are also energy consuming and cost intensive. This book reviews the various ecological concerns and technologies in wastewater treatment.

In Chap. 1, Zaveri et al. review the engineered nanoparticles released into wastewater from various products, their effects at different treatment stages, and their probable transformation and recovery. In Chap. 2, Kaisar Ali and Husain Mir discuss the involvement of microbial ecosystem in improving the environment. In Chap. 3, Maria Santos and Magalhães Pires present the advantages of using microalgal cultures for nutrient recovery from wastewaters, while in Chap. 4, Amita Shakya and Faraz Ahmad emphasize the importance of biochar for heavy metal-contaminated wastewater treatment. In Chap. 5, Arti Srivastava and Ashutosh Srivastava discuss the microplastics and nanoplastics and their emerging threat to the aquatic organisms. Srivastava, in Chap. 6, reviews the impact of sustainable energy resources on healthy environment. In Chap. 7, Kavitha and Inbakanadan discuss eco-friendly methods to produce biopolymer and nanomaterials. Moreover, in Chap. 8, Pabbathi et al. explain the metabolomics in marine toxicology assessment. In Chap. 9, Sahu et al. highlight the causes of epiphytic infestations and its economic implications. Finally, in Chap. 10, Geetanjali et al. discuss the potential applications and practical limitations of microbial fuel cell in wastewater treatment and energy generation (Fig. 1).

Thanks for reading.

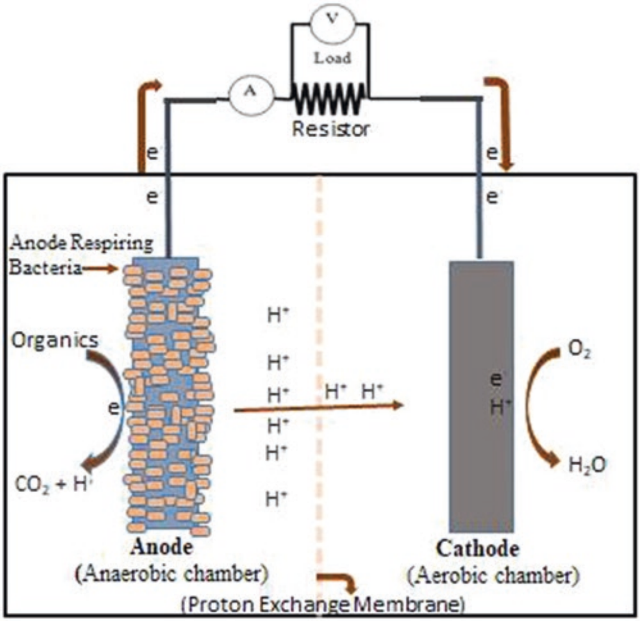


Fig. 1 Basic design of a microbial fuel cell

Vellore, Tamil Nadu, India	K. M. Gothandam
Johannesburg, South Africa	Shivendu Ranjan
Lucknow, Uttar Pradesh, India	Nandita Dasgupta
Aix-en-Provence, France	Eric Lichtfouse

Contents

About the Editors

Dr. K. M. Gothandam is Professor in the Department of Biotechnology, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore. He earned his Ph.D. from Bharathiar University, Coimbatore, and his Postdoctoral Fellowship from the School of Life Sciences and Biotechnology, Korea University, Seoul (2002–2007). He also served as Head of the Department of Biotechnology and Dean of the School of Bio Sciences and Technology (2016 to 2018). His research interest includes functional genomics, plant and microbial metabolites, cancer biology, environmental biotechnology, etc. He has published over 75 scientific research and review articles in international peer-reviewed journals and also refereed many journals of high impact, authored 5 book chapters, edited 2 books, and guided 10 Ph.D. theses and 6 scholars. At present, he is handling two projects funded by DBT and completed five projects funded by DBT, DST, and CSIR.

Dr. Shivendu Ranjan is Director at the Centre for Technological Innovations and Industrial Research, SAIARD (Certified Institute of the Ministry of Micro, Small and Medium Enterprises, Government of India). He is also serving as a Senior Research Associate (visiting) at the Faculty of Engineering and Built Environment, University of Johannesburg, South Africa. His research interests include nanotechnology, nanomedicine, science policy, and diplomacy. He is Associate Editor of *Environmental Chemistry Letters* and Editorial Board Member of several journals of international repute. He has received many awards and honors from national and international organizations. He is Elected Fellow of several scientific societies, such as Indian Chemical Society, Linnean Society (London), Bose Science Society, and Indian Engineering Teachers Association.

Dr. Nandita Dasgupta is working as Assistant Professor in the Department of Biotechnology, Institute of Engineering and Technology, Lucknow, India. She has worked on mesenchymal stem cell-derived exosomes for the treatment of uveitis and has successfully engineered micro-vehicles for model drug molecules. Her areas of interest include nanomaterial fabrication and its applications in medicine, food, and biomedicine. She is the Associate Editor of *Environmental Chemistry Letters* and Elected Fellow of the Linnean Society (London) and Bose Science Society. She has received several awards and recognitions from different national and international organizations.

Dr. Eric Lichtfouse is Geochemist and Professor of Scientific Writing at Aix-Marseille University, France, and Visiting Professor at Xi'an Jiaotong University, China. He has discovered temporal pools of molecular substances in soils, invented carbon-13 dating, and published the book *Scientific Writing for Impact factor Journals*. He is Chief Editor and Founder of the journal *Environmental Chemistry Letters* and the book series Sustainable Agriculture Reviews and Environmental Chemistry for a Sustainable World. He has awards in analytical chemistry and scientific editing. He is World XTERRA Vice-Champion.

Contributors

Roma Agrahari Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, Uttar Pradesh, India

Faraz Ahmad Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India

Md Kaisar Ali School of Life Science, Southwest University, Chongqing, China

Mootapally Chandrashekar Department of Marine Science, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat, India

Indra Ramjibhai Gadhvi Department of Marine Science, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat, India

Geetanjali Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, Uttar Pradesh, India

D. Inbakanadan Centre for Ocean Research (DST-FIST Sponsored Centre), MoES – Earth Science & Technology Cell (Marine Biotechnological Studies), Col. Dr. Jeppiaar Research Park, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

Kapilkumar N. Ingle Department of Ecology, University of Szeged, Szeged, Hungary

G. Kavitha Centre for Ocean Research (DST-FIST Sponsored Centre), MoES – Earth Science & Technology Cell (Marine Biotechnological Studies), Col. Dr. Jeppiaar Research Park, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

Sanjay Kumar School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi, India

Vaibhav A. Mantri Applied Phycology and Biotechnology Division, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar, India

Sajjad Husain Mir School of Chemistry, Advanced Materials and BioEngineering Research Centre (AMBER), Trinity College Dublin, The University of Dublin, Dublin, Ireland

Nasreen S. Munshi Institute of Science, Nirma University, Ahmedabad, Gujarat, India

Neelam M. Nathani Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar, Gujarat, India

Ninian Prem Prashanth Pabbathi Department of Biotechnology, National Institute of Technology (NIT), Warangal, Telangana, India

Rushika Patel Institute of Science, Nirma University, Ahmedabad, Gujarat, India

José Carlos Magalhães Pires LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

Radha Rani Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, Uttar Pradesh, India

Sunil Kumar Sahu BGI-Shenzhen, Shenzhen, China

State Key Laboratory of Agricultural Genomics, China National GeneBank, BGI-Shenzhen, Shenzhen, China

Francisca Maria Santos LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal

Amita Shakya Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Sonipat, Haryana, India

Arti Srivastava Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Noida, India

Ashutosh Srivastava Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Noida, India

Amity Institute of Marine Science and Technology, Amity University, Uttar Pradesh, Noida, India

Rajesh K. Srivastava Department of Biotechnology, Gitam Institute of Technology and Management (GITAM) (Deemed to be a University), Visakhatpatnam, Andhra Pradesh, India

Purvi Zaveri Institute of Science, Nirma University, Ahmedabad, Gujarat, India

Chapter 1
Trickling of Itinerant Nanoparticles in Wastewater Effluents

Purvi Zaveri, Rushika Patel, and Nasreen S. Munshi

Contents

Abstract Engineered nanoparticles have found widespread applications in technology, industry, healthcare and consumer products today. The magnitude of nanoparticles released by various industrial and domestic processes is as high as 2985 tonnes/year into receiving water bodies. Yet the data on release of nanoparticles from different types of products and their interactions in environment are scanty. These nanoparticles also undergo transformations in complex environment such as those of wastewater treatment plants, making their life cycle assessment difficult. Their high concentrations and the transformed products are expected to have adverse effect on functionality and survival of microflora in treatment plant. Silver and gold nanoparticles were found detrimental at even lower concentration of less than 1 ppm. The important microbial activity in wastewater treatment plants including nitrogen removal by nitrifying bacteria was found to be reduced by 85% and biogas production by 33–50% upon exposure to silver nanoparticles. Other processes affected were microbial respiration, chemical oxygen demand reduction, substrate utilization, etc. Changes in structural diversity in presence of silver and

---

P. Zaveri · R. Patel · N. S. Munshi (✉)
Institute of Science, Nirma University, Ahmedabad, Gujarat, India
e-mail: nasreen.munshi@nirmauni.ac.in

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_1

zinc oxide nanoparticles were observed indicating substantial change in microbial community composition and thus function. This review emphasizes on engineered nanoparticles released into wastewater from various products, influence of nanoparticles at different treatment stages, their probable transformation and recovery and most importantly effect on wastewater microbial community and their function.

Keywords Engineered nanoparticles · Wastewater treatment · Microbial community · Structural diversity · Chemical oxygen demand

1.1 Introduction

In today's world, engineered nanoparticles have acquired profound place in a variety of applications including industrial, agricultural and consumer-based products. The global production and use of nanoparticles and their products will be reaching 10^{13} tonnes per year by the year 2020 (Borm et al. 2006). Nanoparticles carry distinct surface charges which induce specific chemical interactions with surrounding chemical milieu and microbial community (Maurer-Jones et al. 2013). The size of engineered nanoparticles being less than 100 nm gives them the advantage of entering any matrix easily and thus have upsurged their applications in various fields, including as drugs and vaccine, antibacterial and antifungal agents in a variety of products, biological probes, etc. (Patel et al. 2017). Moreover, they have enormous usage in fields of textile, cosmetics and electronics and as fuel additives, paints and coatings, plastics and glasses, etc. due to their size specific physicochemical properties (Boyoglu et al. 2013). Nonetheless, the same features have generated a risk for their frequent and unacquainted spread in nature. It is now well-known that majority of the nanoparticles have antimicrobial properties ranging from narrow to broad spectrum toward different groups of microorganisms (Wang et al. 2017a, b). Thus, although they have added values to the products manufactured using these nanoparticles, concerns have increased for environmentalists. To the best of our knowledge, the review published by Baun et al. (2009) was first of its kind disseminating awareness for ecological concerns and related impact of engineered nanoparticles on our environment. Since then, some studies related to nanoparticle release and their interactions at environmental interface have been carried out, but the magnitude of all such products is still not fully covered. It is required to assess their fate in environment after their intended use is over.

According to the physical state of engineered nanoparticles, they may be released in the form of aerosols into the atmosphere or may get precipitated and released into soil and/or surface water (Bystrzejewska et al. 2009). It has been well established that during their life cycle, engineered nanoparticles are bound to encounter wastewater treatment facility and mostly may end up settling in sludge (Brar et al. 2010). It means that to understand the effect of engineered nanoparticles on wastewater microbial community and function, the concentration of nanoparticles released from manufacturing units or after use of individual products needs to be known. Still the data related to nanoparticles released from a variety of sources is fragmentary in nature. Moreover, undefined concentration of nanoparticles in product manufacturing makes it difficult to estimate their release in wastewater and influence on treatment processes.

Some studies investigated the effect of varieties of concentrations of single type of nanoparticles using monoculture or mixed culture of prokaryotic and/or eukaryotic organisms. Widely used nanoparticles, like cadmium, carbon-based nanomaterials, single-walled carbon nanotubes, multiwalled carbon nanotubes, aqueous phase C60, diamond and graphite, cerium dioxide, titanium dioxide, zinc oxide and cadmium-based quantum dots, have been investigated for their cytotoxicity and hazardous implications in the environment (Mahendra et al. 2008; Balmuri et al. 2017; Kang et al. 2009; Zakrzewska et al. 2015; Pelletier et al. 2010; Forest et al. 2017; Frenk et al. 2013; Shah et al. 2017; Zhang et al. 2016; Su et al. 2009; Oh et al. 2016).

Such studies are regarded as poor predictors of community interaction and mass microbial cytotoxicity reactions (Kang et al. 2009). In addition, transformation of engineered nanoparticles in the environment after their release from manufacturing or production units or domestic sources may lead to different types of interactions in environment as compared to that by native nanoparticles. Hence, influence of nanoparticles on complex microbial communities such as those in wastewater treatment plant needs to be addressed effectively.

This review focuses on engineered nanoparticles released into wastewater from various goods and emphasizes on the influence of nanoparticles at different stages of wastewater treatment, their probable transformation and, most importantly, their effects on wastewater microbial community and function.

1.2 Gateways for Entry of Engineered Nanoparticles in Wastewater Streams

The release of nanoparticles from various consumer products creates entry portals of engineered nanoparticles into the environment. Thus, effluents released from industrial manufacturing units or domestic sources become carriers of these moieties, and ultimately wastewater treatment plants become a sink for nanoparticles (Eduok et al. 2013).

Though many articles have summarized the worldwide use of various nanoparticle-based consumer products and derivatives and their release during production (Kunhikrishnan et al. 2015) as well as after usage of these products (Bystrzejewska et al. 2009), it is difficult to get actual data regarding concentrations of such nanoparticles released in receiving wastewaters. In case of unavailability of such release data, probabilistic modelling approach has also been adapted by many, which is also discussed here.

1.2.1 Actual Estimations of Engineered Nanoparticle Releases from Different Sources

Being first of their kinds in this research field, Benn and Westerhoff (2008) studied six types of socks containing up to a maximum of 1360 μg silver nanoparticles/g sock and demonstrated that washing leads to leaching of as high as approximately 50% (650 μg) of silver in 500 ml of distilled water. They indicated the empirical probability of similar amount of silver being released from various consumers worldwide.

Using inductively coupled mass spectrometry, Farkas et al. (2011) studied the effluent produced by nanosilver-producing washing machine. The experimental data suggested that effluent contained silver nanoparticles at an average concentration of 11 μg/l. The silver load released was shown to reduce the bacterial population in wash water emphasizing the effect of silver nanoparticles on domestic wastewater treatment systems and risk associated with sludge produced thereof being used in agricultural fields.

Similarly, Mackevica et al. (2017) analysed release of silver nanoparticles from toothbrush. And the study indicated released quantities to be in nanograms, being 10 ng per toothbrush, and concluded them to be close to negligible for human and environmental exposure but summoned for the requirement to determine the "safe level" from an environmental perspective. The findings were supported by similar studies conducted by Quadros et al. (2013) for the release of silver nanoparticles from clothing material and children's consumer products. The released concentrations were found to be 0.024 ± 0.02 mg Ag/kg from a foam material and 1.6 ± 0.3 mg Ag/kg from baby blankets when they were soaked in tap water. Some of the information about the actual release of engineered nanoparticles from a variety of products including textiles is compiled in Table 1.1.

Apart from these limited findings on nanoparticle release data, no systematic approach was found to be adapted for a myriad of products developed incorporating nanoparticles. Hence, inadequate data of release of nanoparticles from the consumer goods becomes a limiting factor for accurate analysis of their environmental impact.

Table 1.1 Concentration of released engineered nanoparticles from various products reaching into wastewater

Sr. no.	Nanoparticle	Released amount	Description/measured parameter(s)	Reference
1	Ag NPs	0.51 ± 0.04%	Two textiles were studied, released into various body fluids and released after laundry was studied	Stefaniak et al. (2014)
2	Ag NPs	0 mg/kg to about 322 mg/kg	Nanoparticle release from antibacterial fabrics into artificial sweat	Kulthong et al. (2010)
3	Ag NPs	1.5–650 µg	From commercially produced socks after washing cycles	Benn and Westerhoff (2008)
4	TiO_2 NPs	600 µg/l	Ti concentrations in runoff water	Kaegi et al. (2008)
5	TiO_2 NPs	Estimated 230 tonnes/year in France	Release in water from commercialized sunscreens	Botta et al. (2011)
6	Ag NPs	1.7%, 3.0% and 5.6% for 3% (w/v) aqueous acetic acid at 20, 40 and 70 °C	Data is provided also for migration in ethanol	Song et al. (2011)
7	TiO_2 NPs	1.5–15 mg/l of titanium in wastewater	Estimated release from textiles	Lorenz et al. (2012)
8	Ag NPs	11.8 kg per year	Estimated release from washing machine for 20% of Norway population	Farkas et al. (2011)
9	Ag NPs	0.32 and 38.5 mg/ l	Release measured into washing solution from textile	Lorenz et al. (2012)
10	TiO_2 NPs	As high as 5 mg/l	Nanoparticle release during textile washing cycles	Windler et al. (2012)
11	TiO_2 NPs	0.3 mg TiO_2/person/day	Nanoparticle release from food and consumer products	Weir et al. (2012)
12	Ag NPs	0.3–377 µg/g	Release of nanoparticles from nanotextiles during washing	Geranio et al. (2009)
13	Au NPs	124 and 145 µg/kg	Predicted environmental concentration in sludge (estimation for the UK and USA, respectively)	Mahapatra et al. (2015)
14	Nano-SiO_2	0.04 and 0.1 mg/l	Release of nanoparticles in water from paint	Al-Kattan et al. (2015)
15	Ag NPs	45 µg Ag/g product	From products like shirt, detergent, humidifiers, etc. (from 1.4 to 270,000 µg Ag/ g product)	Benn et al. (2016)
16	Ag NPs	0.03–8.4 mg Ag/kg	Plasticized polyvinyl chloride (PVC) nanocomposites	Cushen et al. (2013)
17	Ag NPs	0.003 to 0.005 mg/dm^2	Polyethylene nanocomposites	Cushen et al. (2014)

(continued)

Table 1.1 (continued)

Sr. no.	Nanoparticle	Released amount	Description/measured parameter(s)	Reference
18	Cu NPs	0.024 to 0.049 mg/dm^2	Polyethylene nanocomposites	Cushen et al. (2014)
19	Ag NPs	30 ng/cm^2	Silver released from commercial plastic food containers into different food simulants	Goetz et al. (2013)
20	TiO_2	5 mg/l	Release of titanium in one wash cycle from textile	Windler et al. (2012)

Note: Quantities presented in the table include both estimated and predicted values
Abbreviations used are *Ag NPs* silver nanoparticles, *TiO_2 NPs* titanium dioxide nanoparticles, *Au NPs* gold nanoparticles, *nano-SiO_2* nanoparticles of silicon dioxide, *Cu NPs* copper nanoparticles

1.2.2 Modelling for Estimation of Engineered Nanoparticle Releases

Predicted environmental concentrations of nanoparticles are based on the probability of material flow analysis. Mahapatra et al. (2015) described the flow of gold nanoparticles. They indicated that the second largest flow of gold nanoparticles is from sewage treatment plants and were also able to tabulate their predicted concentrations in various receiving environmental compartments.

More than 30,000 metric tonnes only of zinc oxide-engineered nanomaterials was estimated to be produced globally by Keller et al. (2013). They estimated the release of zinc oxide-engineered nanomaterials to be 170–2985 tonnes/year into receiving water bodies. Using modelling studies, probabilistic estimates were generated for zinc oxide-engineered nanomaterials released through several applications, e.g. plastics, cosmetics, coatings and cleaning agents, batteries and capacitors, paints, consumer electronics, textiles, dietary supplements and research and development (Keller and Lazareva 2013; Keller et al. 2013). In addition, for certain applications such as aerospace and automotive fields, where no actual estimates could be produced regarding nanoparticle release, they estimated from exposed surfaces considering high and low production volumes as well as emission factors. These reports are the finest of the presentations based on the presumed values, and great efforts were put for the compilation of information available from different countries.

Caballero-Guzman and Nowack (2016) mentioned that, apart from actual nanoparticle release information available and even with the best prediction methods, studies related to release of nanoparticles from products cover only 20% of the engineered nanoparticles used industrially and just 36% of the consumer product categories. The report indicates that there are less than 50 research articles available containing release-related information, most of them being only qualitative in nature.

It is clear so far that such studies have been restricted to limited types of nanoparticles, and hence it undoubtedly indicates the need for intense research on release of nanoparticles involving all varieties of nanoparticles and related products.

It is also important to note that all such reports do not necessarily mention mobile phase carrying the nanoparticles for their further dissemination. But it is apparent that most of the time, water/wastewater stream becomes a carrier. Such flowing streams while carrying nanoparticles may not lead to accumulation of substantial amount in it as the released concentrations are generally small. However, these streams growing together and reaching to treatment plant are bound to accumulate them, and hence, it becomes inevitable to speculate the impact on wastewater treatment processes. One of the recently published reports by Wang and Chen (2015) summarized the measured and predicted concentrations of various engineered nanoparticles in wastewater effluents and sludge samples collected from different countries. The report was prepared after extensive study and delivers appreciable information related to this field. It was reported that the concentration of silver, titanium dioxide, zinc oxide and carbon-based nanoparticles and fullerene C60 and fullerene C70 were present in terms of ng to μg/l levels in wastewater effluents, whereas the concentration of the same in sewage sludge was alarmingly very high, i.e. few hundred to thousand parts per million levels. The concentrations of silver, titanium dioxide, zinc oxide and carbon-based nanoparticles and fullerenes in wastewater were found to vary from 1 ng/l to 1.5 μg/l, 1.75 to 4.28 μg/l, 0.3 to 0.441 μg/l, 8.6 to 14.8 ng/l and 0 to 19 μg/l, respectively, whereas sludge generated from wastewater treatment plants was found to contain 0.84 to 1880 mg Ag nanoparticles/kg, 136 to 211 mg TiO_2 nanoparticles/g, 17.1 to 23.2 mg ZnO nanoparticles/kg and 0.062 to 0.069 mg carbon-based nanoparticles/g of biosolids.

Moreover, latest technologies have started using nanoparticles for treatment of wastewater itself, making it amenable for release directly into wastewater stream (Amin et al. 2014; Gehrke et al. 2015).

1.2.3 Nanotechnology in Wastewater Treatment

Application of engineered nanoparticles for wastewater treatment adds a new dimension of entry of nanoparticles in the effluent. Thus, apart from all conventional entry points of engineered nanoparticles from domestic, industrial and incidental release in the environment, varieties of novel water treatment technologies are also probable concern points for bulk entry of nanoparticles.

For treatment of wastewater, use of nanotechnology is based on two basic principles of photocatalysis and nanosorbents (Bora and Dutta 2014).

Photocatalysis Nanostructured visible light photocatalysis uses semiconductor photocatalysis involving titanium dioxide (TiO_2), zinc oxide (ZnO), ferric oxide (Fe_2O_3), zinc sulphide (ZnS) and cadmium sulphide (CdS) nanoparticles. Such semiconductors have proven to be quite useful and efficient for degradation of various chlorinated organic contaminants and phenolic derivatives which are difficult to be removed biologically (Dasgupta et al. 2017). Compounds like tetrachloroethylene, trichloroethylene and carbon tetrachloride which are not easily mineralized biologically were reported to be degraded by the metallo-porphyrinogens, a type of engineered nanoparticle, under anaerobic conditions (Dror et al. 2005; Chen et al. 2005). Antimicrobial potential of engineered nanoparticles has also been explored as a support system to chlorination and ozonation for removal of protozoa from the waste stream. Fullerenes and titanium dioxide nanoparticles produce reactive oxygen species and enhance disinfection capability of ultraviolet radiation applied at the tertiary stage of wastewater treatment. Thus, increased concentration of engineered nanoparticles in the waste stream also forces us to monitor and process for their removal from the effluent stream.

Nanosorbent Heavy metals are one of the major pollutants in wastewater. They are removed from wastewater majorly through sorption onto developing biomass in secondary biological treatment stage. Since this biosorption is physicochemical in nature, there is a limitation on the concentration of heavy metals which can be removed (Zafar et al. 2007). Nano-sized metal oxides, viz. ferric oxides, manganese oxides, aluminium oxides, titanium oxides, magnesium oxides and cerium oxides, are now used for wastewater treatment processes to remove heavy metals (Singh et al. 2013; Sadegh et al. 2017). They provide exceptionally high surface area and specific affinity in their nano-forms to remove heavy metals from aqueous system by faster adsorption rates in water treatment (Sadegh et al. 2016; Dil et al. 2017). With ample amount of surface modification and structural variation, this application has become a popular research field (Hua et al. 2012). Availability of resources and ease of synthesis render nano-sized ferric oxides to be low-cost adsorbents for toxic metal sorption. Direct application of iron oxide nanoparticles in wastewater treatment is a unique and advantageous process involving adsorption of contaminants (heavy metals and organic pollutants) with negligible risks of secondary contamination of nanoparticles (Deliyanni et al. 2004) as they can be removed from the aqueous phase by magnetic separation. In case of polyethylenimine (PEI)-coated magnetic nanoparticles used for wastewater treatment, the majority of engineered nanoparticles are recovered and reused (Lakshmanan et al. 2014); however, concentrations of nanoparticles remaining in wastewater stream after treatment still need to be evaluated. Apart from the mentioned metal oxides, removal of inorganic compounds such as metal ions can be achieved by the dendrimer (poly(amidoamine))-carrying nanoparticles. The dendrimers serve as chelating agents and allow binding of metals to functional groups, such as primary amines, carboxylates and hydroxymates (Diallo et al. 2005). One such accepted example is the application of blended dendrimer, polycitrate-Alumoxane nanoparticles with polyether sulfone (fabricated nanofiltration membrane) in foul prevention and dye removal in wastewater treatment plants (Zinadini 2016). One of the major concerns of wastewater, hexavalent chromium, is also reported to be removed using adsorption and magnetic separation using nanoscale maghemite (Hu et al. 2005), but the remaining nanoparticles' concentration-related information was not provided.

Recently various types of nano-sized materials were reported for their application in degradation of organics. Other nanomaterials are indirectly involved in biodegradation process by enhancement of polyaromatic hydrocarbon bioavailability (Tungittiplakorn et al. 2005; Kumari and Singh 2016).

All these applications developed for the treatment of wastewater imply deliberate addition/exposure of engineered nanoparticles to the effluent stream. The regeneration and retention of engineered nanoparticles are also important aspects of nanotechnology-enabled device design due to both cost and public health concerns.

1.2.4 Recovery of Nanoparticles from Effluent Stream

As seen earlier, photocatalysis of metal nanoparticles is widely used in wastewater treatment process for remediation of non-bio-remediated pollutants. It is very difficult to separate such suspended nanoparticles present in aqueous system. Nowadays, more efforts are put forth at the time of manufacturing of nanoparticles to find solution for recovery of nanoparticles. The coupling of the photocatalysis of titanium dioxide nanoparticles with membrane technology was done to overcome separation issues after completion of treatment. The following are the list of membranes which have incorporated titanium dioxide nanoparticles for recovery purposes: poly(vinylidene fluoride), polyether sulfone, polymethyl methacrylate and poly(amide-imide). A simple filtration technique is efficient to remove such membrane-coupled nanoparticles from the aqueous phase (Lu et al. 2016). Immobilization of various nanoparticles using resins and membranes was done for regeneration of nanoparticles from treatment process, but it significantly reduces the treatment efficiency (Chorawala and Mehta 2015).

Another aspect for removal of titanium dioxide nanoparticles is surface modification at the time of production. Doped titanium dioxide magnetic nanoparticles have been synthesized in a spinning disk reactor, which can be easily recovered using external magnetic field. Nano-sorbent magnetic magnetite (Fe_3O_4) and magnetic maghemite (γ-Fe_2O_4) are widely used in wastewater treatment for removal of various metal ions in wastewater treatment system. These magnetic nanoparticles also could be easily separated and recovered from the system by applying external magnetic field (Lu et al. 2016).

Intensive use of nanoparticles for various product preparations and their unknown release in wastewater streams drive us to the important question of their impact on physiology of microorganism functioning in the treatment plant.

1.3 Engineered Nanoparticles and Wastewater Treatment Processes

In any circumstances, engineered nanoparticles would follow one of the paths to reach to the environment, (1) direct release into any ecosystem and (2) being carried away with effluent stream reaching to various stages of treatment and ending up with effluent disposal and sludge (Fig. 1.1). In the first case, wastewater is not involved in mobile phase, and thus engineered nanoparticles would not encounter wastewater treatment facility and its microbial community, while in the second case, engineered nanoparticles with/without transformation would interact with wastewater microbial community. However, in both cases, they will be influencing the environment via various mechanisms. Such interaction of engineered nanoparticles with wastewater treatment process is discussed here.

Conventional wastewater treatment technologies have been utilizing several physicochemical and biological procedures for removal of organic pollutants, xenobiotic compounds as well as excess inorganic nutrients. The most commonly employed phases include primary, secondary and tertiary treatment stages. Wastewater treatment facilities are designed to either remove or degrade contaminants and pollutants from the aqueous phase. Microbial community plays a very crucial role in the reduction of organic load which is accomplished at the secondary stage of treatment mostly involving activated sludge process or anaerobic digestion

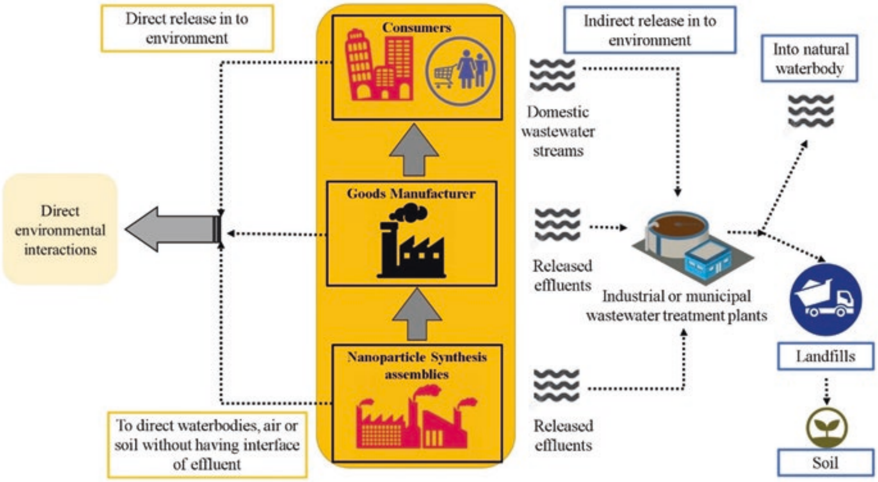


Fig. 1.1 Fate of engineered nanoparticles in the environment. Nanoparticles reach the environment either through direct or indirect routes. Direct route implicates release from manufacturing units and various consumer products through their disposal ultimately reaching air, water bodies or soil, while indirect route indicates their release in wastewater, which carries them finally to natural water bodies where wastewater is disposed after treatment as well as landfills where solids produced from wastewater treatment plants are disposed, ultimately affecting the soil environment

(Bitton 2005). Wastewater treatment not only gives solution for safe disposal of treated water, but it also plays an important role in water recycling.

Keller et al. (2013) indicated wastewater treatment plants as a consistent passing place for engineered nanoparticles before they reach the final destination. It is expected that huge amount of engineered nanoparticles reaching into wastewater treatment plant ultimately becomes part of sludge which is mostly disposed off in landfills.

Separation of solids from the liquid phase is accomplished in the primary treatment process which involves settling and removal of mainly settleable or larger particles. The settling ability of particles depends on the particle size, physicochemical properties and density. Due to their nano-size, engineered nanoparticles have very low settling potential. As gravitational force does not affect nanoparticles, generally they tend to escape the primary treatment process. The experiments conducted by Hou et al. (2012) mentioned retention of silver nanoparticles after primary clarification by 90% of the initial concentration. Thus, in the secondary biological treatment, microbial community, which may be part of suspended or attached growth systems, is bound to encounter nanoparticles.

When nanoparticles enter secondary treatment phase, they can combine with oppositely charged chemicals and form stable super-micellar aggregates (Brar et al. 2010). Such active interaction of nanoparticles is required to be suspected for microbial toxicity and plausible effect on community function.

Apart from settling of nanoparticles, diffusion is a major factor affecting the microbes-nanoparticles interaction. The rate of diffusion of smaller particles is very high as compared to aggregates and bigger particles (≥ 1000 nm) (Eduok et al. 2013).

Moreover, there is still a limitation for determination and monitoring of actual levels of nanoparticles at wastewater treatment plants. Under normal condition, well-developed techniques like scanning electron microscopy, X-ray diffraction, various spectroscopies, service meter, zeta potential, etc. have been used to characterize and quantify nanoparticles (Patel et al. 2017), but their use at wastewater treatment plants has not been developed. The challenge is fostered by interactions of multiple types of engineered nanoparticles present and their transformation by other organics present in wastewater affecting accurate detection of nanoparticles.

Quantitative measurements of nanoparticles, if possible in different matrices, will also find their application in the field of agriculture to detect engineered nanoparticles from soil; will help in elucidation of complete picture of their fate by determination of life cycle assessment with accurate concentrations, in designing of advance material so as to retain nano-catalyst; and would increase probability of recovering, reuse and removal of engineered nanoparticles after their application.

1.4 Probable Transformations of Engineered Nanoparticles in Wastewater

The environment around nanoparticles changes drastically during their life cycle, and hence we can expect a change in the forms of engineered nanoparticles, such as agglomeration (Chaúque et al. 2016). Certain stabilizing agents and coating materials are known to help in stabilizing nanoparticles with respect to their size under aqueous conditions and also in complex systems like wastewater (Kunhikrishnan et al. 2015). Transformation of bare and coated nanoparticles normally differs according to the nature of coating material used.

Interaction of these nanoparticles with other chemical matters present in receiving wastewaters, its interplay with fluids, change in its electrostatic condition, hydraulic conditions and the presence of various natural organic matter may lead to the transformation of engineered nanoparticles. These molecular interactions are expected to play a major role in deciding the fate of nanoparticles under aqueous conditions including wastewaters (Wagner et al. 2014).

It is important to mention that release scenario and transformation for any engineered nanoparticles will depend on various factors mentioned as follows (Caballero-Guzman and Nowack 2016; Reidy et al. 2013):

- Temperature
- pH of surrounding environment
- Humidity of the environment
- Dissolved oxygen
- Composition of wastewater
- Ionic strength of wastewater
- Concentration of dissolved organic matter
- Product type
- Concentration of nanoparticles
- Incorporation type (i.e. embedded or suspended)
- Frequency of exposure to release procedures such as weathering, washing, recycling, etc.

Moreover, toxicity of nanoparticles was found to be altered by humic acids, proteins and other organic ligands that reduce metal bioavailability in a study based on quantum dot weathering (Mahendra et al. 2008). Under certain environmental conditions, silver nanoparticles were converted to reactive species by oxidation and dissolution mechanisms or were found to be responsible for the generation of reactive oxygen species (Reidy et al. 2013), whereas some of the metal oxide nanoparticles, such as cerium dioxide, tend not to undergo a complete transformation, remain as native species and further get accumulated in the sludge (Barton et al. 2015). On the other hand, in sludge treatment process, homo-coagulation or hetero-coagulation of engineered nanoparticles occurs where the nature of coagulation depends on the interaction of nanoparticles with organic chlorides and minerals present in the sludge (Doolette et al. 2013).

Laboratory-based toxicity studies of nanoparticles with or without agglomeration and aggregation of nanoparticles may not be appropriate for evaluation of actual cytotoxicity and genotoxicity of nanoparticles to any living cells in the environment (Thio et al. 2011). Such transformed nanoparticles are bound to influence wastewater microbial community and community functions at various stages.

1.5 Influence of Engineered Nanoparticles on Microbial Community of Wastewater

As the composition of wastewater changes with respect to geographical location, climatic conditions, sources of pollutants, water usage types, frequency, etc., the behaviour of engineered nanoparticles present will also vary with different wastewaters relatively.

In one of the monoculture studies with gold nanoparticles, concentration higher than 1 μM was found to be toxic to bacteria and was also reported to be photomutagenic in combination with citrate (Wang et al. 2016). Palladium nanoparticles which are widely used as a catalyst were found to display size-dependent toxicity to *E. coli* and *S. aureus* at concentrations as low as 10^{-9} M (Adams et al. 2014). But since wastewater treatment employs activity of the whole microbial community, such studies can only provide effect on individual species; nonetheless, the concentration of nanoparticles affecting these environmental species is noteworthy.

The influence of increasing concentration of nanoparticles in wastewater was analysed by García et al. (2012) with studies on cerium dioxide, titanium dioxide, silver and gold nanoparticles in the range of 100 to 1120 ppm on the activity of microbial communities of wastewater. The authors had combined data for aerobic and anaerobic conditions. The techniques employed included respiration rates and biogas production analysis. Major observations comprised cerium dioxide nanoparticle treatment, resulting in complete inhibition of biogas production with 1 h of exposure time, whereas silver nanoparticles led to 33–50% inhibition in biogas production within 4 h of exposure. But it is also important to note that experimental setups designed by García et al. (2012) used concentrations higher than they are observed in actual conditions of wastewater.

The reduction in levels of organic pollutants in wastewater is monitored mostly by determining the chemical oxygen demand from wastewater under treatment where active microbial community oxidizes the organic compounds. Similarly for reduction in inorganic nutrient levels of nitrogen and phosphorus, efforts are made to avoid eutrophication problems due to disposal of treated waters carrying traces of these nutrients in receiving waters. The removal of nitrogen nutrients from wastewater is dependent on nitrification and denitrification activity of microbial community. Hou et al. (2012) studied the effect of silver nanoparticles on various functionalities of wastewater treatment, i.e. reduction in chemical oxygen demand and ammonia levels. The results suggested that exposure to silver nanoparticles did not affect chemical oxygen demand reducing capacity of the microbial community, but in the first exposure itself, the quantities of nanoparticles were sufficient to mellow down the activity of the ammonia oxidizing community of nitrifying bacteria. It was marked very substantially that, looking to the antibacterial properties of such engineered nanoparticles, the effect on environmental bacteria should not be overlooked. They observed that concentration of 1 mg/l of silver nanoparticles reduced respiration of autotrophic nitrifying bacteria by more than 85% when compared to controls, thus reducing ammonia removal efficiency. In the similar type of investigation, Zheng et al. (2011) found the adverse impact of zinc oxide nanoparticles on the biological removal of nitrogen and phosphorous from wastewater. The study suggested that presence of 10 and 50 mg/l of zinc oxide nanoparticles decreased total nitrogen removal efficiencies from 81.5% to 75.6% and 70.8%, respectively.

Hou et al. (2014) reported a study on the effect of zinc oxide nanoparticles on wastewater biofilms and respiration rates determined by oxygen microelectrode. The authors claimed that presence of zinc oxide nanoparticles at a concentration of 50 mg/l was found to be toxic to the microorganisms developed in the biofilms on rotating biological contactors, on the upper layer of about 200 μm. On the effective note, the authors also clarified the fact that 5 mg/l of zinc oxide nanoparticles hardly disturbed the microbial respiration.

In the lab-based reactor experiments, Jeong et al. (2014) studied the difference in the susceptibility of various bacterial communities in wastewater toward silver nanoparticles. As the longer exposures to silver nanoparticles were considered (50 days), the data evaluation was found to be more reliable. It was found that with an increase in the silver nanoparticle concentration, both organic oxidation and nitrification processes were adversely affected, but nitrification was more severely inhibited even at low concentrations of silver nanoparticles (<1 ppm). On long-term exposure, the substrate utilization rate by the microbial community was negatively affected by even a concentration of as low as 0.5 ppm for silver nanoparticles.

The microbial activity for oxidation of organics in secondary treatment stages is accomplished by either *attached growth systems* in the form of biofilms as in the case of rotating biological contactors, trickling filters, etc. or *suspended growth systems* as in the case of activated sludge process, lagoons, oxidation ditches, etc. Techniques like polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) were used to assess the diversity of microbial communities after exposure to silver nanoparticles. This technique gives the idea of component members present in microbial community including both culturable and non-culturable species. They observed a significant loss of microbial diversity. PCR-DGGE analysis showed that *Thiotrichales* was more sensitive to silver nanoparticles than other types of biofilm bacteria in attached growth system for wastewater treatment, while *Bacteroidetes* and *Proteobacteria* were sensitive to silver nanoparticle treatment in activated sludge, i.e. in a suspended condition (Jeong et al. 2014).

Wang and Chen (2015) reported literature regarding the adverse effect of engineered nanoparticles on various enzyme activities involved in important processes of nitrogen (nitrification and denitrification ability) and phosphorus removal as well as sludge anaerobic digestion.

Using X-ray absorption spectroscopy, Clar et al. (2016) described the mechanism of copper nanoparticle-induced toxicity on nitrifying bacteria. When coated and uncoated copper nanoparticles were selected for study, uncoated ions were found to inhibit the specific oxygen uptake rate as compared to treatment by coated one. The mechanism of toxicity was attributed to copper-induced cytotoxicity by the production of reactive oxygen species by ionic copper in the solution released by catalytic reactions of copper nanoparticles.

In an initial report by Brar et al. (2010), details of nanoparticle toxicity and its interactions at various stages and ultimate fate of nanoparticles in the environment have been discussed. Other studies related to impact of engineered nanoparticles on wastewater microbial community function that include effect of silver nanoparticles on biofilm microbial community demonstrated with observed 34.6% decreases in gene number using GeoChip; effect of graphene on activated sludge microbes which was found to be toxic between 50 and 300 mg graphene/l; effect of copper oxide on rotating biological contactor biofilm microbial activity where 50 mg/l coper nanoparticles proved to be toxic; effect of zinc oxide nanoparticles on microbes present in activated sludge with 100 mg/l of zinc oxide nanoparticles leading to decrease of 57.09% reads indicating considerable loss of bacterial abundance; etc. have been reported in the literature (Sheng et al. 2015; Ahmed and Rodrigues 2013; Miao et al. 2016; Meli et al. 2015).

The fixed/attached biofilm systems for wastewater treatment when encountering flow of effluent with engineered nanoparticles face multiple dynamic interactions. Due to the change in ionic conditions upon interaction, aggregates may also be formed. This may lead to hindering the diffusion of oxygen to the surface layer of microbes present. This ultimately may lead to fouling and failure of treatment process on repeated and long-term exposures affecting wastewater treatment efficiency (Eduok et al. 2013).

Thus it is evident that exposure of engineered nanoparticles is affecting negatively the structure and function of microbial communities which are key players in wastewater treatment. Impairment of biological activity of microbial community in wastewater due to the toxicity of nanoparticles is still one of the hidden impacts on the environment. Removal of such nano-particulate matters remains one of the biggest challenges for water and wastewater treatment authorities. As discussed, nanoparticles may affect adversely the biological stage of the treatment process and ultimately decrease the contaminant removal efficiency of the entire process which may lead to failure of wastewater treatment. Challenge in this field of research also lies in bridging the gap between investigated and actual concentrations of nanoparticles available in various effluents and toxicity studies. It is important to note that microbial toxicity studies, if conducted with an actual simulation of treatment plant condition, may lead to a firm conclusion regarding the effect on the performance of treatment plant. Advance microplate arrays are being developed to study monocultures, and the same lineage should be extended for study on microbial community (Wang et al. 2017a, b).

As we can see very clearly, all these research and development have occurred in just the last decade. The flourishing number of products on shelves of market incorporating nanoparticles demands strong change in research themes today. The gap in the research at this stage is observed in nanoparticles-microbes interaction analysis at the microbial community level. Largely various disciplines need to come together for making a significant contribution to generating quantitative data related to exposure of engineered nanoparticles at various stages of wastewater treatment.

Moreover, research findings strongly indicate possibility of biomagnification of engineered nanoparticles in food chains. The study on cadmium selenium quantum dots observed magnification by five times of the nanoparticle concentration in predator, *Tetrahymena thermophila*, of *Pseudomonas aeruginosa* (Werlin et al. 2011). The risk associated with biomagnification of gold nanoparticles was also reported indicating the possibility of potential human exposure (Judy et al. 2011). What if all the species of primary producers are accumulating one or more than one type of nanoparticles at the same time? Multiple such questions need to be answered by extensive research in the field.

Current development in regulatory policy includes amendments to section 8(a) in TSCA (Toxic Substances Control Act) which indicates the requirement of declaration for the amount of engineered nanoparticles used and/or manufactured by the industrial units annually (www.epa.gov). The importance of presence and effect of engineered nanoparticles in wastewater is yet to be realized by environment protection agencies across the world other than the US Environment Protection Agency (USEPA). The speed at which consumers are accepting and utilizing such nano-based products is alarming for policy makers as well. Soon global stand for controlled release, removal of engineered nanoparticles from effluent, treatment guidelines and safe disposal related policies are expected.

1.6 Conclusion

Microbial toxicity studies with engineered nanoparticles have been mostly based on behaviour of monoculture and mixed cultures, but actual community dynamics is not fully clear yet, and such studies are still scanty. Only few treatment processes have been speculated by some researchers, such as adverse effect on nitrification, phosphorus removal, microbial respiration, substrate utilization by microbial community and reduction of chemical oxygen demand. For removal of inorganic nutrients, few microbial species have specialization, and those have been found to be sensitive toward presence of engineered nanoparticles; it seems that we need to worry as far as removal of these important nutrients is concerned. The functionality of enzymes and degradative capabilities of microbial communities exposed to nanoparticles still need to be addressed completely under different environmental and climatic conditions. Improving detection methods and detailed studies on microbial community metabolic responses needs to be taken up by the research community to sustain the wastewater treatment performance and ultimately the environment from adverse cumulative effects of nanoparticles.

References

Adams, CP., P., Walker, KA., Obare, SO., Docherty, K.M. (2014). Size-dependent antimicrobial effects of novel palladium nanoparticles. 9(1). https://doi.org/10.1371/journal.pone.0085981

Ahmed F, Rodrigues DF (2013) Investigation of acute effects of graphene oxide on wastewater microbial community: a case study. J Hazard Mater 256–257:33–39. https://doi.org/10.1016/j.jhazmat.2013.03.064

Al-Kattan A, Wichser A, Vonbank R, Brunner S, Ulrich A, Zuin S, Arroyo Y, Golanski L, Nowack B (2015) Characterization of materials released into water from paint containing nano-SiO_2. Chemosphere 119:1314–1321

Amin MT, Alazba AA, Manzoor U (2014) A review of removal of pollutants from water/wastewater using different types of nanomaterials. Adv Mater Sci Eng 2014:1–24. https://doi.org/10.1155/2014/825910

Balmuri SR, Selvaraj U, Kumar VV, Anthony SP, Tsatsakis AM, Golokhvast KS, Raman T (2017) Effect of surfactant in mitigating cadmium oxide nanoparticle toxicity: implications for mitigating cadmium toxicity in environment. Environ Res 152:141–149. https://doi.org/10.1016/j.envres.2016.10.005

Barton, L. E., Auffan, M., Olivi, L., Bottero, J. and Wiesner, M., R. (2015). Heteroaggregation, transformation and fate of CeO_2 nanoparticles in wastewater treatment. Environ Pollut. 203, 122–129. DOI: https://doi.org/10.1016/j.envpol.2015.03.035

Baun A, Hartmann NB, Grieger KD, Hansen SF (2009) Setting the limits for engineered nanoparticles in European surface waters - are current approaches appropriate? J Environ Monit 11(10):1774–1781. https://doi.org/10.1039/b909730a

Benn TM, Westerhoff P (2008) Nanoparticle silver released into water from commercially available sock fabrics. Environ Sci Technol 42(11):4133–4139. https://doi.org/10.1021/es7032718

Benn T, Cavanagh B, Hristovski K, Posner JD, Westerhoff P (2016) The release of nanosilver from consumer products used in the home. J Environ Qual 39(6):1875–1882. https://doi.org/10.1002/aur.1474.Replication

Bitton G (2005) Waste water microbiology, 3rd edn. Wiley, Hoboken

Bora T, Dutta J (2014) Applications of nanotechnology in wastewater applications of nanotechnology in wastewater treatment—a review. Am Sci Publ 14:613–626. https://doi.org/10.1166/jnn.2014.8898

Borm PJA, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D, Oberdorster E (2006) The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol 3. https://doi.org/10.1186/1743-8977-3-11

Botta C, Labille J, Auffan M, Borschneck D, Miche H, Cabié M, Masion A, Rose J, Bottero J-Y (2011) TiO_2-based nanoparticles released in water from commercialized sunscreens in a life-cycle perspective: structures and quantities. Environ Pollut 159:1543–1550. https://doi.org/10.1016/j.envpol.2011.03.003

Boyoglu C, He Q, Willing G, Boyoglu-Barnum S, Dennis VA, Pillai S, Singh SR (2013) Microscopic studies of various sizes of gold nanoparticles and their cellular localizations. ISRN Nanotechnol 2013:1–13. https://doi.org/10.1155/2013/123838

Brar SK, Verma M, Tyagi RD, Surampalli RY (2010) Engineered nanoparticles in wastewater and wastewater sludge – evidence and impacts. Waste Manag 30(3):504–520. https://doi.org/10.1016/j.wasman.2009.10.012

Bystrzejewska G, Golimowski J, Urban PL (2009) Nanoparticles: their potential toxicity, waste and environmental management. Waste Manag 29(9):2587–2595. https://doi.org/10.1016/j.wasman.2009.04.001

Caballero-Guzman A, Nowack B (2016) A critical review of engineered nanomaterial release data: are current data useful for material flow modeling? Environ Pollut 213:502–517. https://doi.org/10.1016/j.envpol.2016.02.028

Chaúque E, Zvimba J, Ngila J, Musee N (2016) Fate, behaviour, and implications of ZnO nanoparticles in a simulated wastewater treatment plant. Water SA 42(1):72. https://doi.org/10.4314/wsa.v42i1.09

Chen Y, Crittenden JC, Hackney S, Sutter L, Hand DW (2005) Preparation of a novel TiO_2-based p–n junction nanotube photocatalyst. Environ Sci Technol 39(5):1201–1208

Chorawala KK, Mehta MJ (2015) Applications of nanotechnology in wastewater treatment. Int J Innov Emerg Res Eng 2(1):21–26

Clar JG, Li X, Impellitteri CA, Bennett-Stamper C, Luxton TP (2016) Copper nanoparticle induced cytotoxicity to nitrifying Bacteria in wastewater treatment: a mechanistic copper speciation study by X-ray absorption spectroscopy. Environ Sci Technol 50(17):9105–9113. https://doi.org/10.1021/acs.est.6b01910

Cushen M, Kerry J, Morris M, Cruz M, Cummins E (2013) Migration and exposure assessment of silver from a PVC nanocomposite. Food Chem 134(1–4):389–397. https://doi.org/10.1016/j.foodchem.2013.01.045

Cushen M, Kerry J, Morris M, Cruz M, Cummins E (2014) Evaluation and simulation of silver and copper nanoparticle migration from polyethylene nanocomposites to food and an associated exposure assessment. J Agric Food Chem 62(6):1403–1411. https://doi.org/10.1021/jf404038y

Dasgupta N, Ranjan S, Ramalingam C (2017) Applications of nanotechnology in agriculture and water quality management. Environ Chem Let 15(4):591–605. https://doi.org/10.1007/s10311-017-0648-9

Deliyanni EA, Lazaridis NK, Peleka EN, Matis KA (2004) Metals removal from aqueous solution by iron-based bonding agents. Environ Sci Pollut Res 11(1):18–21. 2004

Diallo MS, Christie S, Swaminathan P, Johnson JH, Goddard WA (2005) Dendrimer enhanced ultrafiltration. 1. Recovery of cu(II) from aqueous solutions using PAMAM dendrimers with ethylene diamine core and terminal NH_2 groups. Environ Sci Technol 39(5):1366–1377. https://doi.org/10.1021/es048961r

Dil EA, Ghaedi M, Asfaram A (2017) The performance of nanorods material as adsorbent for removal of azo dyes and heavy metal ions: application of ultrasound wave, optimization and modeling. Ultrason Sonochem 34:792–802

Doolette C, McLaughlin M, Kirby J, Batstone D, Harris H, Ge H, Cornelis G (2013) Transformation of PVP coated silver nanoparticles in a simulated wastewater treatment process and the effect on microbial communities. Chem Cent J 7:46. http://journal.chemistrycentral.com/content/7/1/46

Dror I, Baram D, Berkowitz B (2005) Use of nanosized catalysts for transformation of chloro-organic pollutants. Environ Sci Technol 39(5):1283–1290

Eduok S, Martin B, Villa R, Nocker A, Jefferson B, Coulon F (2013) Evaluation of engineered nanoparticle toxic effect on wastewater microorganisms: current status and challenges. Ecotoxicol Environ Saf 95:1–9. https://doi.org/10.1016/j.ecoenv.2013.05.022

Farkas J, Peter H, Christian P, Gallego Urrea JA, Hassellöv M, Tuoriniemi J, Gustafsson S, Olsson E, Hylland K, Thomas KV (2011) Characterization of the effluent from a nanosilver producing washing machine. Environ Int 37(6):1057–1062. https://doi.org/10.1016/j.envint.2011.03.006

Forest V, Leclerc L, Hochepied J-F, Trouve A, Sarry G, Pourchez J (2017) Impact of cerium oxide nanoparticles shape on their in vitro cellular toxicity. Toxicol in Vitro 38:136–141. https://doi.org/10.1016/j.tiv.2016.09.022

Frenk S, Ben-Moshe T, Dror I, Berkowitz B, Minz D (2013) Effect of metal oxide nanoparticles on microbial community structure and function in two different soil types. PLoS One 8:1–12. https://doi.org/10.1371/journal.pone.0084441

García A, Delgado L, Torà JA, Casals E, González E, Puntes V, Fonta X, Carreraa J, Sánchez A (2012) Effect of cerium dioxide, titanium dioxide, silver, and gold nanoparticles on the activity of microbial communities intended in wastewater treatment. J Hazard Mater 199–200:64–72. https://doi.org/10.1016/j.jhazmat.2011.10.057

Gehrke I, Andreas G, Annette S (2015) Innovations in nanotechnology for water treatment. Nanotechnol Sci Appl 8:1–17. https://doi.org/10.2147/NSA.S43773

Geranio L, Heuberger M, Nowack B (2009) The behavior of silver nanotextiles during washing. Environ Sci Technol 43(21):8113–8118. https://doi.org/10.1021/es9018332

Goetz NV, Fabricius L, Glaus R, Weitbrecht V, Günther D, Hungerbühler K (2013) Migration of silver from commercial plastic food containers and implications for consumer exposure assessment. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 30(3):612–620. https://doi.org/10.1080/19440049.2012.762693

Hou L, Li K, Ding Y, Li Y, Chen J, Wu X, Li X (2012) Removal of silver nanoparticles in simulated wastewater treatment processes and its impact on COD and NH_4 reduction. Chemosphere 87(3):248–252. https://doi.org/10.1016/j.chemosphere.2011.12.042

Hou J, Miao L, Wang C, Wang P, Ao Y, Qian J, Dai S (2014) Inhibitory effects of ZnO nanoparticles on aerobic wastewater biofilms from oxygen concentration profiles determined by microelectrodes. J Hazard Mater 276:164–170. https://doi.org/10.1016/j.jhazmat.2014.04.048

Hu, J., Guohua C. and Irene M., C., L.(2005). Removal and recovery of Cr(VI) from wastewater by maghemite nanoparticles. Water Res*,* 39(18), 4528–4536. DOI: https://doi.org/10.1016/j.watres.2005.05.051

Hua M, Zhang S, Pan B, Zhang W, Lv L, Zhang Q (2012) Heavy metal removal from water/wastewater by nanosized metal oxides: a review. J Hazard Mater 211-212(June):317–331. https://doi.org/10.1016/j.jhazmat.2011.10.016

Jeong E, Im W-T, Kim D-H, Kim M-S, Kang S, Shin H-S, Chae S-R (2014) Different susceptibilities of bacterial community to silver nanoparticles in wastewater treatment systems. J Environ Sci Health A Tox Hazard Subst Environ Eng 49(6):685–693. https://doi.org/10.1080/10934529.2014.865454

Judy, JD. Unrine, J.,M., Bertsch, P.,M. (2011). Evidence for biomagnification of gold nanoparticles within a terrestrial food chain. Environ Sci Technol., 45, 776–781, DOI:https://doi.org/10.1021/es103031a

Kaegi R, Ulrich A, Sinnet B, Vonbank R, Wichser A, Zuleeg S, Simmler H, Brunner S, Vonmont H, Burkhardt M, Boller M (2008) Synthetic TiO2 nanoparticle emission from exterior facades into the aquatic environment. Environ Pollut 156(2):233–239. https://doi.org/10.1016/j.envpol.2008.08.004

Kang S, Mauter MS, Elimelech M (2009) Microbial cytotoxicity of carbon-based nanomaterials: implications for river water and wastewater effluent. Environ Sci Technol 43(7):2648–2653. https://doi.org/10.1021/es8031506

Keller AA, Lazareva A (2013) Predicted releases of engineered nanomaterials: from global to regional to local. Environ Sci Technol Lett 1(1):65–70. https://doi.org/10.1021/ez400106t

Keller AA, McFerran S, Lazareva A, Suh S (2013) Global life cycle releases of engineered nanomaterials. J Nanopart Res 15:1692. https://doi.org/10.1007/s11051-013-1692-4

Kulthong K, Srisung S, Boonpavanitchakul K, Kangwansupamonkon W, Maniratanachote R (2010) Determination of silver nanoparticle release from antibacterial fabrics into artificial sweat. Part Fibre Toxicol 7(1):8. https://doi.org/10.1186/1743-8977-7-8

Kumari B, Singh DP (2016) A review on multifaceted application of nanoparticles in the field of bioremediation of petroleum hydrocarbons. Ecol Eng 97:98–105. https://doi.org/10.1016/j.ecoleng.2016.08.006

Kunhikrishnan A, Shon HK, Bolan NS, El Saliby I, Vigneswaran S (2015) Sources, distribution, environmental fate, and ecological effects of nanomaterials in wastewater streams. Crit Rev Environ Sci Technol 45(4):277–318. https://doi.org/10.1080/10643389.2013.852407

Lakshmanan R, Sanchez M, Matutes JA, Wennmalm S, Rajarao GK (2014) Removal of total organic carbon from sewage wastewater using poly(ethylenimine)-functionalized magnetic nanoparticles. Langmuir 30:1036–1044. https://doi.org/10.1021/la404076n

Lorenz C, Windler L, von Goetz N, Lehmann RP, Schuppler M, Hungerbühler K, Heuberger M, Nowack B (2012) Characterization of silver release from commercially available functional (nano) textiles. Chemosphere 89(7):817–824. https://doi.org/10.1016/j.chemosphere.2012.04.063

Lu H, Wang J, Stoller M, Wang T, Bao Y, Hao H (2016) An overview of nanomaterials for water and wastewater treatment. Adv Mater Sci Eng. Article Id: 4964828. https://doi.org/10.1155/2016/4964828

Mackevica A, Olsson ME, Hansen SF (2017) The release of silver nanoparticles from commercial toothbrushes. J Hazard Mater 322:270–275. https://doi.org/10.1016/j.jhazmat.2016.03.067

Mahapatra I, Sun TY, Clark JRA, Dobson PJ, Hungerbuehler K, Owen R, Nowack B, Lead J (2015) Probabilistic modelling of prospective environmental concentrations of gold nanoparticles from medical applications as a basis for risk assessment. J Nanobiotechnol 13(93):1–14. https://doi.org/10.1186/s12951-015-0150-0

Mahendra S, Zhu HG, Colvin VL, Alvarez PJ, Wang W, Gu B, Mortensen N, Allison D, Joy D, Allison M, Brown S, Phelps T, Doktycz M (2008) Quantum dot weathering results in microbial toxicity. Environ Sci Technol 42(24):9424–9430

Maurer-Jones MA, Gunsolus IL, Murphy CJ, Haynes CL (2013) Toxicity of engineered nanoparticles in the environment. Anal Chem 85:3036–3049. https://doi.org/10.1021/ac303636s

Meli K, Kamika I, Keshri J, Momba MNB (2015) The impact of zinc oxide nanoparticles on nitrification and the bacterial community in activated sludge in an SBR. RSC Adv 5(82):67335–67342. https://doi.org/10.1039/C5RA07106B

Miao L, Wang C, Hou J, Wang P, Ao Y, Li Y, Geng N, Yao Y, Lv B, Yang Y, You G, Xu Y (2016) Aggregation and removal of copper oxide (CuO) nanoparticles in wastewater environment and their effects on the microbial activities of wastewater biofilms. Bioresour Technol 216:537–544. https://doi.org/10.1016/j.biortech.2016.05.082

Oh E, Liu R, Nel A, Gemill KB, Bilal M, Cohen Y, Medintz IL (2016) Meta-analysis of cellular toxicity for cadmium-containing quantum dots. Nat Nanotechnol 11(5):479–486. https://doi.org/10.1038/nnano.2015.338

Patel S, Patel P, Bakshi SR (2017) Titanium dioxide nanoparticles: an in vitro study of DNA binding, chromosome aberration assay, and comet assay. Cytotechnology 69:245. https://doi.org/10.1007/s10616-016-0054-3

Pelletier DA, Suresh AK, Holton GA, Mckeown CK (2010) Effects of engineered cerium oxide nanoparticles on bacterial growth and viability. Appl Environ Microbiol 76:7981–7989

Quadros M, Pierson RI, Tulve N, Willis R, Rogers K, Thomas T, Marr LC (2013) Release of silver from nanotechnology – based consumer products for children. Environ Sci Technol 47:8894–8901

Reidy B, Haase A, Luch A, Dawson KA, Lynch I (2013) Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. Materials 6:2295–2350

Sadegh H, Ghoshekandi RS, Masjedi A, Mahmoodi Z, Kazemi M (2016) A review on carbon nanotubes adsorbents for the removal of pollutants from aqueous solutions. Int J Nano Dimens 7(2):109

Sadegh H, Ali GAM, Gupta VK, Makhlouf ASH, Shahryari-ghoshekandi R, Nadagouda MN, Sillanpää M, Megiel E (2017) J Nanostruct Chem 7:1. https://doi.org/10.1007/s40097-017-0219-4

Shah SNA, Shah Z, Hussain M, Khan M (2017) Hazardous effects of titanium dioxide nanoparticles in ecosystem. Bioinorg Chem Appl 2017:4101735. https://doi.org/10.1155/2017/4101735

Sheng Z, Van Nostrand JD, Zhou J, Liu Y (2015) The effects of silver nanoparticles on intact wastewater biofilms. Front Microbiol 6(Jul):1–11. https://doi.org/10.3389/fmicb.2015.00680

Singh S, Barick K, Bahadur D (2013) Functional oxide nanomaterials and nanocomposites for the removal of heavy metals and dyes. Nanomater Nanotechnol 3:3–20

Song H, Li B, Lin Q-B, Wu H-J, Chen Y (2011) Migration of silver from nanosilver–polyethylene composite packaging into food simulants. Food Addit Contam Part A 49(June 2015):1–5. https://doi.org/10.1080/19440049.2011.603705

Stefaniak AB, Duling MG, Lawrence RB, Thomas TA, LeBouf RF, Wade EE, Abbas Virji M (2014) Dermal exposure potential from textiles that contain silver nanoparticles. Int J Occup Environ Health 20(3):220–234. https://doi.org/10.1179/2049396714Y.0000000070

Su Y, He Y, Lu H, Sai L, Li Q, Li W, Wang L, Shenb P, Huanga Q, Fana C (2009) The cytotoxicity of cadmium-based, aqueous phase – synthesized, quantum dots and its modulation by surface coating. Biomaterials 30:19–25

Thio BJR, Zhou D, Keller AA (2011) Influence of natural organic matter on the aggregation and deposition of Titanium Dioxide nanoparticles. J Hazard Mater 189:1–2, 556–563

Tungittiplakorn W, Cohen C, Lion LW (2005) Engineered polymeric nanoparticles for the bioremediation of hydrophobic contaminants. Environ Sci Technol 39:1354–1358

Wagner S, Gondikas A, Neubauer E, Hofmann T, Kammer F (2014) Spot the difference : engineered and natural nanoparticles in the environment—release, behavior, and fate. Angew Chem Int Ed 53:12398–12419. https://doi.org/10.1002/anie.201405050

Wang D, Chen Y (2015) Critical review of the influences of nanoparticles on biological wastewater treatment and sludge digestion. Crit Rev Biotechnol 36(5):816–828. https://doi.org/10.3109/07388551.2015.1049509

Wang P, Menzies NW, Dennis PG, Guo J, Forstner C, Sekine R, Lombi E, Kappen P, Bertsch PM, Kopittke PM (2016) Silver nanoparticles entering soils via the wastewater-sludge-soil pathway pose low risk to plants but elevated Cl concentrations increase Ag bioavailability. Environ Sci Technol 50(15):8274–8281. https://doi.org/10.1021/acs.est.6b01180

Wang L, Hu C, Shao L (2017a) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine 12:1227–1249

Wang Y, Wu Y, Quadri F, Prox JD, Guo L (2017b) Cytotoxicity of ZnO nanowire arrays on excitable cells. Nano 7:80. https://doi.org/10.3390/nano7040080

Weir A, Westerhoff P, Fabricius L, von Goetz N (2012) Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol 46(4):2242–2250. https://doi.org/10.1021/es204168d

Werlin R, Jackson S, Stoimenov PK, Priester JH, Mielke RE, Kra S, Stucky GD, Cherr GN, Orias E, Holden PA (2011) Biomagnification of cadmium selenide quantum dots in a simple experimental microbial food chain. Nat Nanotechnol 6:65–71. https://doi.org/10.1038/nnano.2010.251

Windler L, Lorenz C, Von Goetz N, Hungerbühler K, Amberg M, Heuberger M, Nowack B (2012) Release of titanium dioxide from textiles during washing. Environ Sci Technol 46(15):8181–8188. https://doi.org/10.1021/es301633b

Zafar S, Farrukh A, Iqbal A (2007) Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour Technol 98(13):2557–2561. https://doi.org/10.1016/j.biortech.2006.09.051

Zakrzewska KE, Samluk A, Wierzbicki M, Jaworski S, Kutwin M, Sawosz E, Chwalibog A, Pijanowska DG, Pluta KD (2015) Analysis of the cytotoxicity of carbon-based nanoparticles, diamond and graphite, in human glioblastoma and hepatoma cell lines. PLoS One 10(3):1–15. https://doi.org/10.1371/journal.pone.0122579

Zhang W, Bao S, Fang T (2016) The neglected nano-specific toxicity of ZnO nanoparticles in the yeast *Saccharomyces cerevisiae*. Nat Publ Group 6:24839. https://doi.org/10.1038/srep24839

Zheng X, Wu R, Chen Y (2011) Effects of ZnO nanoparticles on wastewater biological nitrogen and phosphorus removal. Environ Sci Technol 45(7):2826–2832. https://doi.org/10.1021/es2000744

Zinadini S (2016) Preparation and characterization of high flux PES nanofiltration membrane using hydrophilic nanoparticles by phase inversion method for application in advanced wastewater treatment. J Appl Res Water Wastewater 3(1):233–235

Chapter 2
Microbial Ecosystem and Its Impact on Solving the Environmental Problems: A Molecular Approach

Md Kaisar Ali and Sajjad Husain Mir

Contents

Md. K. Ali
School of Life Science, Southwest University, Chongqing, China

S. H. Mir (✉)
School of Chemistry, Advanced Materials and BioEngineering Research Centre (AMBER), Trinity College Dublin, The University of Dublin, Dublin, Ireland

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_2

Abstract As our admiration for the extent and complexity of microbial ecosystems grow, we can extrapolate how our new comprehension could shape our future. No doubt, microbes can alter their environments and can be harnessed to engineer our planet as well as our health. Indeed, even ecosystem restoration has deep appreciation for the role of microbes in shaping the relevant environment, by decomposition of biomass and recycling of nutrients. Although our present knowledge about evolutionary processes and metabolic mechanisms that fortify most microbial ecosystem dynamics is extremely limited, rapid advances in technologies that help elucidate these processes are set to increase the current rate of knowledge acquisition. Microbial ecology gives a new and safe way to make our environment pollution-free by degrading the contaminants from water to make it useful for humans. It changes the way how human diseases are being treated, by using microbes as prebiotics and probiotics and transfaunation procedures. Similarly, industrial processes that once relied on microbe-based technology to produce bioactive molecules that are extensively used in medical industry, chemical industry, food, and agriculture, as well as biofuel and bioelectricity give an alternative way to fulfill the demand of energy-hungry civilization.

Keywords Microbial ecosystem · Microbial diversity · Molecular evolution · Biogeochemical cycle · Decomposition · Bioremediation · Biofuel · Bioelectricity · Microbial therapeutic and molecular tools

Abbreviations

AOA	Ammonia-oxidizing archaea
AOB	Ammonia-oxidizing bacteria
C	Carbon
CO_2	Carbon dioxide
DCM	Dichloromethane
DNA	Deoxyribonucleic acid
EBPR	Enhanced biological phosphorus removal
ECM	Ectomycorrhizal
GAO	Glycogen-accumulating organisms

N	Nitrogen
NOB	Nitrite-oxidizing bacteria
NPP	Net primary productivity
OTUs	Operational taxonomic unit
P	Phosphorus
PAH	Polycyclic aromatic hydrocarbons
PAO	Polyphosphate-accumulating organisms
RNA	Ribonucleic acid
SOM	Soil organic matter
WWTPs	Wastewater treatment plants

2.1 Introduction

Microbial ecology has historically pinpointed on the relationship of microbes and their biotic and abiotic environments. This relationship either form beneficial link, e.g., nitrogen fixation bacteria present in plant root nodules, or harmful link such as pathogenic relationships between microbes and host (Keymer et al. 2006; Peterson et al. 2008; Taylor et al. 2005). Several interesting features of microbial ecosystems make it as a model system: first, there is rapid growth of species in controlled environments (Ballyk et al. 2001); second, number of individuals in microbial ecosystem is usually so large, meaning the system is useful and justified by physics; and third, classical microbial techniques coupled with molecular techniques make it more accurate to monitor the evolution of individuals' number of different species inside a bioreactor (Bastin and Dochain 1991). Because of these features, ecologists consider the microbial ecosystems as dominant candidates to test hypotheses, principles, and concepts of general ecology (Jessup et al. 2004).

Microbial evolution is defined as the study relation between organisms and genes as well as genetic diversity of evolution in microbes and important tools for the analysis of phylogenic relationships, population genetics, comparative genomics, and genome analyses (Reno et al. 2009; Thompson et al. 2005). To explore the deep microbial phylogeny, researchers focused on comparisons of small subunit ribosomal RNA (16S rRNA) oligonucleotides (Sapp 2007). This depicted sequence data in a phylogenetic tree was reminiscent of Woese's "Universal Phylogenetic Tree." As trees allow microbial ecologists to allocate 16S rRNA gene sequences to phyla, genera, and species and identify the previously unknown groups, diversity became a main theme in microbial ecology (Woese 1987). The comparative analysis of 16rRNA sequences suggested that all of the cellular life belonged to one of the three domains, namely, *Bacteria*, Archaea, and Eukarya (Woese and Fox 1977). This allowed to define the major lineages (phyla or divisions) within these three primary domains (Woese et al. 1990).

Microbial diversity is defined as variability and complexity of microbes at different levels of biological organization, including genetic variations within taxons and richness and relative abundance of functional groups in communities (Fierer et al. 2012; Ghiglione et al. 2012). Recently, the global survey of microbial diversity has been performed by the Earth Microbiome Project, to expose the existence of 5.6 million OTUs (97% similarity) in the first 15,000 samples, which set a new lower bound on the number of bacterial and archaeal phylotypes on earth (Gilbert et al. 2014). Range of factors is responsible for the variation of microbial diversity in the ecosystem, including stress factors such as pH, moisture, pressure, temperature, global warming, and presence of chemical species and disturbance factors that involve rapid changes such as occurrence of plant litter and deadwood, rhizosphere, root exudates, and availability of nutrients in environment (Lladó et al. 2017).

Today, many molecular tools are available to investigate the complexity and structure of microbial communities. Classical methods for determining the complexity of microbial ecosystem were insufficient. New molecular method with high throughput, quantitative, and focus better disclose structure and function of microbial ecosystem in parallel. Moreover, these new tools need to expand beyond the genomics, transcriptomics (study of mRNA as an indicator of gene expression), proteomics (identification, characterization, and quantification of protein and enzymes relate to cellular function), and metabolomics (study of metabolic intermediates of cellular functions) (Rittmann 2006).

Microbial communities are closely linked with animals and plants making necessary nutrients, metals, and vitamins available to their hosts and beneficial for humans and the environment. Microbial ecosystem performs a wide range of services, with reliable, continuous, economic, health, and social benefit. Moreover, microbial communities involved in removal of pollutants from soil, water and air to clean the environment. Moreover, remove and degrade the pesticides and other toxic material using in agricultural practice from the soil, remove and detoxify heavy metal from the environment, involved in nutrient cycle, detoxification of contaminated from wastewater, generate bioelectricity and biofuel in eco-friendly manners and also use as a therapeutics (Rittmann 2006). In this chapter, we discussed the involvement of microbial ecosystem in the betterment of environment in providing for the welfare of the human being.

2.2 Microbial Ecology: Historical Perspective

The word *ecology* originated in the late nineteenth century from two Greek words *oikos* (household) and *logos* (law) to suggest that the study of the relations between organisms comes from "law of the household." Ernst Haeckel (Haeckel 1866), a German biologist, identified ecology as a separate branch of science that deals with the interactions of living organisms with their biotic and abiotic environments. Microbial ecology is the study of microbes and their correlation with the (living and nonliving) environment. Microbes mould the earth's climate, directly and indirectly during the evolution of the earth's atmosphere, which was tightly correlated with evolution of its biota (Kasting and Siefert 2002). However, the term "microbial ecology" was not used commonly until the 1960s when public interest in environmental issues and in the essential roles of microbes in the earth's biosphere led to the emergence of the new field of "microbial ecology" (Rashid and Stingl 2015; Xu 2006).

Microbial ecology has historically focused on the relationship microbes have in host systems as they form beneficial link (e.g., nitrogen-fixing rhizobia in plant root nodules) and pathogenic relationships (e.g., *Pseudomonas aeruginosa* in the lungs of people suffering from cystic fibrosis) with their hosts. Moreover, microbes drive the nutrient cycles that make earth habitable for humans (Keymer et al. 2006; Peterson et al. 2008; Taylor et al. 2005).

Microbial ecosystems represent a number of interesting features. First, it is possible to work with rapid growth species in controlled environments such as the "chemostat" (Ballyk et al. 2001). Second, in any microbial ecosystem, the number of individuals considered is usually so large (i.e., of the order of 10^7–10^{12} individuals/ml) that the formalization using concentrations in an ordinary differential equations' system is meaningful and is justified by physics while it may be highly questionable when working with macroorganisms (as, for instance, lions driving out gazelles), and third, molecular techniques together with the classical microbial techniques allow to accurately monitor the evolution of the individual number of different species inside a biological reactor, at least in a "planktonic" mixed culture (Bastin and Dochain 1991). For these reasons, microbial ecologists claim that microbial ecosystems are good candidates to test hypotheses, principles, and concepts of general ecology (Jessup et al. 2004).

2.3 Molecular Evolutionary of Microbial Communities

This process is a mechanism of evolutionary change, particularly in microbes, because it makes considerable contribution to the instant creation and spread of biological innovation in many lineages that otherwise would have taken millions of years to proceed. The size of genetic material can be transferred horizontally from small gene fragments (Wang and Zhang 2000) to entire operons (Omelchenko et al. 2003) and super-operons that can encode complex biochemical pathways (Igarashi et al. 2001) and in some cases even to whole chromosomes (Akagi et al. 2009).

Evolutionary microbiology can be defined as the study of patterns (relation between genes and organisms) and processes (generating diversity and selecting operating on it) of evolution in microbes. Its main tools are reconstruction of relationships (using phylogenies and networks), population genetics, comparative genomics, and genome analyses. These three major discoveries had an impact on the branches of microbiology as those molecular markers can be used to reconstruct a phylogeny of all organisms (a "Universal Tree of Life"); they followed as a framework to scientific inquiry in microbiology for decades. Another main discovery is that the extent of genetic diversity in microbes is much higher than expected and can vary among species, which conduct into question the universality of species as a unit of diversity or evolution (Reno et al. 2009; Thompson et al. 2005).

Whole genome reconstruction was first accomplished in 1995, with exponential increase in the number of draft genomes reported each subsequent year. There are 30,437 genomes from all three domains of life—*Bacteria*, Archaea, and Eukarya, currently available in the Joint Genome Institute's Integrated Microbial Genomes database (accessed 24 September 2015) (Castelle et al. 2013).

To explore the deep microbial phylogeny, beginning in 1970, Woese and his colleagues at the University of Illinois focused on comparisons of small subunit ribosomal RNA (16S rRNA) oligonucleotides (short fragments of 5–20 or more nucleotides). Although it was not possible to sequence the larger RNA outright in those days, it was possible by using specific ribonucleases (such as T1 ribonuclease) to cleave a large RNA into oligonucleotides with a length of 5–20 bases. These then could be experimentally sequenced and catalogues made (Sapp 2007).

In 1990, two teams of microbial ecologists published the first 16S rRNA gene clone libraries from the Sargasso Sea and hot springs in Yellowstone National Park in the same issue of *Nature* (Giovannoni et al. 1990; Ward et al. 1990). This method was revolutionary, as clone libraries allowed sequencing of 16S rRNA genes amplified directly from environmental DNA, thereby avoiding the requirement for cultivation of their host. The Sargasso Sea study depicted its sequence data in a phylogenetic tree, including sequences from cultured organisms, allowing researchers to identify previously uncultured bacteria from the environment. Their depiction of sequence data in a phylogenetic tree was reminiscent of Woese's "Universal Phylogenetic Tree" (Woese 1987), as was their choice of molecular marker, which was guided by his previous work. As trees allow microbial ecologists to assign 16S rRNA gene sequences to phyla, genera, and occasionally species and reveal previously unknown groups, diversity became a central theme in microbial ecology. Consequently, the 16S rRNA gene is the most sequenced of all genes. Previously unknown groups of organisms can now be studied directly in the environment using molecular techniques and often prove to be abundant, confirming what microbiologists had long suspected: prokaryotes are the unseen majority (Whitman et al. 1998). This approach yields a higher-resolution tree that is obtained from a single gene, such as the widely used 16S rRNA gene (Hug et al. 2013). The use of ribosomal proteins avoids artifacts that would arise from phylogenies constructed using genes with unrelated functions and subject to different evolutionary processes. Another important advantage of the chosen ribosomal proteins is that they tend to be syntonic and co-located in a small genomic region in *Bacteria* and Archaea, reducing binning errors that could substantially perturb the geometry of the tree (Fig. 2.1). Included in this tree is one representative per genus for all genera for which high-quality draft and complete genomes exist (3083 organisms in total).

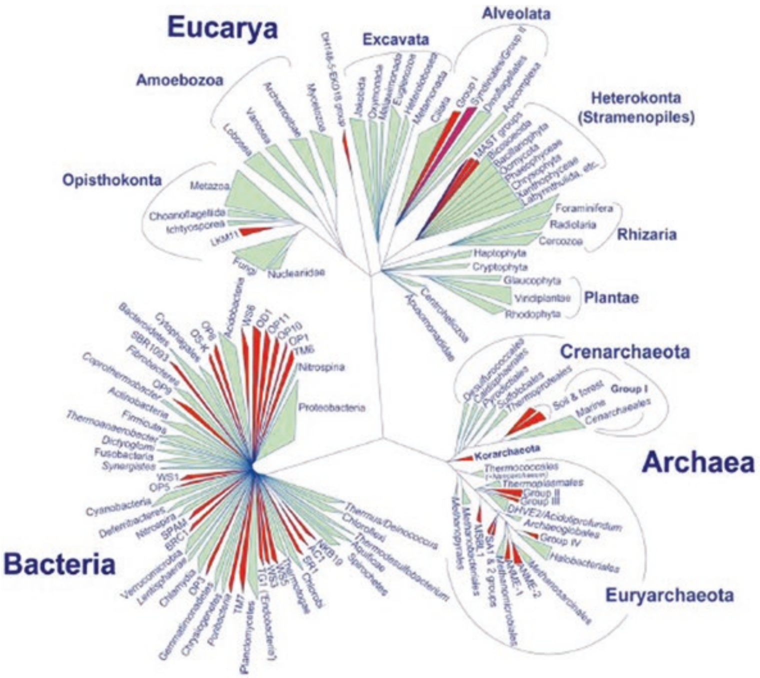


Fig. 2.1 Schematic phylogenic tree of life based on the 16sRNA. Green/light triangles represent phyla, division, or groups of high taxonomic rank for which at least one member has been cultivated and/or properly described. Red/dark triangles represent candidate division or highly divergent lineages without cultivated/described species. The tree is highly simplified, only known eukaryotic phyla are described, and, in the case of bacteria, only phyla and candidate divisions are described

2.4 Microbial Diversity Matter for the Environmental Factors

For the last few decades, microbial ecologists have used a variety of molecular tools to characterize the microbial communities and observe particular organismal assemblages or community characteristics. There have been few reports on the global surveys of microbial diversity, which have revealed dominant physicochemical drivers of microbial community structure using consistent methodologies (Fierer et al. 2012; Ghiglione et al. 2012). To date, the largest global survey of microbial diversity carried out by the Earth Microbiome Project (http://www.earthmicrobiome.org/) exposed the existence of 5.6 million OTUs (97% similarity at the V4 region of the 16S gene; not including singleton OTUs) in the first 15,000 samples, which set a new lower bound on the number of bacterial and archaeal phylotypes on earth. The rapid evolution and speciation in microorganisms can contribute significantly to diversity on ecological timescales (Morris et al. 2014; Raeside et al. 2014). Beyond fine-scale niche partitioning, microbial diversity is also influenced by

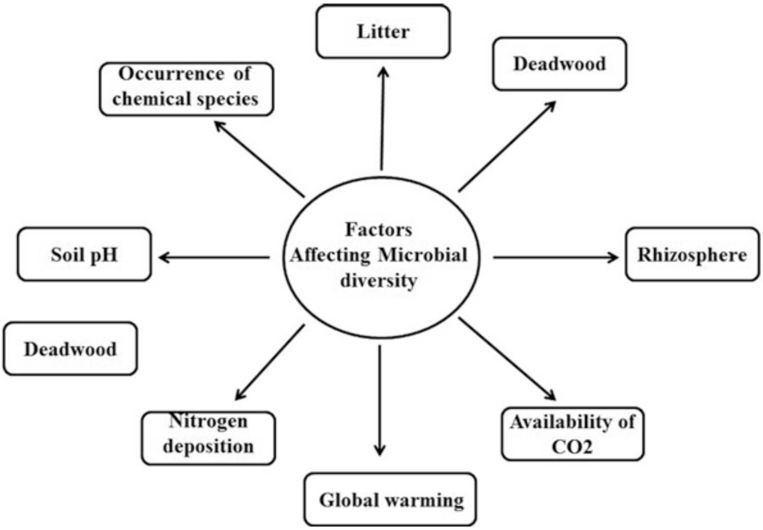


Fig. 2.2 Environmental factors influencing the diversity of microbial communities

stochastic forces, like extremely high rates of dispersal, coupled with the ability of many microorganisms to become dormant when conditions are not conducive to growth (Gibbons et al. 2013). The balance between niche (e.g., pH or temperature driving changes in microbial dominance) and neutral (e.g., stochastic dispersal and dormancy) processes will influence the diversity of microbial ecosystems. Recent work has suggested that increasing environmental heterogeneity or noise disrupts the deterministic coupling between ecosystem properties and ecological diversity, which results in neutrally assembled communities (Fisher and Mehta 2014). There are complex interactions within and between biological and physicochemical parameters that determine how microbial communities assemble in natural ecosystems. Several factors influence the microbial diversity as described in Fig. 2.2.

2.4.1 *Plant Litter*

Due to the activity of primary producers, dead plant biomass—litter—is considered as the main source of C for the forest soil microbes. The estimated 1011 tons of fallen leaf litter that accumulate yearly on the surface of the forest floor transform into other components and contribute in cycling of C and other nutrients in ecosystem (Austin et al. 2014). The diversity of fungal communities is dominant in litter habitat that has traditionally been considered to be the main player in litter decomposition. In addition to fungi, bacteria also actively participate in litter transformation (Štursová et al. 2012). In contrast to fungi, bacteria inhabiting the phyllosphere of living leaves do not seem to be more involved in litter decomposition and replaced by other taxa (Tláskal et al. 2016). The cellulose-C is accumulated mainly by *Betaproteobacteria*, *Bacteroidetes*, and *Acidobacteria* (Eichorst and Kuske 2012). Many of these bacteria belong to the *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Acidobacteria*, but members of other phyla are also active (Kim et al. 2014). Moreover, *Proteobacteria* and *Bacteroidetes* are considered copiotrophs that consume the labile pool of organic C (Fierer et al. 2007) and are thus typical for freshly fallen litter. The bacterial biomass aggrandize gradually during decomposition (Voříšková and Baldrian 2013), as does their diversity (Tláskal et al. 2016). During decomposition, the abundance of mycophagous bacteria co-occurred with the peak of fungal biomass in fresh litter. In this stage of decomposition, potentially mycolytic bacteria in *Quercus* litter represent about 40% of the total microbial community (Tláskal et al. 2016). Several bacterial taxa seem to be associated with decomposing fungal mycelia, including members of genera *Pedobacter*, *Pseudomonas*, *Chitinophaga* (*Bacteroidetes*), *Variovorax*, *Ewingella*, and *Stenotrophomonas* (Brabcová et al. 2016). Indeed, significant differences in the chemical structure of litter and root exudates among tree species influence soil bacterial communities through changes in substrate chemistry (Augusto et al. 2002).

2.4.2 Deadwood

In contrast to litter, coarse deadwood is also a considered a structural component of forest ecosystems, especially in unmanaged forests. Wood debris represents a rich yet recalcitrant source of organic matter, mainly due to its physical impermeability and high content of lignin that is beneficial for filamentous, macroscopic fungi (de Boer et al. 2005), which seem to be dominant decomposers of deadwood. However, bacteria are also important inhabitants of decaying wood, especially during the initial phases of decay (Kielak et al. 2016). The physicochemical composition of wood that is tree specific, such as pH, density, and water and nitrogen content, decide the diversity of bacterial communities (Johnston et al. 2016). The most dominant bacteria belong to the phyla *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Firmicutes* (Hoppe et al. 2015). Because fungal decomposition changes the properties of wood substantially by the removal of easily available C, acidification, and production of fungal mycelia, bacteria that can thrive at an acidic pH and utilize C from fungal mycelia are positively selected (Valášková et al. 2009). The assembly of the bacterial community appears to be a stochastic process during the initial stage of wood decay, and the abundance of bacteria in fresh wood is very low, while during decomposition, the abundance of bacteria gradually increases reaching a high level (Rinta-Kanto et al. 2016). Bacterial genera such as *Burkholderia*, *Phenylobacterium*, and *Methylovirgula*, which are dominant in middle and late stages of wood decomposition, are involved in the degradation of aromatic compounds and use methanol as a main carbon source (Hoppe et al. 2015), while N-fixation bacteria are abundant during the late stages of wood decomposition (such as the members of the *Rhizobiales*, which may account for 25% of all bacteria in this phase), which potentially and mutualistically interact with fungi that provide C via wood decomposition (Hoppe et al. 2015).

2.4.3 Rhizosphere

The roots and rhizospheres of trees as well as ground vegetation associate with fungi to form mycorrhiza (Markkola 1996). The large absorptive area and exudation of labile compounds by ECM hyphae make it important niches for different bacterial communities (Vik et al. 2013). The input of labile C compounds into the rhizosphere selects for bacterial strains with rapid growth and low-affinity substrate enzymes (r-strategists) that are enriched in comparison to the bulk soil profile (Loeppmann et al. 2016). The quantity and quality of exudates select for specific microbial communities and the expression of specific genes and may prime the decomposition of recalcitrant organic matter (Tefs and Gleixner 2012). Considering the seasonal changes in NPP, seasonality is a major factor of microbial community composition and functioning in the rhizosphere (Kaiser et al. 2010).

Recent studies from nonforest environments, including grasslands (Mao et al. 2014; Shi et al. 2015), croplands (Donn et al. 2015), and other ecosystems, observed that the rhizosphere contains a certain subset of bulk soil microbes, which is dominated with phyla *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* (Ai et al. 2015; Donn et al. 2015). Moreover, *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, and *Bacteroidetes* were also observed as dominant members in the rhizospheres of beeches in mountainous forest (Gschwendtner et al. 2016). An enrichment of *Proteobacteria* (e.g., *Burkholderia*, *Rhizobium*, and *Pseudomonas*) and *Actinobacteria* (such as *Streptomyces*) in the mycorrhizosphere was reported based on culture-dependent studies (Tanaka and Nara 2009; Yu et al. 2013). A recent molecular study of *Pinus sylvestris* mycorrhizospheres indicated that the community composition is much more complex and includes both copiotrophic and oligotrophic bacteria (Marupakula et al. 2016).

2.4.4 Carbon Dioxide

Increasing CO_2 concentrations are predicted to boost NPP in ecosystem (Norby et al. 2005); however, their capacity to maintain such high levels of NPP will strongly depend on their ability to overcome nutrient limitations (Luo et al. 2004). It is clear that the increasing level of CO_2 will be offset by C sequestration in forest ecosystems and will strongly depend on the adaptation of both trees and soil microorganisms to the altered environmental conditions. The effects of elevated CO_2 levels do not directly affect soil bacterial populations, because the concentration of CO_2 s in soil pores is normally higher than those in the atmosphere and the response of bacteria to small CO_2 changes is thus negligible (Dunbar et al. 2014). In addition, changes in organic acid exudation by mycorrhizal fungi as a result of the presence of CO_2 will affect the bacterial community composition (Fransson et al. 2016), and this process also depends on the vegetation properties. The expected increase in root exudation should generally be favorable to the development of soil copiotrophic bacteria over specialized oligotrophs. A good example of this response is the decrease in abundance of oligotrophic *Acidobacteria* in various soils following an increase in CO_2 (Dunbar et al. 2014; Fransson et al. 2016).

2.4.5 Global Warming

The rise in temperature is closely associated with increased CO_2 concentration in the atmosphere, because the latter largely causes the former. This long-term scenarios are, however, not clear because multiple long-term events, such as depletion of labile C limiting bacterial growth, thermal adaptation of microbial processes, and shifts in microbial communities or in the expression of their metabolic potential, may attenuate positive feedbacks of warming (Reich et al. 2016). The C balance after warming will chiefly depend on the efficiency of soil microbes by accessing and using C in response to the altered environmental conditions. A very recent work showed that an increase in temperatures can increase tree exudation rates (Schleppi et al. 2012). As a consequence, warming can alter soil drivers through exudation, which in turn might produce different responses of soil rhizosphere bacterial communities, affecting SOM decomposition differently in different environments, depending on the tree-specific exudation (Schleppi et al. 2012). However, it has been suggested that warming affects bacterial diversity and that these changes reflect the magnitude of the temperature change (Wu et al. 2015). These shifts in bacterial communities may affect the C cycling processes that involve bacteria. Members of the *Alphaproteobacteria* and *Acidobacteria* display increased abundances after simulated warming, suggesting a change in soil bacterial lifestyle from copiotrophy to oligotrophy, which coincides with the depletion of labile C while maintaining high levels of respiration (Pold et al. 2015). The warming also impact N cycle process by increase in temperature to accelerate nitrification and denitrification processes, affecting the N cycle in soils that largely influence the bacterial communities (Schütt et al. 2014).

2.4.6 Nitrogen Deposition

Globally, anthropogenic N inputs are estimated to be 30–50% higher than those from natural sources. They have been raised tenfold in the past 150 years and are projected to double during the next century (Galloway et al. 2008). Since N is a limiting nutrient in mostly terrestrial ecosystems, anthropogenic N deposition may largely influence the NPP in these environments by reducing this limitation (Högberg et al. 2014). N deposition also has a range of additional consequences from shifts in the soil C/N ratio, soil acidification, and root exudation to changes in the vegetation and microbiota (Wallenstein et al. 2006). Bacterial soil communities are highly sensitive to elevated level of N itself (Fierer et al. 2011; Frey et al. 2014). It has been observed that N deposition increases soil C sequestration due to the decline in SOM decomposition via the reduction of biomass and activity of soil microbial populations in many different soil environments (Freedman and Zak 2015; Frey et al. 2014; Ramirez et al. 2012; Zak et al. 2008). Although the reduction of biomass following N input was mostly observed in fungi (Frey et al. 2004; Högberg et al. 2014), it was recently noted that the biomass of soil bacteria can be reduced as well, by up to 50% (Long et al. 2012a; Maaroufi et al. 2015). At chronically low levels of N, which are typical of boreal and temperate forest soils, the bacterial biomass may remain stable while the fungal biomass markedly drops. This decrease in the fungal/bacterial biomass ratio due to N addition is consistently found across forest soils, where it ranges from 25% to 70% (Frey et al. 2004; Maaroufi et al. 2015).

N deposition also changes the composition of bacterial communities in forest soils. The abundances of *Acidobacteria* and *Verrucomicrobia* decrease, while those of *Actinobacteria* and *Firmicutes* increase, following the addition of N to a variety of soils (Ramirez et al. 2012). Also, in other cases, the deposition of N significantly affected relative abundances of core bacterial phyla, but it reduced bacterial diversity and altered the bacterial composition at lower taxonomic levels (Freedman and Zak 2015; Frey et al. 2014). When genes of the N cycle (fixation, nitrification, and denitrification) were assessed in N deposition experiments, the metabolic potential of the bacterial community remained stable or even decreased in terms of gene abundance (Long et al. 2012a).

2.4.7 pH

Soil pH is a crucial factor affecting the microbial communities in soil and influences the rates of substrate utilization. The values of substrate utilization by soil microbes were significantly higher in soils with higher pH (Anderson and Joergensen 1997). It has been observed that soil acidity considerably decrease in the availability of carbon to microbial communities (Bååth et al. 1995) and lower the rate of bacterial growth (Bååth 1998). For instance, bacterial communities associated with coniferous forest soils may contain larger proportions of Gram-positive bacteria adapted to the acidifying environment; in contrast, increases in soil pH may result in larger proportions of Gram-negative bacteria (Pennanen 2001). Additionally, a number of bacteria have been shown to decrease in acidified soils (Baath et al. 1980), possibly because bacteria are less adaptive to acidic conditions in soil compared to fungi (Matthies et al. 1997).

2.4.8 Occurrence of Chemical Species

A wide variety of pesticides including herbicides, fungicides, and herbicides are used in agricultural practices in order to improve the yield of various crops. The use of such pesticides beyond recommended concentrations or their accumulation into soil following continuous application leads to changes in soil microbial population in agroecosystem. For instance, the application of endosulfan, profenophos with alphamethrin, and methamidophos has shown considerable inhibition in bacterial population in cotton (*Gossypium hirsutum*) agroecosystem, while monocrotophos and bifenthrin with acetamiprid enhanced the bacterial population of soil. Fungal population was depressed with endosulfan, while monocrotophos, methamidophos, endosulfan with dimethoate, fenpropathrin, bifenthrin with acetamiprid, or with ethion or with a mixture of carbosulfan and chlorpyrifos and profenophos alone or with ethion or cypermethrin or alphamethrin stimulated fungal counts. Another study investigated the effect of 11 pesticides on the populations of bacteria, actinomycetes, fungi, and protozoa by treating a garden soil with their recommended rates (Ekundayo 2003). Wang et al. (2006) showed that both a low and a higher dose of methamidophos (0,S-dimethyl phosphoroamidothioate) in soil significantly decreased microbial biomass C by 41–83% compared to control. In contrast, the respiration activity of the applied soils was significantly higher than the control. It was concluded that methamidophos reduces microbial biomass and enhances functional diversities of soil microbial communities; meanwhile, some species of bacteria may be enriched in soils under methamidophos stress. Similarly, Ratcliff et al. (Ratcliff et al. 2006) reported that commercial formulation of herbicide (glyphosate) applied at the recommended field rate to a clay loam and a sandy loam forest soil has a benign effect on community structure and produced a nonspecific, short-term stimulation of bacteria at a high concentration.

2.5 Microbial Ecosystem for Environmental Betterment

Microbes are recognized as an important component of the earth’s ecosystem and key player for controlling the composition of atmosphere and surface waters, forming the basis of the marine food web and the cycling of chemicals in the ocean, terrestrial ecosystem, agroecosystem, and interaction with human to make several beneficial approaches in the environment. A revolution in microbial ecology has occurred in the past 15–20 years with the advent of rapid methods for discovering and sequencing the genes of uncultivated microbes from natural environments (Ward 2005). In this topic we discuss about the involvement of microbial communities toward the environmental betterment as well as human welfare; an outline is mentioned in Fig. 2.4.

2.5.1 Soil Pollution

Soil pollution is the most problematic in all kinds of environmental pollution, because soil is a point of concentration and recovery of toxic compounds, chemicals, salts, radioactive materials, or disease-causing agents, which have adverse effects on plant growth and animal health. Additionally, soil pollutants can contaminate water, which is a valuable resource that supports cultures and plant life (Ranieri et al. 2016).

Bioremediation is effectively applied in in situ and ex situ treatment of contaminated soil. In situ degradation of soil contaminants in the subsurface depends on the type of contaminants, the type of microorganisms, and the physicochemical conditions. Soil contains several types of microorganisms including bacteria, actinobacteria, cyanobacteria, fungi, microalgae, and protozoa. Usually bacteria are far more numerous than any other soil microbes and are the most focused group for bioremediation, mainly due to their rapid growth and fast metabolic rate (Sana 2014).

Removal of Hydrocarbons from Contaminated Soils

Several strains of microbes have been reported to degrade petroleum hydrocarbons from contaminated soils. Numerous bioremediation techniques were used individually as well as in combination for treatment of hydrocarbon contaminated soil. Laboratory-scale bioremediation of petroleum-contaminated soil has been studied to increase the growth of the indigenous microorganisms of the contaminated site with and without addition of a petroleum-degrading *Pseudomonas aeruginosa* strain (Karamalidis et al. 2010). Several other *Pseudomonas* species are also involved in the bioremediation of petroleum hydrocarbons, polyaromatic hydrocarbons (PAH), and organochlorine pesticides (Kumar et al. 2008; Tang et al. 2013). Bioaugmentation with a defined microbial consortium containing two diesel-degrading strains (*Gordonia alkanivorans* CC-JG39 and *Rhodococcus erythropolis* CC-BC11) and three oil-degrading strains (*Acinetobacter junii CC-FH2*, *Exiguobacterium aurantiacum CC-LSH-4*, and *Serratia marcescens KH1*) removed ∗65% aromatic hydrocarbon after 140 days of treatment (Alisi et al. 2009). The most active microbes were observed in the bioremediation of diesel oil in a co-contaminated soils of genera *Arthrobacter*, *Pseudomonas*, *Rhodococcus*, *Bacillus*, *Exiguobacterium*, and *Delftia* (Sprocati et al. 2012). Bioremediation of a viscous oil-contaminated soil was facilitated by ultraviolet-induced mutation of a microbial consortium constructed with seven oil-degrading microbial strains (including *Acinetobacter* sp. *YC-X2*, *Kocuria* sp. *YC-X4*, and *Kineococcus* sp. *YC-X7*) isolated from crude-oil-contaminated soil (Chen et al. 2011). PAH are one of the most widespread organic pollutants found mainly in soil and sediment. It has been reported that about 36% PAH is removed from a contaminated soil (containing ∗10 mg PAH per kg of dry soil) after 56 days of treatment with a PAH-degrading microbial consortium isolated from a PAH-contaminated site (Mao et al. 2012). Molecular phylogeny identified the most abundant populations of the consortium as close relatives of the *Mesorhizobium*, *Alcaligenes*, and *Bacillus* species (Silva et al. 2009a).

Removal of Agricultural Pesticides from Soil

Indiscriminate use of pesticides in agricultural activity causes continuous contamination of farming lands, which can further pollute surface water as well as underground water when washed out by rain. Several studies were conducted on the microbial biodegradation of chemicals present in pesticides or insecticides. As a consequence, pesticide degradation—whether complete mineralization or partial transformation to elemental substances by the soil microbes—has received ongoing attention from microbiologists over the last 60 years (Dechesne et al. 2014). Many pesticides can be metabolized by bacteria and derive energy, carbon, and other elements (N, P) from their degradation. It has been demonstrated that rapid degradation of phenylurea pesticide in agricultural soils is associated to *Sphingomonas* populations with narrow pH optimum (Bending et al. 2003; Shi and Bending 2007). Frequently used organochlorine pesticide degrade by microbial bioremediation, pesticide, such as lindane was also reported by some researchers. Four lindane-degrading microbial strains have been isolated from the rhizosphere of selected plants at lindane-contaminated site and identified as *Kocuria rhizophila*, *Staphylococcus equorum*, *Microbacterium resistens*, and *Staphylococcus cohnii* (Abhilash et al. 2011). Several endosulfan-degrading bacteria have been isolated from contaminated soil, and by molecular phylogeny some of the isolates were identified as *Ochrobactrum* sp., *Pseudomonas* sp., *Burkholderia* sp., and *Arthrobacter* sp. (Sana 2014).

Removal of Heavy Metals from Soil

Heavy metals are hazardous to human health, and their presence in soil creates a serious concern as they can easily enter the human body through contaminated drinking water and vegetables. Serious heavy metal toxicity may be caused by arsenic, cadmium, chromium, lead, and mercury poisoning, which may cause permanent damage to the skin, teeth, bone, liver, kidney, and nervous system. Study showed that microbial consortia containing *Bacillus subtilis*, *P. aeruginosa*, and *Saccharomyces cerevisiae* victoriously reduced the chromium content by <1% of its original concentration. Another study reported 51 heavy-metal-resistant microbes isolated from three different composts, using different raw materials such as horticulture waste, sewage sludge, and municipal solid waste (del Carmen del Carmen Vargas-García et al. 2012). Most of the microorganisms were able to remove several heavy metals including Cd, Cr, Ni, Pb, and Zn. Some of the isolates were able to remove more than 90% of Pb, and other metals were removed within the range of 20–60%. Molecular phylogeny identified the best performing isolates such as *Graphium putredinis*, *Fusarium solani*, *Fusarium* sp., and

Penicillium chrysogenum. Most metals were removed predominantly by intracellular accumulation within the microorganisms, with the exception of Ni that was equally removed by extracellular absorption and intracellular accumulation (Sana 2014).

2.5.2 Water Pollution

"Water for Life" initiative was declared as the International Decade (2005–2015) for Action for water by the United Nations General Assembly in 2005. Because of the rapid increase in demand for water, and increase in occurrences of pollution of numerous water sources, environmental risks to humans and other life beings are enhanced (Bhuiyan et al. 2013). Groundwater is one of the main sources of potable and drinking water. Many methods are used to reduce the contamination from the groundwater, including MFC technology which has been studied for the removal of nitrate from polluted groundwater (Tang et al. 2013; Virdis et al. 2008). A two-chamber MFC was designed for simultaneous carbon and nitrogen removal using an acetate-adopted microbial consortium obtained from another MFC of a water management center (Virdis et al. 2008).

Mining has an extremely destructive effect on the biosphere as well as source of various contaminants for water pollution. Long-term bioremediation may be useful for eliminating mine-associated pollutants from water. Sulfate and heavy metal content of an acid mine drainage water were successfully reduced in an ex situ bioremediation technique by using sulfate-reducing bacteria (Greben et al. 2009). Another study showed that the biofilm was developed by batch mode for 60 days in biocontactor with microbial consortium; it victoriously removed 20–50% of various heavy metals from the highly contaminated acid mine drainage after 10 weeks of continuous treatment. Removal of various metals was on the order of Cu > Ni > M n > Zn > Sb > Se > Co > Al, and the biofilm was dominated by algae *Ulothrix* species (Orandi et al. 2012).

2.5.3 Control Air Pollution

The air pollution treatment by microbes is the least studied field as compared to soil or water pollution. Airborne phototrophic microorganisms and hydrocarbon-utilizing heterotrophic bacteria were isolated from dust samples collected at 15 m height of Kuwait City air. Three phototrophic microorganisms were identified by molecular phylogeny, including *Leptolyngbya thermalis*, *Nostoc commune*, and a chlorophyte of genus *Gloeotila*, and each of them was correlated with unique consortia of oil-vapor-degrading bacteria that may be involved in in situ bioremediation of atmospheric hydrocarbon pollutants. Novel methods were taken for in situ flue gas bioremediation and simultaneous production of microalgal biomass (Chiu et al.

2011; Douskova et al. 2009). Onsite bioremediation of nitrogen oxide, carbon dioxide, and sulfur dioxide of coke oven flue gas was accomplished by directly passing flue gas through photobioreactor containing a culture of CO_2 and heat-tolerant engineered *Chlorella* species (Chiu et al. 2011). Another study conducted showed that CO_2 present in a municipal waste incineration flue gas was absorbed by *Chlorella vulgaris* and simultaneously reduced biomass production cost (Douskova et al. 2009).

Increasingly stringent environmental legislation is generating a great interest in the industry for the effectiveness of biological waste air treatment techniques. The pollutant vapors and oxygen are transported in humid air by forced convection. Interphase mass transfer occurs, and, provided that the biofilter bed particles are small, interfacial equilibrium is achieved so that gas phase resistance can be neglected. In the biofilm, simultaneous diffusion and biodegradation of the pollutants occur as a result of growing or resting microorganisms. As biodegradation occurs in the biofilm, metabolite formation is possible. If formed, metabolites will undergo the same simultaneous diffusion/biodegradation/sorption processes. Acidic metabolites will be neutralized by limestone or other pH buffer agents generally mixed with the support prior to the packing of the biofilter. The fact that metabolites are seldom observed suggests that they are degraded faster than the primary pollutant either by ancillary strains in direct contact with the primary pollutant degraders (consortia) or directly by the primary pollutant degraders themselves. The carbon dioxide resulting from the oxidation diffuses back and is further transferred to the gas phase. Some of it can also accumulate as carbonate (Deshusses 1997).

2.5.4 Wastewater Treatment

Fresh drinking water is a constant necessity of humans, while wastewater is constantly produced. The provision of drinking water and the management of wastewater have thus been crucial to the success of human civilization. However, when it comes to the purification of sewage water, microbes are superior to humans, and their abilities to degrade the most diverse of organic substances and to cycle elements such as nitrogen, phosphorus, and carbon are unmatched in nature. The successful exploitation of these features has been achieved for almost a century in biological wastewater treatment plants (WWTPs) (Daims et al. 2006).

The removal of nitrogen from wastewater includes three main processes: nitrification, denitrification, and anaerobic ammonium oxidation (anammox). Nitrification consists of two steps that catalyzed sequentially by aerobic chemolithoautotrophic ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). The model organisms for nitrification are ammonia oxidizer *Nitrosomonas europaea* and nitrite-oxidizing *Nitrobacter* species, both of which can be isolated from the most nitrifying activated sludge and biofilm samples (Daims et al. 2006). For decades, these microorganisms are believed to be the main nitrifiers in wastewater treatment. Nitrification has long been one of the wastewater treatment processes that most frequently causes trouble, and because of the slow growth of nitrifiers, nitrification activity does not promptly recur after breakdown events. The genus *Nitrospira*, and not *Nitrobacter*, was identified in mostly wastewater treatment plants by in situ as a main NOB (Daims et al. 2001). Moreover, ammonia-oxidizing archaea, which were only recently discovered in marine and terrestrial habitats also involved in nitrification, occurred in WWTPs (Könneke et al. 2005; Schleper et al. 2005). The chemolithoautotrophic bacteria are responsible for anammox, the direct combination of ammonium and nitrite into dinitrogen gas (Strous et al. 1999). Molecular methods disclosed that these uncultured bacteria present large amounts in WWTPs with nitrogen removal and anaerobic niches (Egli et al. 2001; Schmid et al. 2000).

The biological elimination of phosphorus from WWTP is achieved through EBPR bacteria, by the accumulation of polyphosphate intracellularly; these organisms are selected during sequential anaerobic–aerobic phases (Blackall et al. 2002). The main players in this process are PAO and GAO. The cultivation studies suggested that *Acinetobacter* spp. were PAO responsible for EBPR in activated sludge proved misguided (Wagner et al. 1994), and many new PAO candidates have since emerged, such as uncultivated betaproteobacterium '*Candidatus Accumulibacter phosphatis*'(Hesselmann et al. 1999). The evidence showed that this and other *Rhodocyclus*-related microorganisms are major PAO in both laboratory- (Crocetti et al. 2000) and full-scale (Kong et al. 2004) EBPR systems. Uncultivated gammaproteobacterial GAO (e.g., '*Candidatus Competibacter phosphatis*' have commonly occurred in EBPR plants (Crocetti et al. 2002).

Good settling properties of activated sludge are the main step for the separation of sludge from treated wastewater. Bulking and foaming are the main sludge settling problems that are frequently caused by excess growth of filamentous bacteria (Martins et al. 2004). The filamentous morphotype contains a large number of phylogenetically and physiologically different bacteria, most of which are uncultured and poorly characterized. This part of wastewater microbiology has benefited to particularly high degree from molecular methods compared with traditional, morphology-based approaches (Eikelboom 1975). The filamentous *Microthrix parvicella* is notorious for causing sludge bulking and foaming and has been studied extensively by cultivation-dependent as well as -independent methods. Interestingly, *M. parvicella* has a more hydrophobic cell surface than most other activated sludge bacteria, which might facilitate the uptake of lipids (Nielsen et al. 2002). Addition of polyaluminum chloride selectively suppresses *M. parvicella* (Roels et al. 2002), probably by affecting surface-associated lipases, thereby reducing its ability to metabolize lipids and lowering the competitiveness of this organism (Nielsen et al. 2005).

2.5.5 Biogeochemical Cycles

Carbon Cycle

The flux of C in ecosystem is initiated by the fixation of atmospheric CO_2 by photosynthesis and mediated by the distribution of recalcitrant and simple organic compounds into the soil (Fig. 2.3). When simple organic compounds and plant biomass are decomposed by microorganisms, in this process, some C is returned to the atmosphere via respiration (Xia et al. 2015). Recently, it has been suggested that bacteria are the key players in the transformation of dead plant biomass than was previously assumed and significantly contribute in decomposition processes of litter and soil (López-Mondéjar et al. 2016a). The plant biomass is composed of hemicelluloses in a recalcitrant complex with lignin, cellulose represents the most accessible biopolymer, and its degradation is thus a key step in the C cycle. The enzymes involved in this degradation process are endocellulases, exocellulases, and glucosidases (Rytioja et al. 2014). The 24% of all sequenced bacterial genomes contain cellulases encoding genes (Berlemont and Martiny 2013), and glycosyl hydrolases, which are involve in degradation of other plant structural biopolymers (Berlemont and Martiny 2015). Recently, some cellulolytic bacteria belonging to a variety of phyla isolated from deciduous forest topsoil, demonstrated, for the first time, the presence of cel-

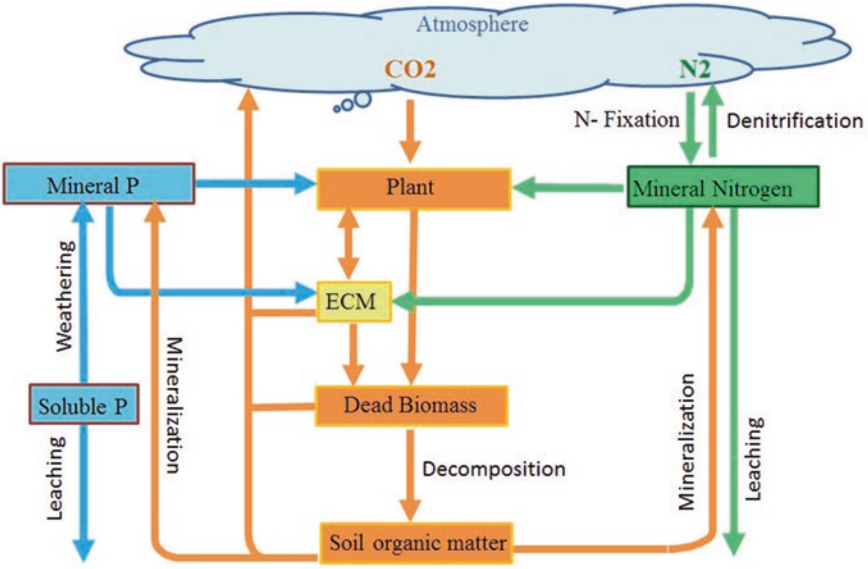


Fig. 2.3 Schematic view of the coupled biogeochemical cycles of phosphorus, carbon, and nitrogen in ecosystems. Colored arrows show the transfer of elements between ecosystem compartments. In colored arrow, P is represented in blue, C in orange, and N in green

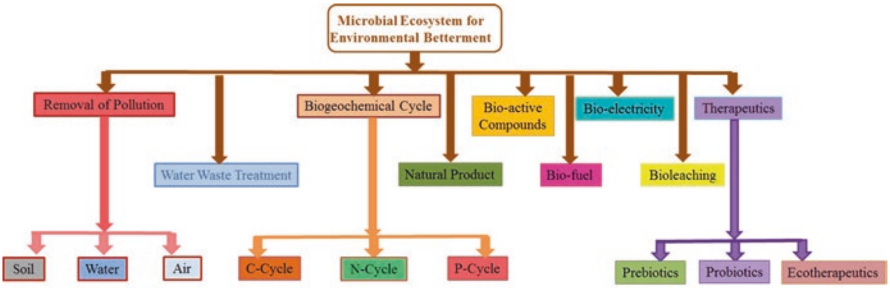


Fig. 2.4 Microbial involve in benefit for environment and human welfare

lulolytic members among some abundant soil genera, e.g., *Mucilaginibacter*, *Luteibacter*, and *Pedobacter* (López-Mondéjar et al. 2016a).

Other studies highlight the important ecological role of strains of the phyla *Acidobacteria* and *Actinobacteria* as degraders of plant biomass polysaccharides in the acidic soils of temperate forests (Větrovský et al. 2014). The rich suite of auxiliary proteins expressed by a *Paenibacillus* sp. strain from a temperate forest soil suggests their important involvement in polysaccharide decomposition (López-Mondéjar et al. 2016b). The fungal mycelia represent the large C and N pool that typically contains chitin and other polysaccharides, including glucomannans and glucans, as well as phenolics, such as melanin (Fernandez and Koide 2014).

Up to 40% of newly photosynthesized C can be allocated to the soil via roots, where it is rapidly respired or incorporated into the microbial biomass (HoÈgberg et al. 2001; Kuzyakov and Cheng 2001). The rhizosphere of roots colonized by mycorrhizal fungi is specific in that a portion of the plant-derived C is released into the soil through the ECM mycelium (Korkama et al. 2007). Methane that occurs in gaseous form of organic C can be oxidized by methanotrophic bacteria under aerobic conditions (Lau et al. 2015), which are the key consumers of atmospheric methane as well as methanogenic archaea-generated methane in waterlogged anaerobic horizons of certain soils (Savi et al. 2016; Shoemaker et al. 2014). Phylogenetically, methanotrophs commonly belong to the bacterial phyla *Alphaproteobacteria* and *Gammaproteobacteria* (Esson et al. 2016; Fig. 2.4).

Nitrogen Cycle

Nitrogen is the most common limiting nutrient that enters into the ecosystem mainly through biological fixation process (Fig. 2.3). This process is dominated by bacteria that are estimated to be responsible for more than 95% of the N input in the ecosystem (Berthrong et al. 2014). It has been observed that *nifH* gene is ubiquitously present in symbiotic as well as free-living N-fixing bacteria, including *Alphaproteobacteria* (*Bradyrhizobium*, *Azospirillum*, *Hyphomicrobium*, and *Gluconacetobacter*) and *Deltaproteobacteria* (*Geobacter* spp.) (VanInsberghe et al. 2015). Nitrification and denitrification cause the N loss from soils in the form of NO, N_2O, and N_2 gas as well as through the leaching of NO_3 if its concentration exceeds to the ecosystem retention capacity (Levy-Booth et al. 2014; Pajares and Bohannan 2016). Nitrification is a multi-enzymatic process for the biological oxidation of ammonia into nitrate. The first step of nitrification is performed by AOB as well as AOA, which contain ammonia oxidase encoded by *amoA* gene. The AOB are more dominant in the soil (Isobe et al. 2011; Long et al. 2012b), belonging to the *Betaproteobacteria* and *Gammaproteobacteria*, and mostly represented under genera *Nitrosomonas*, *Nitrosococcus*, and *Nitrosospira* (Laverman et al. 2001; Szukics et al. 2012). Denitrification is a key step for the global N cycle to manage the reduction of nitrate or nitrite to gaseous nitrogen compounds, including N_2 and nitrous oxide. This process is known as dissimilatory nitrate reduction because nitrate or nitrogen oxides are the final electron acceptors, allowing bacteria to generate energy at the cost of reduced electron donors in the absence of oxygen. In denitrification pathway, different genes are involved in individual enzymatic steps, such as the reduction of nitrate (*narG*), nitrite (*nirK*/*nirS*), nitric oxide (*norB*), and nitrous oxide (*nosZ*) (Jung et al. 2012). Mostly denitrifying bacterial strains with denitrifying genes belong to the phyla *Proteobacteria* and *Firmicutes* (Nelson et al. 2016; Priyanka and Koel 2015; Rösch et al. 2002). In addition to mineral N forms, bacteria also obtain N from a range of organic compounds present in soils, including chitin present in the form of polysaccharides of fungal mycelia and amino acids and proteins from the dead organic matter (Izumi et al. 2008).

Phosphorus Cycle

In contrast of potential source of N—the atmosphere—is theoretically unlimited, the sources of phosphorus (P) is limited in ecosystem, which is mostly supplied by weathering of minerals (Fig. 2.3). Bacteria are key player of P cycle by solubilizing and immobilizing the mineral P in their biomass. The key mechanism involved in the uptake of P by bacteria is through the solubilization and uptake of inorganic P, its most abundant source. The capacity to solubilize inorganic P correlated with the release of organic anions, such as gluconate, citrate, oxalate, and succinate into the soil (Richardson and Simpson 2011). The phosphatase enzymes are responsible for the release of phosphate from organic phosphate esters during organic matter decay. The gene related to P solubilization is dominant in P-rich soil, and those involve in P uptake systems are more dominant in P-limited soil. Additionally, recent metagenomic analysis reveals that members of *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, and *Acidobacteria* are dominant in the processes related to P turnover (Bergkemper et al. 2016a; Bergkemper et al. 2016b).

2.5.6 *Natural Products from Microbes*

Nature has provided some useful products for humans, known as "natural products." These have therapeutic and biotechnological values. Almost half of the therapeutic drugs available in the market have been derived from natural products. Recent findings have suggested that many natural products are of bacterial origin. Most often, bacteria producing natural products live in symbiotic relationship with eukaryotes like fungi, marine invertebrates, insects, or nematodes (Rashid and Stingl 2015).

The phytopathogen *Moniliophthora roreri* and endophyte *Trichoderma harzianum* cohabit in cacao plants. The *T. harzianum* is extensively used as a biocontrol agent and has the ability to antagonize *M. roreri*. The four secondary metabolites (T39 butenolide, sorbicillinol, harzianolide, and an unknown substance) have been identified, with its production phytopathogen dependent and spatially localized in the interaction zone (Tata et al. 2015).

Trichoderma atroviride, commonly used as a biocontrol agent, produces acetic acid-related indole compounds that may promote plant growth. The *T. atroviride* involved stimulate the growth and provide resistance against hemibiotrophic and necrotrophic phytopathogens, by the colonization with Arabidopsis roots (Salas-Marina et al. 2011). Araújo et al. (Araújo et al. 2002) isolated a great number of *Methylobacterium* strains from asymptomatic citrus plants (with *Xylella fastidiosa* but without disease), and then Lacava et al. (Lacava et al. 2004) showed that *Methylobacterium mesophilicum* SR1.6/6 and *Curtobacterium* sp. ER1.6/6 isolated from health and asymptomatic plants inhibited the growth of the phytopathogen *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis.

Hopanoids are essential composition of the cell membrane of some bacteria (Bradley et al. 2010), that perform the similar function of eukaryotes cholesterol (Welander et al. 2009). Knockout of hopanoid biosynthesis genes such as *hnp*F (squalene hopene cyclase-shc) affects the tolerance to various stress conditions, such as extreme acidic environments; toxic compounds as DCM as well as resistance to antibiotics and antimicrobial lipopeptide; involve in bacterial motility and multidrug transport (Braga et al. 2016).

The mycoparasite *S. elegans* release several mycotoxins, mainly trichothecenes and atranones. It has been hypothesized that trichothecenes were triggered by *R. solani* responsible for the alteration in its metabolism, growth, and development (Chamoun et al. 2015). Many microbes secrete siderophores in the environment and are involved in the crucial mechanism to obtain iron as well as its secretion recognized by cell surface receptors and then transported into the microbial cell (Faraldo-Gómez and Sansom 2003). Siderophores can also perform diverse of functions, such as sequesters of metals and even heavy metal toxins, as signaling molecules, regulating oxidative stress regulatory agent, and as antibiotics (Johnstone and Nolan 2015). Some *Pseudomonas* species secrete a group of siderophores called pyoverdines which is involved in the infection and biofilm formation and regulate bacterial growth (Visca et al. 2007). The endosymbiotic bacteria *Burkholderia* spp. secrete the phytotoxin rhizoxin, which are responsible for the rice seedling blight (Partida-Martinez and Hertweck 2005). Moreover, *Burkholderia gladioli* produce enacyloxins in co-culture with *R. microspores* and showed polyketides with potent antibiotic activity. The fungus induces the growth of *B. gladioli* by increasing the production of bongkrekic acid, which inhibited the fungal growth (Ross et al. 2014).

2.5.7 Production of Bioactive Compounds by Microbes

Bioactive compounds are valuable for both industrial and medicinal applications. Microbial processes used in the production of such valuable compounds are not only eco-friendly processes but also are safe and have less disadvantages. These bioactive compounds play dynamic roles in cellular communication processes by attaching to antibodies and other molecules and act as receptors for bacterial and viral particles (Hossain et al. 2016). Some commonly used active compounds produce by microbes are described below.

Amino Sugar

N-Acetylglucosamine and glucosamine are amino sugars derived from substitution of a hydroxyl group of a glucose molecule with an amino group or its acetylated derivative (Liu et al. 2013). They are extensively used in food, pharmaceuticals, and cosmetics industries (Dostrovsky et al. 2011) and produced by the acid hydrolysis of chitin extracted from shrimp shells and crab. However, microbial fermentation by the recombinant strains of *Bacillus subtilis*, *Escherichia coli*, or fungi is an alternative method for the production of these amino sugars (Deng et al. 2005). The expression of N-acetylglucosamine transferase and deletion of *nagE* by using the metabolically engineered *E. coli* increased the production of glucosamine titer by 17 g/L and N-acetylglucosamine titer by 110 g/L (Deng et al. 2005). In *B. subtilis*, the modular pathway was engineered to improve the production of N-acetylglucosamine by fine-tuning synthetic pathways and balancing the metabolism of host cells (Liu et al. 2014).

Oligosugar

Metabolic engineering of microbial cells is an auspicious strategy for the production of adequate amount oligosugars at large scale. Today, a variety of oligosugars were successfully produced by the genetically engineered *E. coli*, *Agrobacterium* sp., *Corynebacterium ammoniagenes*, *C. glutamicum*, and *Pichia pastoris*. The microbial coupling approach was used to obtain high concentration of oligosugars (188 g/L). All bacteria are capable to synthesize UDP-glucose, because it is required for cell wall biosynthesis, and UDP-glucose regeneration is important for the other sugar nucleotides synthesis, including UDP-galactose, which is a main cofactor (Hossain et al. 2016). In *E. coli*, biosynthesis of trehalose through the OtsAB pathway (Giaever et al. 1988) and metabolically engineered *Corynebacterium glutamicum* was also preferred for the trehalose production through heterologous expression of *E. coli* genes *otsA*, *otsB*, and *galU* to improve the capacity of trehalose production (Carpinelli et al. 2006). Another oligosugar, sialyllactose, is produced from sialic acid; CMP-NeuAc was regenerated by the deletion of sialic acid aldolase gene and overexpression of CMP-NeuAc synthase in an *E. coli* K12 strain (Endo et al. 2000).

Hyaluronan

Hyaluronan is a linear polymer of a repeating disaccharide unit of glucuronic acid and N-acetyl glucosamine (Liu et al. 2011). It acts as a lubricant between joint surfaces because of its distinctive viscoelastic properties and lack of immunogenicity and toxicity, which have led to it being used in a wide range of applications in cosmetic and medicinal fields (Widner et al. 2005). The metabolically engineered *E. coli* for hyaluronan biosynthetic was achieved by overexpression of hyaluronic acid synthase gene from *S. pyogenes* (Yu and Stephanopoulos 2008). Moreover, *B. subtilis* was engineered by insertion of two inducible artificial operons containing hyaluronan synthase gene from *Pasteurella multocida* and precursor genes encoding hyaluronan precursor enzymes, which efficiently produce hyaluronan (Jia et al. 2013).

Bacterial Cellulose

Bacterial cellulose nanofibers are some of the hardest organic materials obtained naturally and contain high pure sources of cellulose (Mohite and Patil 2014), and they are extensively used as a biological material for packing edible things, wound dressing materials, medical pads, vascular grafts, artificial skin, tissue engineering scaffolds, artificial blood vessels, dental implants, oil-leakage sponges, toxin-absorbing materials, and optoelectronic materials (Shah et al. 2013). Bacterial cellulose is an extracellular polysugar, mainly obtained by the *Gluconacetobacter xylinus* (Li et al. 2012). Despite its biocompatibility and mechanical properties, the main problems with bacterial cellulose are low productivity and undesirable degradability (Koutinas et al. 2012). To overcome the degradability problem, metabolically engineered *Gluconacetobacter xylinus* was introduced by expressing an operon containing three genes (N-acetylglucosamine kinase, phospho-acetylglucosamine mutase, and UDP-N-acetylglucosamine pyrophosphorylase) from *Candida albicans* for UDP-N-acetylglucosamine synthesis to produce the cellulose (Yadav et al. 2010).

Polysugar

Pullulan is a water-soluble extracellular polysugar, which consists of maltotriose units containing α-1, 4, and α-1,6 linkages, obtained by *Aureobasidium pullulans*. Because of its unique linkage pattern, it showed unique physical properties, including adhesive characteristics and capacity to form fibers, strong oxygen-impermeable films, and compression moldings. Therefore, pullulan has a wide range of economic values in the agricultural, food, chemical, cosmetic, and pharmaceutical industries (Prajapati et al. 2013). Recent research showed that inulinase gene from *Kluyveromyces marxianus* was introduced into the genomic DNA of *Aureobasidium melanogenum* P16, which efficiently produced the pullulan (Ma et al. 2015). Xanthan gum is a water-soluble heteropolysugar that contain repeating units of glucose, mannose, and glucuronic acid and widely used in food industry as a thickener, in agricultural products as an emulsion stabilizer, in pharmaceutical industry, and in the gluten-free baking process. *Xanthomonas campestris* is the main source for xanthan gum production (Hossain et al. 2016; Silva et al. 2009b).

2.5.8 Biofuels from Microbes

In this industrial revolutionary period, with the rising crude oil prices, depletion of resources, environmental challenges, and political instability in producing countries, besides efficiency and intelligent use, only biofuel has the potential to replace the supply of this energy-hungry civilization. Today, different kinds of biofuels are available in the market, of these only biodiesel and bioethanol are presently produced as a fuel on industrial scale.

Bio-hydrogen

Bio-hydrogen is a potential alternative, reliable and renewable source of energy, obtained from microalgae including cyanobacteria. The hydrogen fuel cell technology produces biofuel in an eco-friendly way, since the use of H_2 to generate electricity releases only water as a by-product and can be used in an eco-friendly manner to reduce the emission of global greenhouse gas (Khetkorn et al. 2017). Hydrogen gas has been used to generate electricity using fuel cells, as a fuel in rocket engines including applications in transportation (Ramachandran and Menon 1998). Several photosynthetic and non-photosynthetic microorganisms are able to produce hydrogen gas, namely, green algae, cyanobacteria, photosynthetic bacteria, and dark fermentative bacteria (Khetkorn et al. 2017). Microalgae belonging to the genera *Anabaena*, *Botryococcus*, *Chlamydomonas*, *Chlorococcum*, *Chlorella*, *Nostoc*, *Scenedesmus*, *Synechocystis*, *Tetraspora*, etc. may contain a hydrogenase enzyme for the hydrogen production (Eroglu and Melis 2011). These microbes can need simple nutrient for growth with capacity to fix atmospheric CO_2 as a source of carbon source, and many strains are able to reduce atmospheric N_2 to ammonia as well as utilize sunlight as a source of energy to produce hydrogen**.** Some genetic engineered microalgae strains have been developed to enhance their capability of hydrogen production (Baebprasert et al. 2011; Nyberg et al. 2015).

Bioethanol

Bioethanol was produced on large scale through microbial fermentation process, using enzymatically hydrolyzed starch and batch fermentation with yeast *Saccharomyces cerevisiae* to create ethanol (Antoni et al. 2007). *Zymomonas mobilis* is considered as a workhorse for bacterial fermentation of ethanol and source of enzymes for metabolic engineering of other bacteria toward ethanol production (Weuster-Botz et al. 1993). Worldwide, industrial processes of ethanol production used *Streptococcus fragilis* as in Dansk Gaerings industry process and *Kluyveromyces fragilis* in Milbrew process, whereas *K. fragilis* is used in most commercial plants. At the industrial scale, bioethanol was also produced by the fermentation of syngas (a CO/H_2 mixture from gasified biomass) by using *C. ljungdahlii* (Antoni et al. 2007).

Biodiesel

Biodiesel is a monoalkyl ester of fatty acids derived from vegetable oil. Instead of using vegetable oil, microalgae could be grown in photobioreactors for the production of suitable oil. The possible future fuel completely obtained by bacteria is known as microdiesel. Metabolically engineered *E. coli* contain pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase (*adhB*) genes from *Zymomonas mobilis* for the ethanol production. The gene *atfA* for an unspecific acyltransferase from *Acinetobacter baylyi* was introduced to esterify ethanol with the acyl moieties of CoA thioesters of fatty acids (Antoni et al. 2007; Kalscheuer et al. 2006).

n-Butanol and Acetone

Acetone was produced by wood since the World War I; supply of wood became insufficient at the start of war because acetone demand increased in line with the manufacture of cordite, a cartridge and shell propellant in which acetone was an essential ingredient. An alternative method was developed by Russian chemist C. Weizmann. Later Israeli President, Abe did fermentation process for acetone production, using bacterium *C. acetobutylicum* (Jones and Woods 1986). In this process, acetone is produced together with butanol and ethanol (ABE 3:6:1) (Antoni et al. 2007). Today, the emerging uses of butanol are replacement of diesel and kerosene, as silage preserver, biocide and C4 compound for chemical industry (Schwarz et al. 2006). The bacterial butanol fermentation was carried by BP and DuPont to finance development to introduce the butanol metabolic pathway from one of the solvent producing clostridia to another bacterium which is more tolerant of the products. However, bio-butanol production again started recently after decades of inactivity (Antoni et al. 2007).

2.5.9 Bioelectricity

Microbial fuel cells (MFCs) are bioelectrochemical systems that use bacterial metabolism to generate electrical current from a variety of organic substrates (ElMekawy et al. 2013). The main advantage of this technology is the use of microbes as a catalyst to convert organic materials directly into electricity; thus it produces bioenergy even from wastes. If oxygen is available (i.e., aerobic respiration), they are used as the terminal electron acceptor, because oxygen provides the greatest Gibbs free energy difference and leads to generate the highest level of energy, and microbes usually maximize their energy gain by selecting electron acceptor with the highest potential. In anaerobic condition, different electron acceptors including nitrate, nitrite, ferric iron, sulfate, carbon dioxide, and small organic molecules are used (Szöllősi et al. 2015).

Classic MFCs are designed with cathode and anode chambers separated by a proton exchange membrane (PEM). The organic substrates are oxidized at anode by biocatalyzed reactions to release electrons. The released electrons are transferred to the cathode, to generate current, via an external electrical circuit that connects both electrodes. Suitable electrocatalysts (including some of biological origin) complete the reduction reactions at cathode, where the protons are transported via PEM and combined with electrons and oxygen to form water. The driving force of a typical MFC using glucose as fuel can be articulated at anode and cathode, respectively, as follows (Grzebyk and Poźniak 2005):

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O$$

Interaction between microbes and electrodes can happen via DET or MET. In DET, microbial enzymes are set according to the electronic states in surface material and enzyme active center overlap, to increasing the chances of electron transfer across the interface (Dominguez-Benetton et al. 2012). Several microbes, including iron-reducing bacteria (*Geobacter sulfurreducens*, *Geobacter metallireducens*, *Geobacter toluenoxydans (*Caccavo et al. 1994*)*, *Rhodoferax ferrireducens* (Chaudhuri and Lovley 2003), *Shewanella putrefaciens* (Kim et al. 2002), *Shewanella japonica*, *Shewanella* algae (Ivanova et al. 2001)), have been reported for use in MFCs to transfer electrons out of the cell membrane (Luu and Ramsay 2003).

2.5.10 Bioleaching Microbes

Microbial ecology impacts mineral industry by providing microbes with the wide range of physicochemical conditions under which bioleaching is economically viable and competitive. Ecological studies reveal that *L. ferrooxidans* and *T. ferrooxidans* have been obtained from laboratory- and commercial-scale mineral processing leachates (De Wulf-Durand et al. 1997). Based on the nature of interaction occurring between acidophiles, it can be possible to design microbial consortia for specific purposes. For example, a mixed mesophilic consortium composed of iron-oxidising chemolithotroph e.g. *L. ferrooxidans*., to effect primary mineral oxidation, a sulphur-oxidiser e.g. *T. caldus*., to eliminate RSCs and generate acidity, and a heterotrophic acidophile to remove problematic organic materials, constitute a highly efficient and powerful mesophilic bioleaching system (Clark and Norris 1996). It is possible that cold-adapted bacteria used for leaching of sulphidic ores at low temperature, e.g. for heap leaching and in situ biomining in the high latitudes. Although some early studies casted doubt on the existence of psychrophilic iron-oxidisers, a recent report (Langdahl and Ingvorsen 1997) has described microorganisms which have optimum temperature for sulphide ore dissolution of 21 °C, and that are capable of leaching minerals at 0 °C. At lowering pH, effective mineral bioleaching is a possibility. Mesophilic iron-oxidizing bacteria that have been isolated have an optimum pH of around 1.5 and grow at pH 1.0 with 7 h culture doubling time (Johnson 1999). This study considered microbial diversity in uranium mine waste heaps in Romania, found that several moderately acidophilic, sulphur-oxidising thiobacilli occurred in samples, suggested that suitable substrates for these bacteria Streamwater Conveyance Structures (RSCs). Later, laboratory studies of pyrite oxidation using *T. ferrooxidans*, *L. ferrooxidans*, and an abiotic control ferric iron proved conclusively that thiosulfate is the first sulfur product formed during pyrite oxidation, but, due to its chemical instability in acidic liquors containing ferric iron, this RSC is rapidly oxidized to tetrathionate, pentathionate, and sulfate. Both tetrathionate and pentathionate are stable in sterile, acidic, ferric iron-containing solutions, but in the presence of pyrite, both of these RSCs were degraded (Schippers et al. 1995).

2.5.11 Microbial Therapeutic

Microbes are living in and on the human body. Thus, human health depends on the health of the microbial ecosystem. The microbial world is not one of good versus evil, but rather one of community; thus optimization includes not only minimizing pathogens, but also enhancing beneficial organisms. Options for optimization include judicious antibiotic use, administration of supplements such as prebiotics or probiotics, and transfaunation procedures such as fecal microbial transplant or microbial ecosystem therapeutics (Grady et al. 2016).

Prebiotics

Nondigestible food ingredients are known as prebiotics that selectively invigorate the growth and activity of anaerobic/microaerophilic flora in the mammal's colon. The commonly occurring prebiotics are oligosaccharides, prevalent in human milk in the form of milk proteins lactoferrin and lactalbumin that promote the specific growth of bifidobacteria and are described as "bifidogenic factors" (Patel and Denning 2013). Benefits of using prebiotics include ease of oral administration and support of the selective growth of beneficial bacteria that are endogenous to the gastrointestinal tract rather than the introduction of new bacterial species (Indrio et al. 2009). Prebiotics are a native constituent of human milk and are now being added to many commercially available infant formulas. Although prebiotic therapy has not been associated with any serious adverse effects in previous studies, it has been associated with some gastrointestinal side effects, such as bloating, flatulence, and diarrhea, which all stopped with the termination of treatment [34].

Probiotics

The probiotic supplements normally contain one or few bacterial species that directly alter the host microbiome, to fulfill the missing bacterial species and confer a health benefit. In probiotic therapy, lactobacilli and bifidobacteria are used bacterial. Several explanations of the mechanism of probiotic effect on the neonatal microbiome and disease susceptibility include competitive exclusion of pathogenic bacteria, improvement of epithelial barrier function within the intestines, bacteriocin secretion, and direct anti-inflammatory effects on epithelial signaling pathways (Panigrahi 2014; Patel and Denning 2013). It has been suggested that probiotics can improve the growth and enteral feeding in preterm infants, as well as other feeding-related symptoms such colic and reflux (Sung et al. 2014). Probiotics may be helpful to prevent necrotizing enterocolitis (NEC) in a multifactorial manner through intestinal maturation and normalization of gastrointestinal colonization (Patel and Denning 2013). Probiotics are regulated as a dietary supplement in many countries including the USA rather than as a pharmaceutical drug or biological agent (Ohishi et al. 2010).

Ecotherapeutics

In this therapy, it utilizes whole bacterial community directly derived from the human gastrointestinal tract to maintain or restore human health (Petrof et al. 2012). These days, two kinds of ecotherapeutics are used to alter the intestinal microbiome on a larger scale, including FMT and MET. In FMT, the fecal samples have been directly taken from the gastrointestinal tract of healthy donors, transplanted into the patient. It is most widely used for the treatment of antibiotic refractory or recurrent *Clostridium difficile* infection (CDI) (Petrof and Khoruts 2014) with mean cure rate of 87–90% (Kelly et al. 2015). FMT is also being used for the treatment of other conditions related to dysbiosis, including metabolic syndrome, obesity, food allergies, inflammatory bowel disease (IBD), and irritable bowel syndrome (IBS) (Kelly et al. 2015). In MET, selected bacteria from the donor fecal sample are isolated and maintained in culture, producing a stable and well-defined ecosystem for transplantation to donor (Petrof and Khoruts 2014), to reduce the risk of unknown infectious agents being transmitted to the recipients. Two studies have been employed of MET for the treatment of CDI, developing communities of 10 or 33 bacterial strains (Petrof et al. 2013).

2.6 Molecular Tools to Understand Microbial Ecosystem

To explore the complexity of microbial life in the environment have recognized many methods, many of them combined with high-throughput DNA sequencing, has allowed this diversity to be disclose in ever-greater detail. Although a strong science push comes from the explosion of new molecular tools, existing tools remain inadequate for elaborating the complexity of microbial ecosystems and supporting the design and operation of innovative processes in microbial ecology (Rittmann 2006). In this topic, we discuss about new molecular tools used for the deep study of microbial ecosystem.

2.6.1 Quantitative PCR (qPCR)

Quantitative PCR is based on the measurement of DNA/RNA concentration, number of copies of specific gene, or genetic region in a sample, relative to a known set of standard DNA/RNA concentrations, and this is accomplished through real-time assessments of the amount of DNA/RNA during a PCR reaction. DNA/RNA replication can be measured by the incorporation of fluorescent nucleic acid stain, such as SYBR Green or a fluorescently labeled probe, during the PCR reaction (Emerson et al. 2017). This technique is also applied for the number of studies of air and the built environment for measuring microbial concentrations (Emerson et al. 2015). Although, universal 16S rRNA gene primers are used for the detection of bacteria from environmental sample (Checinska et al. 2015). Some limitations of qPCR techniques including, the probe-based qPCR is likely to be problematic for study of complex community studies because probe-based techniques rely on sequence conservation. Therefore, nonspecific DNA-intercalating dyes are likely to be a more appropriate choice for qPCR of microbial communities (Emerson et al. 2015).

2.6.2 DNA Microarrays

Existing tools are inadequate for elaborating the complexity of microbial ecosystems. New molecular tools such as DNA microarrays have higher throughput, quantitative, and focus better on revealing structure and function in parallel. A DNA microarray is a rapid and versatile tool for microbial ecologists to characterize rapidly many samples for the presence and (with certain limitations) the abundance of many target organisms (Wagner et al. 2007).

Today, DNA microarrays are mainly applied in microbial ecology to infer the microbial community compositions or of defined guilds. Additionally, it will become necessary for the highly parallel analyses of in situ function of microbes and their interactions with each other. It is expected that in the near future, DNA microarrays coupled with other recently developed techniques, such as composition of light and heavy DNA or RNA fractions obtained from stable isotope probing experiments, will make more rapid, versatile, and efficient tools for deep structural and functional analysis of microbial communities with defined ecophysiology (Manefield et al. 2002; Radajewski et al. 2000). Today, several microarray-based techniques have been developed (Hazen et al. 2013). The PhyloChip and GeoChip are two most commonly used microarray technologies. PhyloChip is a 16S rRNA-based microarray, able to probe the diversity of 10,993 subfamilies in 147 phyla, while GeoChip is a functional gene-based microarray, able to probe the diversity of 152,414 genes from 410 gene categories (Hazen et al. 2010).

2.6.3 Metagenomics

Metagenomic approaches are applied in two forms—targeted metagenomics and shotgun metagenomics. In targeted metagenomics—also known as microbiomics—the diversity of a single gene is probed to identify the full complement of sequences of a specific gene in the environment. This approach is most often applied for the investigation of both the phylogenetic diversity and relative abundance of a particular gene in the sample as well as investigate the impact of environmental contaminants in altering microbial community structure (Techtmann and Hazen 2016). In this approach, small subunit rRNA sequences (16S/18S rRNA) are regularly used to investigate the diversity in a sample (Techtmann and Hazen 2016). In shotgun metagenomics approach, the total genomic complement of an environmental community is probed through genomic sequencing. In this approach, the extracted environmental DNA is fragmented to prepare the sequencing library to determine the total genomic content in the sample. This is a powerful technique to identify the microbial community, while often most limited by the depth of sequencing (Delmont et al. 2012).

2.6.4 Metatranscriptomics

Metatranscriptomics approach has been applied for identification of the actively expressed genes in a microbial community and are thus indicators for the microbial functions being expressed under the conditions at the time of sampling. In this approach, extracted environmental RNA is converted into cDNA and then sequenced in a similar fashion to metagenomics. This approach provides an inventory of the actively expressed genes in a sample (Techtmann and Hazen 2016).

2.6.5 Metaproteomics

Compared to metatranscriptomics, metaproteomics does not involve nucleic acid sequencing, but rather high-resolution mass spectrometry coupled with enzymatic digests of proteins and liquid chromatography. This approach provides the complement of proteins found in an environmental sample, including posttranslational modifications in proteins and their activity (Techtmann and Hazen 2016).

2.7 Conclusion

From a fundamental perspective, this chapter summarizes the current knowledge of the structural and functional ecology for the human welfare and betterment of environment in terms of its microbial ecology. Topics covered in this chapter include historical prospective, evolution of microbes in molecular basis, microbial community diversity, microbial community for environmental betterment, as well as tools for identification and analysis of complexity of microbial community.

The microbial evolution makes the considerable contribution to the instant creation and spread of biological innovation in many lineages that otherwise would have taken millions of years to proceed. Microbial evolution provides the major tools to reconstruct the relationships, population genetics, comparative genomics and genome analyses.

It has been suggested that microbial diversity plays a vital role in the long-term stability of an ecosystem. It has long been recognized that microbial communities are main player in intrinsic role in residue decomposition and nutrient cycling. Several factors have been suggested to affect the microbial communities in an ecosystem. The presence of litter and deadwood, considered as a primary source of C for the microbes in forest soil, fungi, and bacteria, actively participates in its transformation (Štursová et al. 2012) to maintain the nutrient cycle in ecosystem. Moreover, differences in the chemical composition of litter and deadwood among tree species influence the diversity of bacterial communities (Augusto et al. 2002).

The roots and rhizospheres of trees as well as ground vegetation associate with fungi to form mycorrhiza. The large absorptive area and exudation of labile compounds by mycorrhizal hyphae make important niches for different bacterial communities (Vik et al. 2013). Because of higher availability of C in the rhizosphere, the microbial abundance and activity are higher (Brzostek et al. 2013; Drake et al. 2013). The CO_2 concentrations and N availability are important factors for the diversity of microbial community. Increasing CO_2 level is an indicator to boost in NPP (Norby et al. 2005) and offset by C sequestration in ecosystems is strongly depend on the adaptation of both trees and microorganisms to the altered environmental conditions. A good example of this response is the decrease in abundance of oligotrophic *Acidobacteria* in various soils following an increase in CO_2 level (Dunbar et al. 2014; Fransson et al. 2016). Diversity of bacterial communities is highly sensitive to elevation of N level itself (Fierer et al. 2011; Frey et al. 2014). The N deposition significantly affected the relative abundances of core bacterial phyla, but it reduced bacterial diversity and altered the bacterial composition at lower taxonomic levels (Freedman and Zak 2015; Frey et al. 2014). Soil pH is an important factor affecting the diversity of microbial communities. Decreasing soil pH, means decrease in the carbon availability to the microbial communities (Bååth et al. 1995), and to slower the bacterial growth rate (Bååth 1998). Moreover, acidifying environment favors Gram-positive bacteria, while higher pH favors Gram-negative bacteria (Pennanen 2001). Global warming is long-term scenario, associated with CO2 concentration and increase in temperature that affects bacterial diversity in the magnitude of temperature change, specially the bacterial communities associated with C cycling processes (Wu et al. 2015).

Microbes are important components of the earth's ecosystem and are the main players for controlling the composition of the ecosystem. Its interaction with environment is beneficial as well as good for human welfare. Microbial communities are involved in the removal of environmental pollutions and hydrocarbon contaminants, like viscous oil contaminants from soil by bioremediation technique. It is also involved in the elimination of agricultural waste such as pesticides and toxic heavy metals from the soil. In contrary of participation of microbes in removal of pollutant from water, including elimination of nitrate from polluted groundwater by including MFC technology, elimination of mine-associated pollutant by long-term bioremediation and waste water treatment plant. Air pollution treatment by microbes is associated with in situ bioremediation of atmospheric hydrocarbon pollutants. Increasingly stringent environmental legislation is generating interest in industry for the effectiveness of biological waste air treatment techniques. Biofilter bed particles are small, interfacial equilibrium is achieved so that gas-phase resistance can be neglected and biodegradation of the pollutants occurs as a result of growing or resting microorganisms.

Microbes are also involved in maintaining the integrity and stability of the ecosystem by renewal and recycling of nutrients through biogeochemical cycle, including C, N, and P cycle (Lladó et al. 2017). Microbial communities are indirectly associated with the flux of C in an ecosystem by the transformation of dead plant biomass. Up to 40% of newly photosynthesized C can be allocated to the soil via roots, where it is rapidly respired or incorporated into the microbial biomass (HoÈgberg et al. 2001; Kuzyakov and Cheng 2001). Nitrogen cycle is dominated by bacteria, responsible for more than 95% of N input in the ecosystem (Berthrong et al. 2014). Gene responsible for N fixation, namely, *nifH,* is ubiquitously present in symbiotic as well as free-living N-fixing bacteria. Moreover, bacteria are key player of P cycle by the uptake of P by bacteria through solubilization and also uptake of inorganic P, which is most abundant source.

Microbial interaction is response by the gene expression toward the environmental stimulus, which is responsible for the production of molecules involved in these interactions. These molecules are secondary metabolites and bioactive compounds. These compounds have wide range of economic values, extensively use in medicine, cosmetics, chemical industry, food, agriculture and much other human welfare. Moreover, amino sugar, oligosugar, and polysugars are important for both medicinal and industrial applications. The metabolically engineered microbial strains are used to deliver production strategies for these valuable sugars, which were previously difficult to manufacture by other means, in necessary amounts to support their applications. In this industrial period, with the rising crude oil prices, depletion of resources, environmental challenges, and political instability in producing countries, besides efficiency and intelligent use, only biofuel has the potential to replace the supply of this energy-hungry civilization. Biofuel produced through microbial technology is a safe and eco-friendly approach to fill the energy requirements. Today, bio-hydrogen, bioethanol, and biodiesel are successfully produced through the microbial technology. Microbes also produce bioelectricity through the MFCs, a bioelectrochemical system that uses bacterial metabolism to generate electrical current from a variety of organic substrates. More recently, microbial ecosystem therapeutics is a new approach for the treatment of several diseases in human, in the form of administration of supplements such as prebiotics or probiotics and transfaunation procedures (Grady et al. 2016).

Because of complexity in structure and function of microbial ecosystem, a classical method was inadequate. The culture-based techniques have some limitations such as, in many cases, the impetus, facility, and time necessary for appropriate cultivation technique, which may not be available and takes longer time for detection for the live cells. Membrane integrity-based method also has limitations, the result in overestimation of a "snapshot" of viable cells because lethal stress may not lead to immediate cell membrane disintegration, and the dyes typically employed to assess membrane integrity may be ineffective against cells with a hardy membrane or cell wall, such as spores (Emerson et al. 2017). In quantitative PCR-based technique, universal 16S rRNA gene primers are used for detecting bacteria from environmental samples but with some disadvantages; it is problematic for the study of complex community studies because probe-based techniques rely on sequence conservation. In contrary to traditional methods, new developed molecular techniques have high throughput, quantitative, convenient and focus on better reveal of microbial ecosystem complexity. The most advanced technologies in this quest are DNA microarrays, single-cell genomics, and NGS. DNA microarrays approach provides a fast and high-throughput parallel detection of microbes from environmental samples. The development of new-generation sequencing technologies challenged the use of DNA microarrays in microbial community studies. It showed that NGS technology is the most promising approach in low to medium ecosystems complexity, while in highly complex ecosystems, it suffers with random sampling, under sampling, and rRNA interference. DNA microarrays techniques such as PhyloChip and GeoChip are perfect tools for the analysis of highly complex ecosystem (Nikolaki and Tsiamis 2013). Metagenomics is good to study the microbial community in terms of taxonomic diversity and metabolic potential. It provides the way to discover new genes and proteins or even the complete genomes of non-cultivable organisms in less time with better accuracy than classical microbiology or other molecular methods (Escobar-Zepeda et al. 2015).

Acknowledgments I am thankful to the international college of Southwest University and school of life science of Southwest University, China. I am also thankful to my friends and professors of Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi, India.

References

Abhilash PC, Srivastava S, Singh N (2011) Comparative bioremediation potential of four rhizospheric microbial species against lindane. Chemosphere 82:56–63

Ai C, Liang G, Sun J, Wang X, He P, Zhou W, He X (2015) Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. Soil Biol Biochem 80:70–78

Akagi Y, Akamatsu H, Otani H, Kodama M (2009) Horizontal chromosome transfer, a mechanism for the evolution and differentiation of a plant-pathogenic fungus. Eukaryot Cell 8:1732–1738

Alisi C, Musella R, Tasso F, Ubaldi C, Manzo S, Cremisini C, Sprocati AR (2009) Bioremediation of diesel oil in a co-contaminated soil by bioaugmentation with a microbial formula tailored with native strains selected for heavy metals resistance. Sci Total Environ 407:3024–3032

Anderson T-H, Joergensen RG (1997) Relationship between SIR and FE estimates of microbial biomass C in deciduous forest soils at different pH. Soil Biol Biochem 29:1033–1042

Antoni D, Zverlov VV, Schwarz WH (2007) Biofuels from microbes. Appl Microbiol Biotechnol 77:23–35

Araújo WL, Marcon J, Maccheroni W, van Elsas JD, van Vuurde JWL, Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with Xylella fastidiosa in citrus plants. Appl Environ Microbiol 68:4906–4914

Augusto L, Ranger J, Binkley D, Rothe A (2002) Impact of several common tree species of European temperate forests on soil fertility. Ann For Sci 59:233–253

Austin AT, Vivanco L, González-Arzac A, Pérez LI (2014) There's no place like home? An exploration of the mechanisms behind plant litter–decomposer affinity in terrestrial ecosystems. New Phytol 204:307–314

Bååth E (1998) Growth rates of bacterial communities in soils at varying pH: a comparison of the thymidine and leucine incorporation techniques. Microb Ecol 36:316–327

Baath E, Berg B, Lohm U, Lundgren B, Lundkvist H, Thomas R, Soderstrom B, Wirén A (1980) Effects of experimental acidification and liming on soil organisms and decomposition in a Scots pine forest. Pedobiologia 20:85–100

Bååth E, Frostegård Å, Pennanen T, Fritze H (1995) Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biol Biochem 27:229–240

Baebprasert W, Jantaro S, Khetkorn W, Lindblad P, Incharoensakdi A (2011) Increased H2 production in the cyanobacterium Synechocystis sp. strain PCC 6803 by redirecting the electron supply via genetic engineering of the nitrate assimilation pathway. Metab Eng 13:610–616

Ballyk MM, Jones DA, Smith HL (2001) Microbial competition in reactors with Wall attachment. Microb Ecol 41

Bastin G, Dochain D (1991) On-line estimation and adaptive control of bioreactors. Elsevier, Amsterdam, 1990 (ISBN 0–444–88430-0). xiv+ 379 pp. Price US $146.25/Dfl. 285.00. Analytica Chimica Acta 243: 324

Bending GD, Lincoln SD, Sørensen SR, Alun W Morgan J, Aamand J, Walker A (2003) In-field spatial variability in the degradation of the phenyl-urea herbicide isoproturon is the result of interactions between degradative Sphingomonas spp. and soil pH. Appl Environ Microbiol 69:827–834

Bergkemper F, Kublik S, Lang F, Krüger J, Vestergaard G, Schloter M, Schulz S (2016a) Novel oligonucleotide primers reveal a high diversity of microbes which drive phosphorous turnover in soil. J Microbiol Methods 125:91–97

Bergkemper F, Schöler A, Engel M, Lang F, Krüger J, Schloter M, Schulz S (2016b) Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. Environ Microbiol 18:1988–2000

Berlemont R, Martiny AC (2013) Phylogenetic distribution of potential cellulases in bacteria. Appl Environ Microbiol 79:1545–1554

Berlemont R, Martiny AC (2015) Genomic potential for polysaccharide deconstruction in bacteria. Appl Environ Microbiol 81:1513–1519

Berthrong ST, Yeager CM, Gallegos-Graves L, Steven B, Eichorst SA, Jackson RB, Kuske CR (2014) Nitrogen fertilization has a stronger effect on soil nitrogen-fixing bacterial communities than elevated atmospheric CO2. Appl Environ Microbiol 80:3103–3112

Bhuiyan AB, Mokhtar MB, Toriman ME, Gasim MB, Ta GC, Elfithri R, Razman MR (2013) The environmental risk and water pollution: a review from the river basins around the world. Am Eurasian J Sustain Agric 7:126–136

Blackall LL, Crocetti GR, Saunders AM, Bond PL (2002) A review and update of the microbiology of enhanced biological phosphorus removal in wastewater treatment plants. Antonie Van Leeuwenhoek 81:681–691

Brabcová V, Nováková M, Davidová A, Baldrian P (2016) Dead fungal mycelium in forest soil represents a decomposition hotspot and a habitat for a specific microbial community. New Phytol 210:1369–1381

Bradley AS, Pearson A, Sáenz JP, Marx CJ (2010) Adenosylhopane: the first intermediate in hopanoid side chain biosynthesis. Org Geochem 41:1075–1081

Braga RM, Dourado MN, Araújo WL (2016) Microbial interactions: ecology in a molecular perspective. Braz J Microbiol 47:86–98

Brzostek ER, Greco A, Drake JE, Finzi AC (2013) Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. Biogeochemistry 115:65–76. https://doi.org/10.1007/s10533-012-9818-9

Caccavo F, Lonergan DJ, Lovley DR, Davis M, Stolz JF, McInerney MJ (1994) Geobacter sulfurreducens sp. nov., a hydrogen-and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl Environ Microbiol 60:3752–3759

Carpinelli J, Krämer R, Agosin E (2006) Metabolic engineering of Corynebacterium glutamicum for trehalose overproduction: role of the TreYZ trehalose biosynthetic pathway. Appl Environ Microbiol 72:1949–1955

Castelle CJ, Hug LA, Wrighton KC, Thomas BC, Williams KH, Wu D, Tringe SG, Singer SW, Eisen JA, Banfield JF (2013) Extraordinary phylogenetic diversity and metabolic versatility in aquifer sediment. Nat Commun 4

Chamoun R, Aliferis KA, Jabaji S (2015) Identification of signatory secondary metabolites during mycoparasitism of Rhizoctonia solani by Stachybotrys elegans. Front Microbiol 6:353

Chaudhuri SK, Lovley DR (2003) Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat Biotechnol 21:1229

Checinska A, Probst AJ, Vaishampayan P, White JR, Kumar D, Stepanov VG, Fox GE, Nilsson HR, Pierson DL, Perry J (2015) Microbiomes of the dust particles collected from the international Space Station and Spacecraft Assembly Facilities. Microbiome 3:50

Chen J, Yang Q, Huang T, Zhang Y, Ding R (2011) Enhanced bioremediation of soil contaminated with viscous oil through microbial consortium construction and ultraviolet mutation. World J Microbiol Biotechnol 27:1381–1389

Chiu S-Y, Kao C-Y, Huang T-T, Lin C-J, Ong S-C, Chen C-D, Chang J-S, Lin C-S (2011) Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using Chlorella sp. cultures. Bioresour Technol 102:9135–9142

Clark DA, Norris PR (1996) Acidimicrobium ferrooxidans gen. nov., sp. nov.: mixed-culture ferrous iron oxidation with Sulfobacillus species. Microbiology 142:785–790. https://doi.org/10.1099/00221287-142-4-785

Crocetti GR, Hugenholtz P, Bond PL, Schuler A, Keller J, Jenkins D, Blackall LL (2000) Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. Appl Environ Microbiol 66:1175–1182

Crocetti GR, Banfield JF, Keller J, Bond PL, Blackall LL (2002) Glycogen-accumulating organisms in laboratory-scale and full-scale wastewater treatment processesb. Microbiology 148:3353–3364

Daims H, Nielsen JL, Nielsen PH, Schleifer K-H, Wagner M (2001) In situ characterization of nitrospira-like nitrite-oxidizing bacteria active in wastewater treatment plants. Appl Environ Microbiol 67:5273–5284

Daims H, Taylor MW, Wagner M (2006) Wastewater treatment: a model system for microbial ecology. Trends Biotechnol 24:483–489

de Boer W, Folman LB, Summerbell RC, Boddy L (2005) Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol Rev 29:795–811

De Wulf-Durand P, Bryant LJ, Sly LI (1997) PCR-mediated detection of acidophilic, bioleaching-associated bacteria. Appl Environ Microbiol 63:2944–2948

Dechesne A, Badawi N, Aamand J, Smets BF (2014) Fine scale spatial variability of microbial pesticide degradation in soil: scales, controlling factors, and implications. Front Microbiol 5:667

del Carmen Vargas-García M, López MJ, Suárez-Estrella F, Moreno J (2012) Compost as a source of microbial isolates for the bioremediation of heavy metals: in vitro selection. Sci Total Environ 431:62–67

Delmont TO, Simonet P, Vogel TM (2012) Describing microbial communities and performing global comparisons in the 'omic era. ISME J 6:1625

Deng M-D, Severson DK, Grund AD, Wassink SL, Burlingame RP, Berry A, Running JA, Kunesh CA, Song L, Jerrell TA (2005) Metabolic engineering of Escherichia coli for industrial production of glucosamine and N-acetylglucosamine. Metab Eng 7:201–214

Deshusses MA (1997) Biological waste air treatment in biofilters. Curr Opin Biotechnol 8:335–339. doi: S0958-1669(97)80013-4 [pii]

Dominguez-Benetton X, Sevda S, Vanbroekhoven K, Pant D (2012) The accurate use of impedance analysis for the study of microbial electrochemical systems. Chem Soc Rev 41:7228–7246

Donn S, Kirkegaard JA, Perera G, Richardson AE, Watt M (2015) Evolution of bacterial communities in the wheat crop rhizosphere. Environ Microbiol 17:610–621

Dostrovsky NR, Towheed TE, Hudson RW, Anastassiades TP (2011) The effect of glucosamine on glucose metabolism in humans: a systematic review of the literature. Osteoarthr Cartil 19:375–380. https://doi.org/10.1016/j.joca.2011.01.007

Douskova I, Doucha J, Livansky K, Machat J, Novak P, Umysova D, Zachleder V, Vitova M (2009) Simultaneous flue gas bioremediation and reduction of microalgal biomass production costs. Appl Microbiol Biotechnol 82:179–185

Drake JE, Darby BA, Giasson M-A, Kramer MA, Phillips RP, Finzi AC (2013) Stoichiometry constrains microbial response to root exudation-insights from a model and a field experiment in a temperate forest. Biogeosciences 10:821

Dunbar J, Steven B, Mueller R, Hesse C, Zak DR, Kuske CR (2014) Surface soil fungal and bacterial communities in aspen stands are resilient to eleven years of elevated CO2 and O3. Soil Biol Biochem 76:227–234

Egli K, Fanger U, Alvarez PJJ, Siegrist H, van der Meer JR, Zehnder AJB (2001) Enrichment and characterization of an anammox bacterium from a rotating biological contactor treating ammonium-rich leachate. Arch Microbiol 175:198–207

Eichorst SA, Kuske CR (2012) Identification of cellulose-responsive bacterial and fungal communities in geographically and edaphically different soils by using stable isotope probing. Appl Environ Microbiol 78:2316–2327

Eikelboom DH (1975) Filamentous organisms observed in activated sludge. Water Res 9:365–388

Ekundayo EO (2003) Effect of common pesticides used in the Niger delta basin of southern Nigeria on soil microbial populations. Environ Monit Assess 89:35–41

ElMekawy A, Hegab HM, Dominguez-Benetton X, Pant D (2013) Internal resistance of microfluidic microbial fuel cell: challenges and potential opportunities. Bioresour Technol 142:672–682. https://doi.org/10.1016/j.biortech.2013.05.061

Emerson JB, Keady PB, Brewer TE, Clements N, Morgan EE, Awerbuch J, Miller SL, Fierer N (2015) Impacts of flood damage on airborne bacteria and fungi in homes after the 2013 Colorado front range flood. Environ Sci Technol 49:2675–2684

Emerson JB, Adams RI, Román CMB, Brooks B, Coil DA, Dahlhausen K, Ganz HH, Hartmann EM, Hsu T, Justice NB (2017) Schrödinger's microbes: tools for distinguishing the living from the dead in microbial ecosystems. Microbiome 5:86

Endo T, Koizumi S, Tabata K, Ozaki A (2000) Large-scale production of CMP-NeuAc and sialylated oligosaccharides through bacterial coupling. Appl Microbiol Biotechnol 53:257–261. https://doi.org/10.1007/s002530050017

Eroglu E, Melis A (2011) Photobiological hydrogen production: recent advances and state of the art. Bioresour Technol 102:8403–8413

Escobar-Zepeda A, Vera-Ponce de León A, Sanchez-Flores A (2015) The road to metagenomics: from microbiology to DNA sequencing technologies and bioinformatics. Front Genet 6:348

Esson KC, Lin X, Kumaresan D, Chanton JP, Colin Murrell J, Kostka JE (2016) Alpha-and gammaproteobacterial methanotrophs codominate the active methane-oxidizing communities in an acidic boreal peat bog. Appl Environ Microbiol 82:2363–2371

Faraldo-Gómez JD, Sansom MSP (2003) Acquisition of siderophores in gram-negative bacteria. Nat Rev Mol Cell Biol 4:105

Fernandez CW, Koide RT (2014) Initial melanin and nitrogen concentrations control the decomposition of ectomycorrhizal fungal litter. Soil Biol Biochem 77:150–157

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354–1364

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2011) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J 6:1007. https://doi.org/10.1038/ismej.2011.159

Fierer N, Leff JW, Adams BJ, Nielsen UN, Bates ST, Lauber CL, Owens S, Gilbert JA, Wall DH, Caporaso JG (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proc Natl Acad Sci U S A 109:21390–21395. https://doi.org/10.1073/pnas.12152101101215210110[pii]

Fisher CK, Mehta P (2014) The transition between the niche and neutral regimes in ecology. Proc Natl Acad Sci U S A 111:13111–13116. https://doi.org/10.1073/pnas.14056371111405637111[pii]

Fransson P, Andersson A, Norström S, Bylund D, Bent E (2016) Ectomycorrhizal exudates and pre-exposure to elevated CO2 affects soil bacterial growth and community structure. Fungal Ecol 20:211–224

Freedman ZB, Zak DR (2015) Atmospheric N deposition alters connectance, but not functional potential among saprotrophic bacterial communities. Mol Ecol 24:3170–3180

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. For Ecol Manag 196:159–171

Frey SD, Ollinger S, Nadelhoffer K, Bowden R, Brzostek E, Burton A, Caldwell BA, Crow S, Goodale CL, Grandy AS, Finzi A, Kramer MG, Lajtha K, LeMoine J, Martin M, McDowell WH, Minocha R, Sadowsky JJ, Templer PH, Wickings K (2014) Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry 121:305–316. https://doi.org/10.1007/s10533-014-0004-0

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320:889–892

Ghiglione JF, Galand PE, Pommier T, Pedros-Alio C, Maas EW, Bakker K, Bertilson S, Kirchmanj DL, Lovejoy C, Yager PL, Murray AE (2012) Pole-to-pole biogeography of surface and deep marine bacterial communities. Proc Natl Acad Sci U S A 109:17633–17638. https://doi.org/10.1073/pnas.12081601091208160109[pii]

Giaever HM, Styrvold OLAFB, Kaasen INGA, Strøm AR (1988) Biochemical and genetic characterization of osmoregulatory trehalose synthesis in Escherichia coli. J Bacteriol 170:2841–2849

Gibbons SM, Caporaso JG, Pirrung M, Field D, Knight R, Gilbert JA (2013) Evidence for a persistent microbial seed bank throughout the global ocean. Proc Natl Acad Sci U S A 110:4651–4655. https://doi.org/10.1073/pnas.12177671101217767110[pii]

Gilbert JA, Jansson JK, Knight R (2014) The earth microbiome project: successes and aspirations. BMC Biol 12:69

Giovannoni SJ, Britschgi TB, Moyer CL, Field KG (1990) Genetic diversity in Sargasso Sea bacterioplankton. Nature 345:60–63

Grady NG, Petrof EO, Claud EC (2016) Microbial therapeutic interventions. In: Microbial therapeutic interventions, Seminars in Fetal and Neonatal Medicine. Elsevier, pp 418–423

Greben HA, Baloyi J, Sigama J, Venter SN (2009) Bioremediation of sulphate rich mine effluents using grass cuttings and rumen fluid microorganisms. J Geochem Explor 100:163–168

Grzebyk M, Poźniak G (2005) Microbial fuel cells (MFCs) with interpolymer cation exchange membranes. Sep Purif Technol 41:321–328

Gschwendtner S, Engel M, Lueders T, Buegger F, Schloter M (2016) Nitrogen fertilization affects bacteria utilizing plant-derived carbon in the rhizosphere of beech seedlings. Plant Soil 407:203–215

Haeckel E (1866) Generelle Morphologie der Organismen allgemeine Grundzuge der organischen Formen-Wissenschaft, mechanisch begrundet durch die von Charles Darwin reformirte Descendenz-Theorie von Ernst Haeckel: Allgemeine Entwickelungsgeschichte der Organismen kritische Grundzuge der mechanischen Wissenschaft von den entstehenden Formen der Organismen, begrundet durch die Descendenz-Theorie: Verlag von Georg Reimer

Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N, Jansson JK, Probst A, Borglin SE, Fortney JL (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330:204–208

Hazen TC, Rocha AM, Techtmann SM (2013) Advances in monitoring environmental microbes. Curr Opin Biotechnol 24:526–533

Hesselmann RPX, Werlen C, Hahn D, van der Meer JR, Zehnder AJB (1999) Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge. Systematic and Applied Microbiology 22:454–465. https://doi.org/10.1016/S0723-2020(99)80055-1

HoÈgberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, HoÈgberg MN, Nyberg G, Ottosson-LoÈfvenius M, Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature 411:789

Högberg MN, Blaško R, Bach LH, Hasselquist NJ, Egnell G, Näsholm T, Högberg P (2014) The return of an experimentally N-saturated boreal forest to an N-limited state: observations on the soil microbial community structure, biotic N retention capacity and gross N mineralisation. Plant Soil 381:45–60. https://doi.org/10.1007/s11104-014-2091-z

Hoppe B, Krüger D, Kahl T, Arnstadt T, Buscot F, Bauhus J, Wubet T (2015) A pyrosequencing insight into sprawling bacterial diversity and community dynamics in decaying deadwood logs of Fagussylvatica and Piceaabies. Sci Rep 5:9456

Hossain GS, Shin H-d, Li J, Wang M, Du G, Chen J, Liu L (2016) Metabolic engineering for amino-, oligo-, and polysugar production in microbes. Appl Microbiol Biotechnol 100:2523–2533

Hug LA, Castelle CJ, Wrighton KC, Thomas BC, Sharon I, Frischkorn KR, Williams KH, Tringe SG, Banfield JF (2013) Community genomic analyses constrain the distribution of metabolic traits across the Chloroflexi phylum and indicate roles in sediment carbon cycling. Microbiome 1:22

Igarashi N, Harada J, Nagashima S, Matsuura K, Shimada K, Nagashima KVP (2001) Horizontal transfer of the photosynthesis gene cluster and operon rearrangement in purple bacteria. J Mol Evol 52:333–341

Indrio F, Riezzo G, Raimondi F, Bisceglia M, Cavallo L, Francavilla R (2009) Effects of probiotic and prebiotic on gastrointestinal motility in newborns. J Physiol Pharmacol 60:27–31

Isobe K, Koba K, Otsuka S, Senoo K (2011) Nitrification and nitrifying microbial communities in forest soils. J For Res 16:351

Ivanova EP, Sawabe T, Gorshkova NM, Svetashev VI, Mikhailov VV, Nicolau DV, Christen R (2001) Shewanella japonica sp. nov. Int J Syst Evol Microbiol 51:1027–1033

Izumi H, Cairney JWG, Killham K, Moore E, Alexander IJ, Anderson IC (2008) Bacteria associated with ectomycorrhizas of slash pine (Pinus elliottii) in South-Eastern Queensland, Australia. FEMS Microbiol Lett 282:196–204

Jessup CM, Kassen R, Forde SE, Kerr B, Buckling A, Rainey PB, Bohannan BJM (2004) Big questions, small worlds: microbial model systems in ecology. Trends Ecol Evol 19:189–197

Jia Y, Zhu J, Chen X, Tang D, Su D, Yao W, Gao X (2013) Metabolic engineering of Bacillus subtilis for the efficient biosynthesis of uniform hyaluronic acid with controlled molecular weights. Bioresour Technol 132:427–431

Johnson DB (1999) Importance of microbial ecology in the development of new mineral technologies. In: Amils R, Ballester A (eds) Importance of microbial ecology in the development of new mineral technologies, Process metallurgy. Elsevier, pp 645–656

Johnston SR, Boddy L, Weightman AJ (2016) Bacteria in decomposing wood and their interactions with wood-decay fungi. FEMS Microbiol Ecol 92

Johnstone TC, Nolan EM (2015) Beyond iron: non-classical biological functions of bacterial siderophores. Dalton Trans 44:6320–6339

Jones DT, Woods DR (1986) Acetone-butanol fermentation revisited. Microbiol Rev 50:484

Jung J, Yeom J, Han J, Kim J, Park W (2012) Seasonal changes in nitrogen-cycle gene abundances and in bacterial communities in acidic forest soils. J Microbiol 50:365–373

Kaiser C, Koranda M, Kitzler B, Fuchslueger L, Schnecker J, Schweiger P, Rasche F, Zechmeister-Boltenstern S, Sessitsch A, Richter A (2010) Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. New Phytol 187:843–858

Kalscheuer R, Stölting T, Steinbüchel A (2006) Microdiesel: Escherichia coli engineered for fuel production. Microbiology 152:2529–2536

Karamalidis AK, Evangelou AC, Karabika E, Koukkou AI, Drainas C, Voudrias EA (2010) Laboratory scale bioremediation of petroleum-contaminated soil by indigenous microorganisms and added Pseudomonas aeruginosa strain Spet. Bioresour Technol 101:6545–6552

Kasting JF, Siefert JL (2002) Life and the evolution of Earth9s atmosphere. Science 296:1066–1068

Kelly CR, Kahn S, Kashyap P, Laine L, Rubin D, Atreja A, Moore T, Wu G (2015) Update on fecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. Gastroenterology 149:223–237

Keymer JE, Galajda P, Muldoon C, Park S, Austin RH (2006) Bacterial metapopulations in nanofabricated landscapes. Proc Natl Acad Sci 103:17290–17295

Khetkorn W, Rastogi RP, Incharoensakdi A, Lindblad P, Madamwar D, Pandey A, Larroche C (2017) Microalgal hydrogen production – a review. Bioresour Technol 243:1194–1206. https://doi.org/10.1016/j.biortech.2017.07.085

Kielak AM, Scheublin TR, Mendes LW, Van Veen JA, Kuramae EE (2016) Bacterial community succession in pine-wood decomposition. Front Microbiol 7:231

Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzym Microb Technol 30:145–152

Kim M, Kim W-S, Tripathi BM, Adams J (2014) Distinct bacterial communities dominate tropical and temperate zone leaf litter. Microb Ecol 67:837–848

Kong Y, Nielsen JL, Nielsen PH (2004) Microautoradiographic study of Rhodocyclus-related polyphosphate-accumulating bacteria in full-scale enhanced biological phosphorus removal plants. Appl Environ Microbiol 70:5383–5390

Könneke M, Bernhard AE, José R, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543

Korkama T, Fritze H, Pakkanen A, Pennanen T (2007) Interactions between extraradical ectomycorrhizal mycelia, microbes associated with the mycelia and growth rate of Norway spruce (Picea abies) clones. New Phytol 173:798–807

Koutinas AA, Sypsas V, Kandylis P, Michelis A, Bekatorou A, Kourkoutas Y, Kordulis C, Lycourghiotis A, Banat IM, Nigam P (2012) Nano-tubular cellulose for bioprocess technology development. PLoS One 7:e34350

Kumar M, Lakshmi CV, Khanna S (2008) Microbial biodiversity and in situ bioremediation of endosulfan contaminated soil. Indian J Microbiol 48:128–133

Kuzyakov Y, Cheng W (2001) Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol Biochem 33:1915–1925

Lacava PT, Araújo WL, Marcon J, Maccheroni W, Azevedo JL (2004) Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria Xylella fastidiosa, causal agent of citrus-variegated chlorosis. Lett Appl Microbiol 39:55–59

Langdahl BR, Ingvorsen K (1997) Temperature characteristics of bacterial iron solubilisation and 14C assimilation in naturally exposed sulfide ore material at Citronen Fjord, North Greenland (83°N). FEMS Microbiol Ecol 23:275–283. https://doi.org/10.1016/S0168-6496(97)00032-9

Lau E, Dillard ZW, Dague RD, Semple AL, Wentzell WL (2015) High throughput sequencing to detect differences in methanotrophic Methylococcaceae and Methylocystaceae in surface peat, forest soil, and Sphagnum moss in Cranesville Swamp Preserve, West Virginia, USA. Microorganisms 3:113–136

Laverman AM, Speksnijder AGCL, Braster M, Kowalchuk GA, Verhoef HA, Van Verseveld HW (2001) Spatiotemporal stability of an ammonia-oxidizing community in a nitrogen-saturated forest soil. Microb Ecol 42:35–45

Levy-Booth DJ, Prescott CE, Grayston SJ (2014) Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. Soil Biol Biochem 75:11–25

Li Y, Tian C, Tian H, Zhang J, He X, Ping W, Lei H (2012) Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and sodium citrate involved. Appl Microbiol Biotechnol 96:1479–1487. https://doi.org/10.1007/s00253-012-4242-6

Liu L, Liu Y, Li J, Du G, Chen J (2011) Microbial production of hyaluronic acid: current state, challenges, and perspectives. Microb Cell Factories 10:99

Liu L, Liu Y, Shin H-d, Chen R, Li J, Du G, Chen J (2013) Microbial production of glucosamine and N-acetylglucosamine: advances and perspectives. Appl Microbiol Biotechnol 97:6149–6158

Liu Y, Zhu Y, Ma W, Shin H-d, Li J, Liu L, Du G, Chen J (2014) Spatial modulation of key pathway enzymes by DNA-guided scaffold system and respiration chain engineering for improved N-acetylglucosamine production by Bacillus subtilis. Metab Eng 24:61–69. https://doi.org/10.1016/j.ymben.2014.04.004

Lladó S, López-Mondéjar R, Baldrian P (2017) Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. Microbiol Mol Biol Rev 81:e00063–00016

Loeppmann S, Blagodatskaya E, Pausch J, Kuzyakov Y (2016) Substrate quality affects kinetics and catalytic efficiency of exo-enzymes in rhizosphere and detritusphere. Soil Biol Biochem 92:111–118

Long X, Chen C, Xu Z, Linder S, He J (2012a) Abundance and community structure of ammonia oxidizing bacteria and archaea in a Sweden boreal forest soil under 19-year fertilization and 12-year warming. J Soils Sediments 12:1124–1133

Long X, Chen C, Xu Z, Oren R, He J-Z (2012b) Abundance and community structure of ammonia-oxidizing bacteria and archaea in a temperate forest ecosystem under ten-years elevated CO2. Soil Biol Biochem 46:163–171

López-Mondéjar R, Zühlke D, Becher D, Riedel K, Baldrian P (2016a) Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. Sci Rep 6:25279

López-Mondéjar R, Zühlke D, Větrovský T, Becher D, Riedel K, Baldrian P (2016b) Decoding the complete arsenal for cellulose and hemicellulose deconstruction in the highly efficient cellulose decomposer Paenibacillus O199. Biotechnol Biofuels 9:104

Luo Y, Su BO, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren RAM, Parton WJ (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. AIBS Bull 54:731–739

Luu YS, Ramsay JA (2003) Review: Microbial mechanisms of accessing insoluble Fe (III) as an energy source. World J Microbiol Biotechnol 19:215–225

Ma Z-C, Liu N-N, Chi Z, Liu G-L, Chi Z-M (2015) Genetic modification of the marine-isolated yeast Aureobasidium melanogenum P16 for efficient pullulan production from inulin. Mar Biotechnol 17:511–522

Maaroufi NI, Nordin A, Hasselquist NJ, Bach LH, Palmqvist K, Gundale MJ (2015) Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils. Glob Chang Biol 21:3169–3180

Manefield M, Whiteley AS, Griffiths RI, Bailey MJ (2002) RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. Appl Environ Microbiol 68:5367–5373

Mao J, Luo Y, Teng Y, Li Z (2012) Bioremediation of polycyclic aromatic hydrocarbon-contaminated soil by a bacterial consortium and associated microbial community changes. Int Biodeterior Biodegradation 70:141–147

Mao Y, Li X, Smyth EM, Yannarell AC, Mackie RI (2014) Enrichment of specific bacterial and eukaryotic microbes in the rhizosphere of switchgrass (Panicum virgatum L.) through root exudates. Environ Microbiol Rep 6:293–306. https://doi.org/10.1111/1758-2229.12152

Markkola AM (1996) Resource allocation in ectomycorrhizal symbiosis in Scots pine affected by environmental changes. Oulun Yliopisto, Oulun

Martins AMP, Pagilla K, Heijnen JJ, van Loosdrecht MCM (2004) Filamentous bulking sludge—a critical review. Water Res 38:793–817

Marupakula S, Mahmood S, Finlay RD (2016) Analysis of single root tip microbiomes suggests that distinctive bacterial communities are selected by Pinus sylvestris roots colonized by different ectomycorrhizal fungi. Environ Microbiol 18:1470–1483

Matthies C, Erhard H-P, Drake HL (1997) Effects of pH on the comparative culturability of fungi and bacteria from acidic and less acidic forest soils. J Basic Microbiol 37:335–343

Mohite BV, Patil SV (2014) A novel biomaterial: bacterial cellulose and its new era applications. Biotechnol Appl Biochem 61:101–110

Morris JJ, Papoulis SE, Lenski RE (2014) Coexistence of evolving bacteria stabilized by a shared Black Queen function. Evolution 68:2960–2971. https://doi.org/10.1111/evo.12485

Nelson MB, Martiny AC, Martiny JBH (2016) Global biogeography of microbial nitrogen-cycling traits in soil. Proc Natl Acad Sci 113:8033–8040

Nielsen PH, Roslev P, Dueholm TE, Nielsen JL (2002) Microthrix parvicella, a specialized lipid consumer in anaerobic–aerobic activated sludge plants. Water Sci Technol 46:73–80

Nielsen PH, Kragelund C, Nielsen JL, Tiro S, Lebek M, Rosenwinkel K-H, Gessesse A (2005) Control of Microthrix parvicella in activated sludge plants by dosage of polyaluminium salts: possible mechanisms. CLEAN–Soil, Air, Water 33:255–261

Nikolaki S, Tsiamis G (2013) Microbial diversity in the era of omic technologies. Biomed Res Int 2013

Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R (2005) Forest response to elevated CO2 is conserved across a broad range of productivity. Proc Natl Acad Sci U S A 102:18052–18056

Nyberg M, Heidorn T, Lindblad P (2015) Hydrogen production by the engineered cyanobacterial strain Nostoc PCC 7120 ΔhupW examined in a flat panel photobioreactor system. J Biotechnol 215:35–43

Ohishi A, Takahashi S, Ito Y, Ohishi Y, Tsukamoto K, Nanba Y, Ito N, Kakiuchi S, Saitoh A, Morotomi M (2010) Bifidobacterium septicemia associated with postoperative probiotic therapy in a neonate with omphalocele. J Pediatr 156:679–681

Omelchenko MV, Makarova KS, Wolf YI, Rogozin IB, Koonin EV (2003) Evolution of mosaic operons by horizontal gene transfer and gene displacement in situ. Genome Biol 4:R55

Orandi S, Lewis DM, Moheimani NR (2012) Biofilm establishment and heavy metal removal capacity of an indigenous mining algal-microbial consortium in a photo-rotating biological contactor. J Ind Microbiol Biotechnol 39:1321–1331

Pajares S, Bohannan BJM (2016) Ecology of nitrogen fixing, nitrifying, and denitrifying microorganisms in tropical forest soils. Front Microbiol 7:1045

Panigrahi P (2014) Probiotics and prebiotics in neonatal necrotizing enterocolitis: new opportunities for translational research. Pathophysiology 21:35–46

Partida-Martinez LP, Hertweck C (2005) Pathogenic fungus harbours endosymbiotic bacteria for toxin production. Nature 437:884

Patel RM, Denning PW (2013) Therapeutic use of prebiotics, probiotics, and postbiotics to prevent necrotizing enterocolitis: what is the current evidence? Clin Perinatol 40:11–25. https://doi.org/10.1016/j.clp.2012.12.002 S0095-5108(12)00135-2[pii]

Pennanen T (2001) Microbial communities in boreal coniferous forest humus exposed to heavy metals and changes in soil pH—a summary of the use of phospholipid fatty acids, Biolog® and 3H-thymidine incorporation methods in field studies. Geoderma 100:91–126

Peterson CN, Day S, Wolfe BE, Ellison AM, Kolter R, Pringle A (2008) A keystone predator controls bacterial diversity in the pitcher-plant (Sarracenia purpurea) microecosystem. Environ Microbiol 10:2257–2266

Petrof EO, Khoruts A (2014) From stool transplants to next-generation microbiota therapeutics. Gastroenterology 146:1573–1582

Petrof EO, Claud EC, Gloor GB, Allen-Vercoe E (2012) Microbial ecosystems therapeutics: a new paradigm in medicine? Benefic Microbes 4:53–65

Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K, Allen-Vercoe E (2013) Stool substitute transplant therapy for the eradication of Clostridium difficile infection:'RePOOPulating'the gut. Microbiome 1:3

Pold G, Melillo JM, DeAngelis KM (2015) Two decades of warming increases diversity of a potentially lignolytic bacterial community. Front Microbiol 6:480

Prajapati VD, Jani GK, Khanda SM (2013) Pullulan: an exopolysaccharide and its various applications. Carbohydr Polym 95:540–549

Priyanka J, Koel M (2015) Diversity study of nitrate reducing bacteria from soil samples-a metagenomics approach. J Comp Sci Syst Biol 8:191

Radajewski S, Ineson P, Parekh NR, Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. Nature 403:646

Raeside C, Gaffe J, Deatherage DE, Tenaillon O, Briska AM, Ptashkin RN, Cruveiller S, Medigue C, Lenski RE, Barrick JE, Schneider D (2014) Large chromosomal rearrangements during a long-term evolution experiment with Escherichia coli. MBio 5:e01377–01314. https://doi.org/10.1128/mBio.01377-14e01377-14

Ramachandran R, Menon RK (1998) An overview of industrial uses of hydrogen. Int J Hydrogen Energy 23:593–598. https://doi.org/10.1016/S0360-3199(97)00112-2

Ramirez KS, Craine JM, Fierer N (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Glob Chang Biol 18:1918–1927

Ranieri E, Bombardelli F, Gikas P, Chiaia B (2016) Soil pollution prevention and remediation. Appl Environ Soil Sci 2016:2. https://doi.org/10.1155/2016/9415175

Rashid M, Stingl U (2015) Contemporary molecular tools in microbial ecology and their application to advancing biotechnology. Biotechnol Adv 33:1755–1773

Ratcliff AW, Busse MD, Shestak CJ (2006) Changes in microbial community structure following herbicide (glyphosate) additions to forest soils. Appl Soil Ecol 34:114–124

Reich PB, Sendall KM, Stefanski A, Wei X, Rich RL, Montgomery RA (2016) Boreal and temperate trees show strong acclimation of respiration to warming. Nature 531:633

Reno ML, Held NL, Fields CJ, Burke PV, Whitaker RJ (2009) Biogeography of the Sulfolobus islandicus pan-genome. Proc Natl Acad Sci 106:8605–8610

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability update on microbial phosphorus. Plant Physiol 156:989–996

Rinta-Kanto JM, Sinkko H, Rajala T, Al-Soud WA, Sørensen SJ, Tamminen MV, Timonen S (2016) Natural decay process affects the abundance and community structure of Bacteria and archaea in Picea abies logs. FEMS Microbiol Ecol 92:fiw087

Rittmann BE (2006) Microbial ecology to manage processes in environmental biotechnology. Trends Biotechnol 24:261–266

Roels T, Dauwe F, Van Damme S, De Wilde K, Roelandt F (2002) The influence of PAX-14 on activated sludge systems and in particular on Microthrix parvicella. Water Sci Technol 46:487–490

Rösch C, Mergel A, Bothe H (2002) Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl Environ Microbiol 68:3818–3829

Ross C, Opel V, Scherlach K, Hertweck C (2014) Biosynthesis of antifungal and antibacterial polyketides by Burkholderia gladioli in coculture with Rhizopus microsporus. Mycoses 57:48–55

Rytioja J, Hildén K, Yuzon J, Hatakka A, de Vries RP, Mäkelä MR (2014) Plant-polysaccharide-degrading enzymes from basidiomycetes. Microbiol Mol Biol Rev 78:614–649

Salas-Marina MA, Silva-Flores MA, Uresti-Rivera EE, Castro-Longoria E, Herrera-Estrella A, Casas-Flores S (2011) Colonization of Arabidopsis roots by Trichoderma atroviride promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. Eur J Plant Pathol 131:15–26

Sana B (2014) Bioresources for control of environmental pollution. In Bioresources for control of environmental pollution. In: Biotechnological applications of biodiversity. Springer, Berlin, pp 137–183

Sapp J (2007) The structure of microbial evolutionary theory. Stud Hist Philos Sci Part C: Stud Hist Philos Biol Biomed Sci 38:780–795

Savi F, Di Bene C, Canfora L, Mondini C, Fares S (2016) Environmental and biological controls on CH4 exchange over an evergreen Mediterranean forest. Agric For Meteorol 226–227:67–79. https://doi.org/10.1016/j.agrformet.2016.05.014

Schippers A, Hallmann R, Wentzien S, Sand W (1995) Microbial diversity in uranium mine waste heaps. Appl Environ Microbiol 61:2930–2935

Schleper C, Jurgens G, Jonuscheit M (2005) Genomic studies of uncultivated archaea. Nat Rev Microbiol 3:479

Schleppi P, Bucher-Wallin I, Hagedorn F, Körner C (2012) Increased nitrate availability in the soil of a mixed mature temperate forest subjected to elevated CO2 concentration (canopy FACE). Glob Chang Biol 18:757–768. https://doi.org/10.1111/j.1365-2486.2011.02559.x

Schmid M, Twachtmann U, Klein M, Strous M, Juretschko S, Jetten M, Metzger JW, Schleifer K-H, Wagner M (2000) Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. Syst Appl Microbiol 23:93–106

Schütt M, Borken W, Spott O, Stange CF, Matzner E (2014) Temperature sensitivity of C and N mineralization in temperate forest soils at low temperatures. Soil Biol Biochem 69:320–327

Schwarz WH, Gapes JR, Zverlov VV, Antoni D, Erhard W, Slattery M (2006) Personal communication and demonstration at the TU Muenchen (Campus Garching and Weihenstephan) in June 2006

Shah N, Ul-Islam M, Khattak WA, Park JK (2013) Overview of bacterial cellulose composites: a multipurpose advanced material. Carbohydr Polym 98:1585–1598

Shi S, Bending GD (2007) Changes to the structure of Sphingomonas spp. communities associated with biodegradation of the herbicide isoproturon in soil. FEMS Microbiol Lett 269:110–116

Shi S, Nuccio E, Herman DJ, Rijkers R, Estera K, Li J, da Rocha UN, He Z, Pett-Ridge J, Brodie EL (2015) Successional trajectories of rhizosphere bacterial communities over consecutive seasons. MBio 6:e00746–e00715

Shoemaker JK, Keenan TF, Hollinger DY, Richardson AD (2014) Forest ecosystem changes from annual methane source to sink depending on late summer water balance. Geophys Res Lett 41:673–679

Silva ÍS, dos Santos E d C, de Menezes CR, de Faria AF, Franciscon E, Grossman M, Durrant LR (2009a) Bioremediation of a polyaromatic hydrocarbon contaminated soil by native soil microbiota and bioaugmentation with isolated microbial consortia. Bioresour Technol 100:4669–4675

Silva MF, Fornari RCG, Mazutti MA, de Oliveira D, Padilha FF, Cichoski AJ, Cansian RL, Di Luccio M, Treichel H (2009b) Production and characterization of xantham gum by Xanthomonas campestris using cheese whey as sole carbon source. J Food Eng 90:119–123

Sprocati AR, Alisi C, Tasso F, Marconi P, Sciullo A, Pinto V, Chiavarini S, Ubaldi C, Cremisini C (2012) Effectiveness of a microbial formula, as a bioaugmentation agent, tailored for bioremediation of diesel oil and heavy metal co-contaminated soil. Process Biochem 47:1649–1655

Strous M, Fuerst JA, Kramer EHM, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MSM (1999) Missing lithotroph identified as new planctomycete. Nature 400:446

Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P (2012) Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. FEMS Microbiol Ecol 80:735–746

Sung V, Hiscock H, Tang MLK, Mensah FK, Nation ML, Satzke C, Heine RG, Stock A, Barr RG, Wake M (2014) Treating infant colic with the probiotic Lactobacillus reuteri: double blind, placebo controlled randomised trial. BMJ 348:g2107

Szöllősi A, Rezessy-Szabó JM, Hoschke Á, Nguyen QD (2015) Novel method for screening microbes for application in microbial fuel cell. Bioresour Technol 179:123–127. https://doi.org/10.1016/j.biortech.2014.12.004

Szukics U, Hackl E, Zechmeister-Boltenstern S, Sessitsch A (2012) Rapid and dissimilar response of ammonia oxidizing archaea and bacteria to nitrogen and water amendment in two temperate forest soils. Microbiol Res 167:103–109

Tanaka M, Nara K (2009) Phylogenetic diversity of non-nodulating rhizobium associated with pine ectomycorrhizae. FEMS Microbiol Ecol 69:329–343

Tang J, Feng T, Cui C, Feng Y (2013) Simultaneous biodegradation of phenanthrene and oxidation of arsenite by a dual-functional bacterial consortium. Int Biodeterior Biodegradation 82:173–179

Tata A, Perez C, Campos ML, Bayfield MA, Eberlin MN, Ifa DR (2015) Imprint desorption electrospray ionization mass spectrometry imaging for monitoring secondary metabolites production during antagonistic interaction of fungi. Anal Chem 87:12298–12305

Taylor MW, Schupp PJ, De Nys R, Kjelleberg S, Steinberg PD (2005) Biogeography of bacteria associated with the marine sponge Cymbastela concentrica. Environ Microbiol 7:419–433

Techtmann SM, Hazen TC (2016) Metagenomic applications in environmental monitoring and bioremediation. J Ind Microbiol Biotechnol 43:1345–1354

Tefs C, Gleixner G (2012) Importance of root derived carbon for soil organic matter storage in a temperate old-growth beech forest–evidence from C, N and 14C content. For Ecol Manag 263:131–137

Thompson JR, Pacocha S, Pharino C, Klepac-Ceraj V, Hunt DE, Benoit J, Sarma-Rupavtarm R, Distel DL, Polz MF (2005) Genotypic diversity within a natural coastal bacterioplankton population. Science 307:1311–1313

Tláskal V, Voříšková J, Baldrian P (2016) Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. FEMS Microbiol Ecol 92:fiw177

Valášková V, De Boer W, Gunnewiek PJAK, Pospíšek M, Baldrian P (2009) Phylogenetic composition and properties of bacteria coexisting with the fungus Hypholoma fasciculare in decaying wood. ISME J 3:1218

VanInsberghe D, Maas KR, Cardenas E, Strachan CR, Hallam SJ, Mohn WW (2015) Non-symbiotic Bradyrhizobium ecotypes dominate north American forest soils. ISME J 9:2435

Větrovský T, Steffen KT, Baldrian P (2014) Potential of cometabolic transformation of polysaccharides and lignin in lignocellulose by soil Actinobacteria. PLoS One 9:e89108

Vik U, Logares R, Blaalid R, Halvorsen R, Carlsen T, Bakke I, Kolstø A-B, Økstad OA, Kauserud H (2013) Different bacterial communities in ectomycorrhizae and surrounding soil. Sci Rep 3:3471

Virdis B, Rabaey K, Yuan Z, Keller J (2008) Microbial fuel cells for simultaneous carbon and nitrogen removal. Water Res 42:3013–3024

Visca P, Imperi F, Lamont IL (2007) Pyoverdine siderophores: from biogenesis to biosignificance. Trends Microbiol 15:22–30

Voříšková J, Baldrian P (2013) Fungal community on decomposing leaf litter undergoes rapid successional changes. ISME J 7:477

Wagner M, Erhart R, Manz W, Amann R, Lemmer H, Wedi D, Schleifer KH (1994) Development of an rRNA-targeted oligonucleotide probe specific for the genus Acinetobacter and its application for in situ monitoring in activated sludge. Appl Environ Microbiol 60:792–800

Wagner M, Smidt H, Loy A, Zhou J (2007) Unravelling microbial communities with DNA-microarrays: challenges and future directions. Microb Ecol 53:498–506

Wallenstein MD, McNulty S, Fernandez IJ, Boggs J, Schlesinger WH (2006) Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. For Ecol Manag 222:459–468. https://doi.org/10.1016/j.foreco.2005.11.002

Wang Y, Zhang Z (2000) Comparative sequence analyses reveal frequent occurrence of short segments containing an abnormally high number of non-random base variations in bacterial rRNA genes. Microbiology 146:2845–2854

Wang M-C, Gong M, Zang H-B, Hua X-M, Yao J, Pang Y-J, Yang Y-H (2006) Effect of methamidophos and urea application on microbial communities in soils as determined by microbial biomass and community level physiological profiles. J Environ Sci Health B 41:399–413

Ward BB (2005) Molecular approaches to marine microbial ecology and the marine nitrogen cycle. Annu Rev Earth Planet Sci 33:301–333

Ward DM, Weller R, Bateson MM (1990) 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. Nature 345:63–65

Welander PV, Hunter RC, Zhang L, Sessions AL, Summons RE, Newman DK (2009) Hopanoids play a role in membrane integrity and pH homeostasis in Rhodopseudomonas palustris TIE-1. J Bacteriol 191:6145–6156

Weuster-Botz D, Aivasidis A, Wandrey C (1993) Continuous ethanol production by Zymomonas mobilis in a fluidized bed reactor. Part II: process development for the fermentation of hydrolysed B-starch without sterilization. Appl Microbiol Biotechnol 39:685–690

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. Proc Natl Acad Sci 95:6578–6583

Widner B, Behr R, Von Dollen S, Tang M, Heu T, Sloma A, Sternberg D, DeAngelis PL, Weigel PH, Brown S (2005) Hyaluronic acid production in Bacillus subtilis. Appl Environ Microbiol 71:3747–3752

Woese CR (1987) Bacterial evolution. Microbiol Rev 51:221

Woese CR, Fox GE (1977) Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci 74:5088–5090

Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci 87:4576–4579

Wu J, Xiong J, Hu C, Shi Y, Wang K, Zhang D (2015) Temperature sensitivity of soil bacterial community along contrasting warming gradient. Appl Soil Ecol 94:40–48

Xia M, Talhelm AF, Pregitzer KS (2015) Fine roots are the dominant source of recalcitrant plant litter in sugar maple-dominated northern hardwood forests. New Phytol 208:715–726

Xu J (2006) Invited review: microbial ecology in the age of genomics and metagenomics: concepts, tools, and recent advances. Mol Ecol 15:1713–1731

Yadav V, Paniliatis BJ, Shi H, Lee K, Cebe P, Kaplan DL (2010) Novel in vivo-degradable cellulose-chitin copolymer from metabolically engineered Gluconacetobacter xylinus. Appl Environ Microbiol 76:6257–6265

Yu H, Stephanopoulos G (2008) Metabolic engineering of Escherichia coli for biosynthesis of hyaluronic acid. Metab Eng 10:24–32

Yu H-X, Wang C-Y, Tang M (2013) Fungal and bacterial communities in the rhizosphere of Pinus tabulaeformis related to the restoration of plantations and natural secondary forests in the Loess Plateau, Northwest China. Sci World J 2013

Zak DR, Holmes WE, Burton AJ, Pregitzer KS, Talhelm AF (2008) Simulated atmospheric NO_3^- deposition increases soil organic matter by slowing decomposition. Ecol Appl 18:2016–2027

Chapter 3
Microalgae Cultivation in Wastewater to Recycle Nutrients as Biofertilizer

Francisca Maria Santos and José Carlos Magalhães Pires

Contents

Abstract Nitrogen and phosphorus are two macronutrients present in chemical fertilizers applied to agricultural practices. Nitrogen is usually produced from the Haber–Bosch synthesis process, which converts atmospheric nitrogen into ammonia using natural gas. Consequently, it generated substantial amounts of CO_2, which is the main contributor to global warming. Phosphorus is obtained from nonrenewable phosphate-based minerals using chemical processes with sulfuric acid. This method produces hazardous substances, which have a risk to both human health and the environment. Besides the environmental impacts from the production processes, the nutrient uptake efficiency by the cultures may be very low. Nitrogen can be easily lost to the environment due to denitrification, volatilization, and/or leaching. Phosphate may be converted into insoluble compounds after chemical reaction with soil minerals, which decreases the availability of this nutrient. These losses have major impacts on the environment, polluting the soil, water, and air.

F. M. Santos · J. C. M. Pires (✉)
LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Porto, Portugal
e-mail: franciscalfs@fe.up.pt; jcpires@fe.up.pt

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_3

With the increasing tendency of the fertilizer demand for agricultural practices, it is imperative (i) to find sustainable alternatives to chemical fertilizers (minimizing their world market cote) and (ii) to develop technologies that enhance nutrient uptake efficiency, reducing simultaneously the environmental impacts. Nutrient recycling from wastewaters represents a sustainable solution. These effluents have been proposed as sources of nitrogen and phosphorus for the culture of microalgae, with the simultaneous benefit of nitrogen and phosphorus removal (avoiding the environmental negative impacts with their discharge). Then, microalgal biomass can have several applications, including the production of biofertilizers. This process will enable nutrient recycling, reducing the requirement of fertilizers produced in a non-environmentally friendly way. This chapter aims to present the advantages (and research needs) of using microalgal cultures for nutrient recovery from wastewaters.

Keywords Nutrient recycling · Wastewater · Microalgae · Sustainability · Process integration · Nitrogen · Phosphorus · Biofertilizer · Circular economy

3.1 Introduction

The United Nations Report 2017 predicts the world's population of 8.6 billion in 2030 and 9.8 billion in 2050 (United Nations 2017). The continuous growth of population and consumption represent a greater demand for food, fish, and meat. This adds a huge tension on the food system, which increases the intensive farming practices to meet the food demand (Godfray et al. 2010). Intensive agricultural practices use a great amount of land, water, and energy. Besides these, and to increase crop production yields, excessive amounts of fertilizers and pesticides are applied (Tilman et al. 2002). However, these agricultural practices are the cause of many negative environmental impacts: (i) land clearing causes losses on biodiversity and emits a great amount of greenhouse gases and (ii) excessive fertilization causes the degradation of water, air, and soil (Tilman et al. 2011).

Inefficient use of fertilizers leads to an increase of the amount of nutrients in groundwater and surface waters, causing eutrophication. It is also the main cause of the change in the nitrogen and phosphorus global cycles (Rockström et al. 2009; Tilman et al. 2002). Anthropogenic activities, mainly the fertilizer industry, convert atmospheric nitrogen into reactive forms. This new reactive form, mainly nitrogen oxides (NO_x), ammonia (NH_3), nitrous oxide (N_2O), and nitrate (NO_3^-), ends up accumulated in land, polluting the watercourses and the atmosphere. Regarding the phosphorus, this nutrient can be found on fossil minerals and it is achieved through mining. However, almost half of the mined phosphorus returns into the oceans, increasing the ocean anoxic events, which potentially explains some extinctions of marine life. Ocean anoxic events are caused by the algae bloom that grows due to the waters loaded with nitrogen and phosphate. When algae die and sink to the bottom of the ocean, bacteria feed on it and deplete all the oxygen, which result in a dead zone (Rockström et al. 2009).

Besides the environmental impacts of fertilizers, the world food production relies on these compounds to meet its demand. The application of nitrogen and phosphorus fertilizers can improve the quality of the soil until the point where higher levels of addition decline the crop uptake efficiency. Crops only take approximately 31–49% of the added nitrogen (Cassman et al. 2002) and 45% of the added phosphorus (Smil 2000). Therefore, a significant amount of supplied nutrients is lost from the fields. Consequently, besides the search of alternatives to produce fertilizers, it is extremely important to improve the nutrient uptake efficiency and to practice more sustainable agricultural methods without environment damage, trying to respond to the increased global food demand (Tilman et al. 2002). This chapter aims to describe the potential of microalgal cultures to recover nitrogen and phosphorus from wastewaters. This technology will promote nutrient recycling (reducing the requirement of chemical production). In addition, the biofertilizers produced with microalgal biomass have a high content of organic matter, which can retain the inorganic nutrients in the soil, enhancing their uptake efficiency by crops.

3.2 Current Processes of Fertilizer Production

In 2015, the world demand for nitrogen and phosphorus fertilizers was 110.03 and 41.2 million tons, respectively. With an expected growth of 1.5% and 2.2% annually, between 2015 and 2020, the demand of nitrogen and phosphorus is expected to be 118.8 and 45.9 million tons, in 2020 (FAO 2017). Hence, the worldwide production of fertilizer will increase to meet the increased demand. The current fertilizer production relies on two key chemicals: NH_3 and phosphoric acid. Figure 3.1 shows a scheme of the fertilizer production. The next sections describe the processes for chemical fertilizer production.

3.2.1 Nitrogen-Based Fertilizers

The main chemical for manufacturing nitrogen-based fertilizers is NH_3, which is synthesized by the Haber process (also called the Haber–Bosch process). A mixture of hydrogen and nitrogen (molar ratio of 3:1) reacts with an iron catalyst at high temperatures (to achieve high reaction kinetics) and pressures. The nitrogen is supplied from the air, where the hydrogen can be obtained from several hydrocarbon sources; the most common is natural gas (Appl 2006; Pellegrino 2000). The extraction of nitrogen from the air is made through three steps: (i) filtration, (ii) liquefaction, and (iii) fractional distillation. First, the air is filtered to remove dust. Second, it is cooled to −200 °C, the temperature in which air turns liquid. Last, the nitrogen

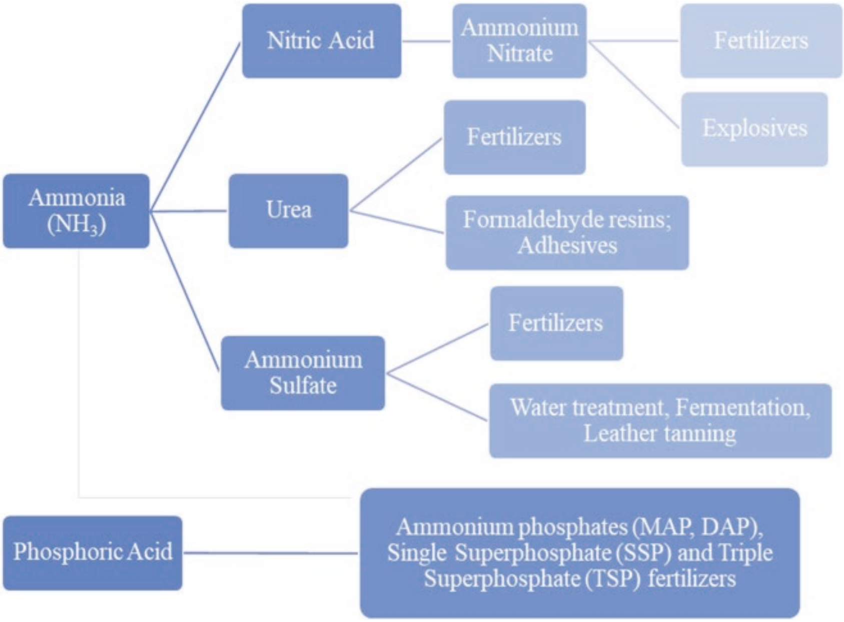


Fig. 3.1 Current chain for nitrogen and phosphorus fertilizer production (Pellegrino 2000). Note: *DAP* diammonium phosphate, *MAP* monoammonium phosphate, *SSP* single superphosphate, *TSP* triple superphosphate

is separated from the rest of air constituents through fractional distillation. On the distillation column, the cooled liquid is gently warmed, and when the temperature rises to −196 °C, the nitrogen boils off and is collected (Smith and Klosek 2001). The production of H_2 can occur under two reactions: a catalyst with steam reforming or a non-catalytic reaction with oxygen, called partial oxidation. The technology applied depends on the hydrocarbon feedstock composition. Natural gas has a higher content of hydrogen and therefore is considered the best basic feedstock for the steam-reforming route, while in the partial oxidation, the raw synthesis gas has a high CO content. Moreover, the first technology can only be used for light hydrocarbons; heavy hydrocarbons (e.g., fuel oil) contain a great amount of sulfur and a small quantity of heavy metals that would contaminate the sensitive reforming catalyst. Thus, any sulfur compounds have to be extracted by hydrodesulfurization with a mixture of cobalt–molybdenum and zinc oxide catalysts (Appl 2006).

After production, NH_3 can be either used to produce solid fertilizers or directly applied into the soil. The solid fertilizers can be distinguished in (i) urea, (ii) ammonium nitrate, (iii) ammonium sulfate, and (iv) ammonium phosphate (production process described in Sect. 3.2.2) (Appl 2006). The urea (also known as carbamide or carbonyl diamide) is synthesized by the reaction of NH_3 with carbon dioxide (CO_2) under high pressure and moderate temperature. This fertilizer is found in both liquid and solid forms (prills or granules). The production process of ammonium nitrate is the neutralization of nitric acid with NH_3. The final product can be liquid or solid, where high-density solids are generally used as fertilizers. Ammonium sulfate can be produced from different ways: (i) direct synthesis with NH_3 and sulfuric acid, (ii) a by-product of the production of caprolactam (an aromatic compound), or (iii) a coke oven by-product. In the first, an exothermic reaction between anhydrous ammonia and sulfuric acid occurs producing ammonium sulfate and a bisulfate solution. After, the solution circulates through an evaporator and a centrifuge, where the ammonium sulfate crystals are separated from the solution. Caprolactam is produced by the reaction of cyclohexanone and hydroxylamine, where sulfuric or phosphoric acid is used as a catalyst. Hence, the treatment with NH_3 origins ammonium sulfate or ammonium phosphate, depending on the catalyst (Pellegrino 2000). Lastly, in the production of coke, a solid carbonaceous residue (with a high content of H_2) generates NH_3 that forms the ammonium sulfate in contact with a gas containing sulfuric acid (Razzaq et al. 2013).

3.2.2 Phosphorus-Based Fertilizers

Phosphorus is fundamental in growing food. However, this element is extracted from a limited, nonrenewable resource that may be exhausted in 50–100 years (Cordell et al. 2009). The primary resource of phosphorus is a phosphate rock called apatite (Chien and Menon 1995). The rock is treated through a wet chemical process with 93% sulfuric acid, producing the commercial phosphoric acid (Pellegrino 2000). Then, the phosphoric acid can be used to produce the water-soluble phosphate salts. These fertilizers can be distinguished in four different types: (i) single superphosphate (SSP), (ii) triple superphosphate (TSP), (iii) monoammonium phosphate (MAP), and (iv) diammonium phosphate (DAP). The SSP is made from the reaction of the phosphate rock with of 65–75% sulfuric acid. The TSP is formed from the reaction of phosphate rock or limestone with low concentration phosphoric acid. Finally, the ammonium phosphate fertilizers (MAP and DAP) are produced from the combination of the phosphoric acid with anhydrous ammonia (Pellegrino 2000). SSP has a phosphorus content of 7–9.5% and TSP has 19–23%; however, from the SSP production results a product called gypsum. This product can be beneficial for soils limited by sulfur (S). MAP and DAP are the most popular fertilizers because these combine the two essential nutrients: nitrogen and phosphorus (Chien et al. 1990).

3.2.3 Environmental Impacts and Costs of Fertilizers

The inefficient use of fertilizers in agricultural fields has negative impacts on different environments. Volatilization, denitrification, leaching, and runoff losses cause air, water, and soil pollution. Volatilization and denitrification of nitrogen result in the atmospheric emissions of nitrous oxide (N_2O), nitric oxide, and NH_3. N_2O is partly responsible for the depletion of the stratospheric ozone layer and global warming and can affect human health. The deposition of nitric oxide and NH_3 in the soil and water bodies contributes to their acidification and eutrophication, which threatens biodiversity. The leaching of nitrate (NO_3^-) indirectly influences the soil pH and causes groundwater toxicity that can origin human health problems (Choudhury and Kennedy 2005). Also, the runoff of phosphorus to surface water bodies is the main cause of eutrophication (Syers et al. 2008).

Besides the impacts of the application on fields, the production has several negative impacts on the environment. Around 85% of the NH_3 globally produced is used in the nitrogen-based fertilizer industry (Pellegrino 2000). The worldwide NH_3 production is responsible for the emission of approximately 3.5 tons of CO_2 to the atmosphere and for the consumption of 52.8 MJ of energy throughout the manufacture of 1 ton of NH_3. The main cause of the carbon footprint is due to H_2 production that also generates H_2S and CO emission (Kool et al. 2012). However, the nitrogen generation by fractional distillation of air is also an energy-intensive process and contributes to the heavy carbon footprint of NH_3 production (Froehlich 2013). Moreover, in nitric acid production, nitrous oxide is a co-product. As previously mentioned, N_2O has a significant global warming potential, and it is considered as an ozone-depleting substance (Kool et al. 2012).

The data related to the emissions from phosphate rock mining is missing from the literature (Tartakovsky et al. 2016). The environmental impacts from the extraction of phosphate rock are still unknown as well as the impact of this raw material in the lifecycle of phosphorus fertilizers. Tartakovsky et al. (2016) evaluated indirectly several emission factors from mining activities in surface phosphate mines through air dispersion modeling. Air quality monitoring around two open mines showed that the particle matter with 10 μm or less in diameter (PM_{10}) concentration was 300–488 $\mu g \cdot m^{-3}$ at a distance of 300 m from the mine and at inhabited areas (7.5 km from the mine) was 2.3–7.5 $\mu g \cdot m^{-3}$. Additionally, the particle matter with 2.5 μm or less in diameter ($PM_{2.5}$) was 57–92 $\mu g \cdot m^{-3}$ in the first situation and 0.7–2.3 $\mu g \cdot m^{-3}$ in the second situation. The permanent exposure to PM can result in respiratory illness to workers and the population that lives close to the mines. World Health Organization (2005) guidelines of the annual mean values for PM_{10} and $PM_{2.5}$ are 20 $\mu g \cdot m^{-3}$ and 10 $\mu g \cdot m^{-3}$, respectively. Furthermore, the phosphoric acid (main compound of phosphorus fertilizers) relies on sulfuric acid production. Approximately, 65–70% of this acid is used in phosphoric acid production. Sulfuric acid is made through a contact method, an oxidation process where elemental sulfur (brimstone) is burned with dry air (Pellegrino 2000). This process is highly exothermic which often results in a net export of energy, producing around 3.0 GJ per ton of sulfuric acid (Kool et al. 2012). However, it also emits a large quantity of sulfur dioxide and acid mist emissions.

In the production of phosphoric acid, other environmental concern is the by-product phosphogypsum (calcium sulfate hydrate, $CaSO_4 \cdot 2H_2O$). From each ton of phosphate processed, it generated 5 tons of phosphogypsum. This compound contains high toxic impurities such as toxic metals and radioactive elements (e.g., uranium and thorium), which can represent a treat to human health (Cordell et al. 2009; Pérez-López et al. 2010). Cadmium (Cd) is the toxic metal present in phosphorus fertilizers that represent an increasing concern. Through plant uptake, this heavy metal can easily enter the human food chain (Chien et al. 2011). The main effect of the Cd in human health is on the kidney or renal cortex, but it also affects the pulmonary, cardiovascular, and musculoskeletal systems. Moreover, it is considered a human carcinogen; in other words, it is a substance that can promote the formation of cancer (Roberts 2014).

3.3 Nutrient Efficiency in Agricultural Crops

In agricultural crops, the availability of nutrients in the soil determined the quality and quantity of what it produced (Timilsena et al. 2015). Therefore, the main goal of the application of fertilizers is to supply nutrients to plants and to enhance or sustain optimal crop yield (Chien et al. 2009). However, approximately 35–60% of nitrogen and 75–85% of phosphorus applied are lost to the environment (Fixen et al. 2015; Umesha et al. 2017). These losses contribute not only to waste of valuable nutrients but also to the severe pollution of the environment. Nitrogen is mainly lost through leaching, mineralization, erosion, and denitrification, while phosphorus losses are due to runoff and mineralization (Fig. 3.2) (Timilsena et al. 2015).

3.3.1 Suitable Soil Properties to Improve Nutrient Efficiency

Physicochemical properties of the soil have a strong influence on nutrient availability to crops. Regarding nitrogen, soil pH influences the availability of NH_3. Higher pH values convert ammonium (NH_4^+) into NH_3 (chemical equilibrium), which may be removed from the water as gas (Choudhury and Kennedy 2005). Temperature also influences the volatilization of NH_3, where warm temperatures increased the rate of ammonium conversion to NH_3 gas (Jones et al. 2013). However, low temperatures do not guarantee reduced NH_3 volatilization. The NH_3 formation rate and the conversion to nitrate are also reduced. Moreover, the soil stays moist, which influences indirectly the volatilization. These factors cause the retention of NH_3 in solution for a longer period. In addition, the soil properties like texture, organic matter, and bicarbonate content can influence the volatilization. Highly buffered soils (in other words, high soil organic matter, bicarbonate content, and clay content) have a lower risk of volatilization. Moreover, soils with high cation exchange capacity (CEC) can retain more NH_4^+ and reduce the volatilization. Silt or clay-dominated soils have a higher CEC where sandier soils have a lower CEC. Additionally, the higher CEC soils often contain high levels of exchangeable calcium (Ca), which can affect the pH, and consequently influence nitrogen losses (Jones et al. 2013).

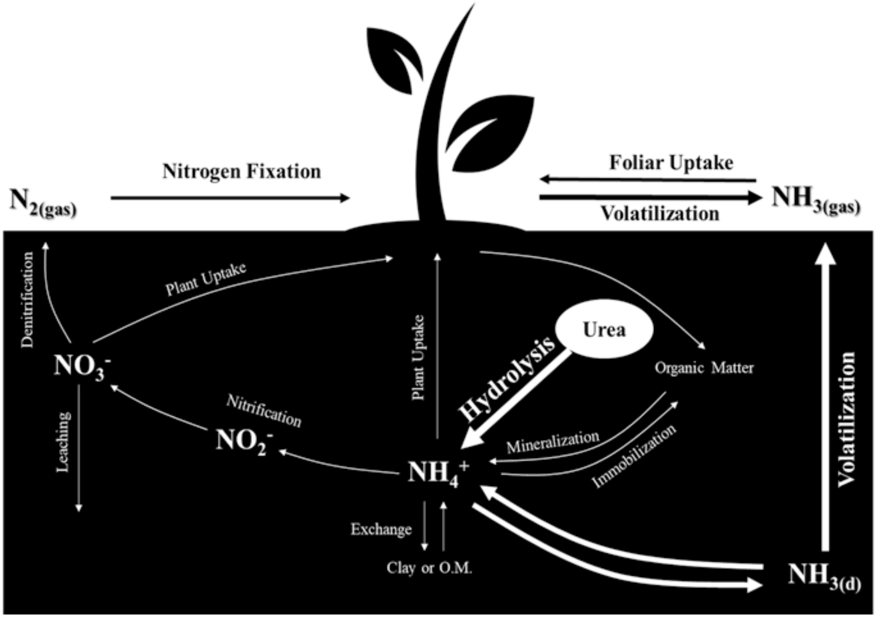


Fig. 3.2 Nitrogen losses in the soil (Jones et al. 2013). Note: NO_2^- nitrite; NO_3^- nitrate; $N_{2(gas)}$ nitrogen gas; $NH_{3(d)}$ ammonia dissolved; $NH_{3(gas)}$ ammonia gas; NH_4^+ ammonium; OM organic matter

However, soils with high CEC tend not to hold anions, which may result on leaching of nitrate.

Phosphorus fertilizers are more susceptible to losses through surface runoff and mineralization (Timilsena et al. 2015). The factors that affect phosphorus availability are similar to the nitrogen availability with addition of biological factors. Soil texture and mineralogy affect the ability of the soil to fix fertilizer phosphorus. Likewise in N fixation, clay soils increase the amount of available phosphorus. In addition, soil constituents like iron (Fe), aluminum (Al), and Ca also affect this availability (Amstrong 1999). In acidic soils, Fe and Al are found naturally, and the Fe–Al oxides can convert phosphorus in the soil solution to water-insoluble Fe–Al–P, due to their strong chemical bond with phosphorus (Chien et al. 2009; Chien et al. 2011). In calcareous soils, $CaCO_3$ can adsorb the phosphorus in the solution. Furthermore, free phosphorus can precipitate with all of these cations, decreasing phosphorus availability over time (Chien et al. 2011). Soil pH has an influence on the release and dissolution of Al from clay minerals. At low pH, the large concentration of free Al thickens the root tips and lateral roots, and, consequently, phosphorus uptake is reduced. Microbial activity in the rhizosphere can control the Fe and Al binding and can adjust the pH, which increase phosphorus availability (Syers et al. 2008). Temperature influences phosphorus uptake by plants, where lower temperatures affect the root growth and therefore phosphorus absorption (Mackay and

Barber 1984). In summary, increasing organic matter, controlling soil pH, and increasing the microbial activity can improve nutrient efficiency without the need to increase the amount of fertilizers that is applied.

3.4 Nutrient Recycling from Wastewater Using Microalgae

As previously mentioned, the population is growing exponentially, and therefore, the demand for food and nutrients to sustain food production is increasing. Algae already provide many products for our consumption (e.g., nutritional supplements, cosmetics, etc.) (Spolaore et al. 2006), but for large-scale production, nutrient recycling can be an extremely important application. It is well-known that wastewaters are loaded with nutrients. Specifically, domestic and agro-industrial wastewaters are rich in phosphorus and nitrogen. The disposal of these without treatment is directly accountable for eutrophication of rivers, lakes, and seas, being, therefore, a permanent threat to the freshwater reserves on a global scale. Most wastewater treatment technologies comprise chemical, physical, and biological methods, which application to the agricultural wastewater is not economically viable (Cai et al. 2013; De-Bashan and Bashan 2004). In the traditional wastewater treatment, the primary phase removes materials that are floating or settled by gravity; in the secondary stage, anaerobic digestion occurs. Then, the effluent is treated with ultraviolet radiation, oxygen, or ozone to remove any microorganisms (e.g., virus or bacteria), and, lastly, it is discharged. However, the final effluent composition has great amounts of nitrogen and phosphors. In fact, these wastewaters containing heavy loads of nutrients (after primary and secondary treatment) can be used as a culture medium for algae. Several studies showed the microalgae' ability to effectively remove nitrogen and phosphorus from different effluents. Lim et al. (2010) studied the use of *Chlorella vulgaris* for bioremediation of textile wastewater. The investigation was carried out in an outdoor pond, and the initial concentration of nitrogen and phosphorus was 6.50 and 7.14 $mg{\cdot}L^{-1}$, respectively. *C. vulgaris* removed 45.1% of nitrogen and 33.3% of phosphorus from the effluent. Martınez et al. (2000) studied the removal of nitrogen and phosphorus from urban wastewater by the microalgae *Scenedesmus obliquus*. Urban wastewater was treated by a secondary sewage treatment before the microalgae cultivation, and the initial concentration of nitrogen and phosphorus was 27.4 $mg{\cdot}L^{-1}$ and 11.8 $mg{\cdot}L^{-1}$, respectively. The highest percentage of removal was 98% for phosphorus and 100% for nitrogen, where this last removal percentage was influenced by two factors: microalgae' growth and desorption of NH_3. González et al. (1997) evaluated the nitrogen and phosphorus removal from agro-industrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. Secondary treatment of wastewater was performed before the cultivation of microalgae. The initial nitrogen concentration was 36.3 mg $NH_3{\cdot}L^{-1}$ and 1.9 mg $NO_3^{-}{\cdot}L^{-1}$, and the initial phosphorus concentration was 111.8 $mg{\cdot}L^{-1}$. The efficiency of nitrogen removal was higher for *S. dimorphus* during the cycle; however, in the end, both microalgae obtained removal rates of 90%. Regarding the phosphorus removal, the obtained efficiencies were similar for the two microalgae with results of 55%. Li et al. (2011) evaluated the nutrient removal from municipal wastewater by *Chlorella* sp. The tests were performed in two stages with different culture media: (i) first stage, autoclaved effluent (AE), and (ii) second stage, raw effluent (RE). Additionally, on the first stage, a control experiment with TAP media was used to assess the performance in the AE. The initial nitrogen concentration was (i) AE, 85.9 mg $NH_3·L^{-1}$ and 132.3 mg $N·L^{-1}$, and (ii) RE, 82.5 mg $NH_3·L^{-1}$ and 116.1 mg $N·L^{-1}$. The initial phosphorus concentration was (i) AE, 212.0 mg PO_4^{3-}-$P·L^{-1}$, and (ii) RE, 215.1 mg PO_4^{3-}-$P·L^{-1}$. The highest efficiency removal rates were obtained in the RE, where *Chlorella* sp. was able to remove 93.9%, 89.1%, and 80.9% of NH_3, total nitrogen, and total phosphorus, respectively.

The microalgae' ability to remove nutrients from wastewaters has been proven. This represents a win–win solution for wastewater treatment and also offers the potential to reduce the need for chemical fertilizers and their environmental impacts. However, the real potential of using wastewaters needs to be explored because research is still limited, especially at large scale. Only 30% of the published studies use real wastewaters as a nutrient source, while 70% of the lab-scale studies use chemical fertilizers because they are easy to access and prepare (Lam and Lee 2012).

3.5 Microalgal Production Technology

3.5.1 Microalgal Culture

Microalgae can be cultivated in three different systems: open, closed, and hybrid systems. The hybrid systems are a combination of open and closed systems in a two-stage cultivation system. In all of these three systems, suspended cultures are used; however, it is also possible to use immobilized cultures to cultivate microalgae. In the immobilization technology, the microalgae are fixed to a solid medium, which allows exchanges of substrates and products through the pores. High nutrient removal rates and simplified subsequent processes (harvesting and water recycling) are some advantages of this technology over the suspended cultivation. Yet, there are some limitations regarding the high cell densities and the high cost, which affect the application of the immobilization technology in wastewater treatment (Cai et al. 2013).

The open cultivation systems include natural (e.g., lakes and ponds) or artificial ones (e.g., raceway pond). The algae cultivation is commonly carried out in a raceway pond, where the culture recirculated in a closed track with 0.3 m depth allowing the photosynthesis. The mixture promoted by the paddlewheels prevents the sedimentation and enables the constant movement of the culture throughout the pond. The simple construction and operation represent an economic advantage in comparison to closed systems. However, the high dependence on external factors makes the control of the growth conditions, such as light and nutrients, extremely difficult.

Contamination problems and water evaporation reduce the productivity and cause variability of biomass (Cai et al. 2013; Lam and Lee 2012).

On the other hand, closed systems reduce contamination and water losses. Additionally, the growth conditions as pH, temperature, air supply, and O_2 and CO_2 concentration can be controlled, enabling the achievement of higher biomass concentrations (Gonçalves 2017). Three categories can be distinguished from these cultivation systems: tubular photobioreactors, flat plate reactors, and bag systems. The first two reactors are extensively used and were conceived to guarantee an optimum light arrangement and gas exchange. Sunlight received can be maximized by altering the configuration of the reactor. The bag systems consist in, as the name suggests, a large plastic bag fitted with aeration systems. Despite the enhancement of the biomass productivity, the cost to install and maintain these reactors is a major setback on the commercialization of closed systems (Kim et al. 2013). Lam and Lee (2012) performed a life cycle assessment to the microalgae technology and found that based on the current technology, PBRs are not ready for be commercialized until significant changes are made to reduce the energy consumption. The authors found that the operation of a tubular photobioreactor consumes 350% more energy compared to a raceway pond. In addition to the systems and its maintenance costs, downstream processing can represent a great impact on the total economic cost of production. The high production cost is derived from the lack of an inexpensive process that incorporates multiple steps related to harvest, conversion, and purification of biomass (Kim et al. 2013).

3.5.2 Microalgal Harvesting and Drying

In all microalgal cultivation process, the biomass needs to be separated from the medium by more than one solid–liquid separation step. Harvesting of biomass may occur through sedimentation, centrifugation, or filtration, which occasionally needs an extra flocculation step (Grima et al. 2004). Sedimentation is the technique where the hanging particles accumulate on the bottom of the tank by the action of gravity. It is an energy-efficient method and widely applied for the separation of microalgae (Pragya et al. 2013). Almost all microalgae can be separated from the diluted medium by centrifugation. Centrifugation is a sedimentation tank that involves the application of gravitational force to improve the sedimentation rate. Thus, the harvest efficiency is improved, and the concentration of microalgal biomass is increased in a shorter period (Grima et al. 2004; Kim et al. 2013). Filtration is the separation technique where the culture goes across the filters and holds the algae, while the water passes through. The process is simple and continuous until the filter gets saturated; in other words, the thick film of algae does not allow the medium to go across (Pragya et al. 2013). Flocculation is the cells' aggregation achieved by adding polymers that form precipitates, which enhance the clustering process and sedimentation. The negative surface of the microalgal cells is neutralized by the cationic polymers, which also reduce the repulsive forces (Kim et al. 2013). This induces bridging mechanisms that bind cells together and stimulate flocculation. Despite the addition of polymers, flocculation can be caused by a modification in pH levels, for instance, altering the values for a range between 11.8 and 12 causes extensive flocculation (Grima et al. 2004).

The selection criteria for the harvesting methods depend on the quality of the product. For instance, if microalgal cultures are used for wastewater treatment, the large volume that is processed excludes the use of centrifuges or filter, at least on the primary steps. Therefore, sedimentation by gravity action combined with flocculants is recommended for sewage-based work sedimentation. On the other hand, if algae are produced to be used in food or aquaculture applications, the use of centrifuges is recommended. These avoid bacterial contamination and can be easily cleaned and sterilized (Grima et al. 2004).

After harvesting, the algal slurry must be processed to not get spoiled. Dehydrating can be done by several methods as spray-drying, drum-drying, freeze-drying, and sun-drying. Spray-drying is a quick and continuous method that uses pressure, centrifugal atomizers, or gas–liquid jets to form a thin spray of solution droplets that are in continuous contact with hot air in a large chamber. The large droplet surface area and the small droplet size guarantee a high evaporation rate, which allows the drying process to be in seconds. The freeze-drying or lyophilization is a gentle drying method that freezes the biomass, and the ice crystals are sublimed by slight warming without thawing. The exposure to a partial pressure of water vapor below 4.6 mmHg, the triple point of water, directly sublimes the crystals into vapor. However, this has high-energy requirements and combined with the high capital cost of equipment is only recommended for high market-value products. Since it is frozen, the elements of the algal material are preserved, maintaining the biochemical composition. Drying has a major problem related to the high water content of the algal biomass, which makes sun-drying difficult and spray-drying expensive for some algal applications (Grima et al. 2004).

3.5.3 Microalgal Biomass as Biofertilizer

The commercial application of microalgae is extensive. It can be a raw material to produce cosmetics, biofuels, and food and feed supplements because of their biochemical composition. Microalgae are rich in proteins, lipids (e.g., fatty acids like ω3 and ω6), pigments (e.g., carotenoids and chlorophyll), and vitamins (Spolaore et al. 2006). Besides these widely studied applications, the microalgal biomass can be used as a biofertilizer, because it has the potential to prevent losses, enhance nutrient efficiency, and consequently increase crop yields. Garcia-Gonzalez and Sommerfeld (2016) evaluated the potential of using the biomass of the microalgae *Acutodesmus dimorphus* as a biofertilizer in the tomato plant production. The authors concluded that the treatments with biofertilizer trigger faster germination and enhanced plant growth, namely, a higher number of branches and flowers. Kholssi et al. (2018) studied the effect of the application of *Chlorella sorokiniana* biomass on the growth of wheat. The authors concluded that the treatments with *Chlorella sorokiniana* biomass increased the plant length and the dry biomass aboveground and underground by 30%, 22%, and 51%, respectively. Additionally, the chemical composition of microalgae, particularly the high-value molecules (e.g., pigments), improved the fruit and vegetable quality (Coppens et al. 2016). In a study performed by Coppens et al. (2016), the use of *Nannochloropsis* biomass as a fertilizer increased the sugar and carotenoid levels of the tomatoes. Additionally, the authors performed an economic evaluation and confirmed the economic feasibility of microalgae-based fertilizers compared to conventional fertilizers.

The combination of algal biomass production and wastewater treatment closes the nutrient cycle. Nowadays, the nutrients have a linear economy; in other words, they are produced or extracted, used, and finally disposed (Vollaro et al. 2016). With this solution, nutrients wasted in wastewaters can be reused and applied in soil, reducing the cost of wastewater treatment and the costs and the environmental impacts of fertilizer production.

3.6 Conclusion

The predicted increase of world population will lead to a new sustainability problem: food production. The climate change, the distribution of water resources, and the degradation of soils will reduce agricultural production. Moreover, the synthesis and use of chemical fertilizers are other concerning issues in terms of environmental impact. A sustainable solution is the nitrogen and phosphorus recovery from wastewater by microalgae. Microalgae has been studied for other environmental purposes (CO_2 capture, wastewater treatment, and biofuel production), but these photosynthetic microorganisms may play also an important role in the nutrient recycling, reducing the impact of human activities in the nitrogen and phosphorus cycles. The introduction of microalgal cultures in wastewater treatment will promote the recovery of nutrients, as the resultant biomass can be used as biofertilizer. This biofertilizer has high organic matter content, which favors nutrient retention in the soils and consequently enhances the uptake efficiency by crops. Therefore, microalgae may improve the environmental sustainability of fertilizer production.

Acknowledgments This work was financially supported by: (i) Project UID/EQU/00511/2019-Laboratory for Process Engineering, Environment, Biotechnology and Energy (LEPABE) funded by national funds through FCT/MCTES (PIDDAC); and (ii) Project POCI-01-0145-FEDER-031736-PIV4Algae (Process Intensification for microalgal production and Valorisation) funded by FEDER funds through COMPETE2020-Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES. J.C.M. Pires acknowledges the FCT Investigator 2015 Programme (IF/01341/2015).

References

Amstrong D (1999) Important factors affecting crop response to phosphorus. Better Crops 83:16–19

Appl M (2006) Ammonia, vol 1. Wiley-VCH, Weinheim. https://doi.org/10.1002/14356007

Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew Sust Energ Rev 19:360–369. https://doi.org/10.1016/j.rser.2012.11.030

Cassman KG, Dobermann A, Walters DT (2002) Agroecosystems, nitrogen-use efficiency, and nitrogen management. AMBIO J Hum Environ 31:132–140. https://doi.org/10.1639/0044-7447(2002)031[0132:anuean]2.0.co;2

Chien S, Menon R (1995) Factors affecting the agronomic effectiveness of phosphate rock for direct application. Fertilizer Res 41:227–234. https://doi.org/10.1007/bf00748312

Chien S, Sale P, Hammond L (1990) Comparison of the effectiveness of phosphorus fertilizer products. In: Phosphorus requirements for sustainable agriculture in Asia and Oceania Int Rice res Inst. International Rice Research Institute, Manila, pp 143–156

Chien S, Prochnow L, Cantarella H (2009) Recent developments of fertilizer production and use to improve nutrient efficiency and minimize environmental impacts. Adv Agron 102:267–322. https://doi.org/10.1016/s0065-2113(09)01008-6

Chien S, Prochnow L, Tu S, Snyder C (2011) Agronomic and environmental aspects of phosphate fertilizers varying in source and solubility: an update review. Nutr Cycl Agroecosyst 89:229–255. https://doi.org/10.1007/s10705-010-9390-4

Choudhury A, Kennedy I (2005) Nitrogen fertilizer losses from rice soils and control of environmental pollution problems. Commun Soil Sci Plant Anal 36:1625–1639. https://doi.org/10.1081/css-200059104

Coppens J, Grunert O, Van Den Hende S, Vanhoutte I, Boon N, Haesaert G, De Gelder L (2016) The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J Appl Phycol 28:2367–2377. https://doi.org/10.1007/s10811-015-0775-2

Cordell D, Drangert J-O, White S (2009) The story of phosphorus: global food security and food for thought. Glob Environ Chang 19:292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009

De-Bashan LE, Bashan Y (2004) Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003). Water Res 38:4222–4246. https://doi.org/10.1016/j.watres.2004.07.014

FAO (2017) World fertilizer trends and outlook to 2020. Food and Agriculture Organization of the United Nations, Rome

Fixen P, Brentrup F, Bruulsema T, Garcia F, Norton R, Zingore S (2015) Nutrient/fertilizer use efficiency: measurement, current situation and trends. In: Drechsel P, Heffer P, Magen H, Mikkelsen R, Wichelns D (eds) Managing water and fertilizer for sustainable agricultural intensification. International Fertilizer Industry Association, Paris, pp 8–38

Froehlich P (2013) A sustainable approach to the supply of nitrogen. Parker Balston. https://www.parker.com/literature/Balston%20Filter/IND/IND%20Technical%20Articles/PDFs/Sustainable%20Approach%20to%20N2%20Supply.pdf. Accessed Apr 2018

Garcia-Gonzalez J, Sommerfeld M (2016) Biofertilizer and biostimulant properties of the microalga *Acutodesmus dimorphus*. J Appl Phycol 28:1051–1061. https://doi.org/10.1007/s10811-015-0625-2

Godfray HCJ et al (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818. https://doi.org/10.1126/science.1185383

Gonçalves ALC (2017) Microalgal cultivation for biomass production, carbon dioxide capture and nutrients uptake. Faculdade de Engenharia da Universidade do Porto

González LE, Cañizares RO, Baena S (1997) Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. Bioresour Technol 60:259–262. https://doi.org/10.1016/s0960-8524(97)00029-1

Grima EM, Fernández FA, Medina AR (2004) 10 downstream processing of cell-mass and products. In: Handbook of microalgal culture: biotechnology and applied phycology, pp 215–252. https://doi.org/10.1002/9780470995280.ch10

Jones C, Brown B, Engel R, Horneck D, Olson-Rutz K (2013) Factors affecting nitrogen fertilizer volatilization

Kholssi R, Marks EA, Miñón J, Montero O, Debdoubi A, Rad C (2018) Biofertilizing effect of *Chlorella sorokiniana* suspensions on wheat growth. J Plant Growth Regul:1–6. https://doi.org/10.1007/s00344-018-9879-7

Kim J et al (2013) Methods of downstream processing for the production of biodiesel from microalgae. Biotechnol Adv 31:862–876. https://doi.org/10.1016/j.biotechadv.2013.04.006

Kool A, Marinussen M, Blonk H (2012) LCI data for the calculation tool feedprint for greenhouse gas emissions of feed production and utilization. GHG Emissions of N, P and K fertilizer production

Lam MK, Lee KT (2012) Microalgae biofuels: a critical review of issues, problems and the way forward. Biotechnol Adv 30:673–690. https://doi.org/10.1016/j.biotechadv.2011.11.008

Li Y et al (2011) Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour Technol 102:5138–5144. https://doi.org/10.1016/j.biortech.2011.01.091

Lim S-L, Chu W-L, Phang S-M (2010) Use of *Chlorella vulgaris* for bioremediation of textile wastewater. Bioresour Technol 101:7314–7322. https://doi.org/10.1016/j.biortech.2010.04.092

Mackay A, Barber S (1984) Soil temperature effects on root growth and phosphorus uptake by corn. Soil Sci Soc Am J 48:818–823. https://doi.org/10.2136/sssaj1984.03615995004800040024x

Martınez M, Sánchez S, Jimenez J, El Yousfi F, Munoz L (2000) Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. Bioresour Technol 73:263–272. https://doi.org/10.1016/s0960-8524(99)00121-2

Pellegrino JL (2000) Energy and environmental profile of the US Chemical Industry prepared by Energetics Incorporated Columbia. Maryland, USA. https://doi.org/10.2172/1218625

Pérez-López R, Nieto JM, López-Coto I, Aguado JL, Bolívar JP, Santisteban M (2010) Dynamics of contaminants in phosphogypsum of the fertilizer industry of Huelva (SW Spain): from phosphate rock ore to the environment. Appl Geochem 25:705–715. https://doi.org/10.1016/j.apgeochem.2010.02.003

Pragya N, Pandey KK, Sahoo P (2013) A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renew Sust Energ Rev 24:159–171. https://doi.org/10.1016/j.rser.2013.03.034

Razzaq R, Li C, Zhang S (2013) Coke oven gas: availability, properties, purification, and utilization in China. Fuel 113:287–299. https://doi.org/10.1016/j.fuel.2013.05.070

Roberts TL (2014) Cadmium and phosphorus fertilizers: the issues and the science. Procedia Eng 83:52–59. https://doi.org/10.1016/j.proeng.2014.09.012

Rockström J et al (2009) A safe operating space for humanity. Nature 461:472

Smil V (2000) Phosphorus in the environment: natural flows and human interferences. Annu Rev Energy Environ 25:53–88. https://doi.org/10.1146/annurev.energy.25.1.53

Smith A, Klosek J (2001) A review of air separation technologies and their integration with energy conversion processes. Fuel Process Technol 70:115–134. https://doi.org/10.1016/s0378-3820(01)00131-x

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96. https://doi.org/10.1263/jbb.101.87

Syers J, Johnston A, Curtin D (2008) Efficiency of soil and fertilizer phosphorus use: reconciling changing concepts of soil phosphorus behaviour with agronomic information vol 18. Food and Agriculture Organization of the United Nations (FAO). https://doi.org/10.1017/s0014479708007138

Tartakovsky D, Stern E, Broday DM (2016) Indirect estimation of emission factors for phosphate surface mining using air dispersion modeling. Sci Total Environ 556:179–188. https://doi.org/10.1016/j.scitotenv.2016.02.207

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671. https://doi.org/10.1038/nature01014

Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. Proc Natl Acad Sci 108:20260–20264

Timilsena YP, Adhikari R, Casey P, Muster T, Gill H, Adhikari B (2015) Enhanced efficiency fertilisers: a review of formulation and nutrient release patterns. J Sci Food Agric 95:1131–1142. https://doi.org/10.1002/jsfa.6812

Umesha C, Sridhara C, Kumarnaik A (2017) Recent forms of fertilizers and their use to improve nutrient use efficiency and to minimize environmental impacts. Int J Pure App Biosci 5:858–863. https://doi.org/10.18782/2320-7051.2739

United Nations (2017) World population prospects: the 2017 revision, methodology of the United Nations population estimates and Projections. New York

Vollaro M, Galioto F, Viaggi D (2016) The circular economy and agriculture: new opportunities for re-using phosphorus as fertilizer. Bio-based Appl Econ 5:267

World Health Organization (2005) WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulphur dioxide. Global Update 2005. Summary of risk assessment. World Health Organization

Chapter 4
Biochar: A Growing Sanguinity as a Combinatorial Tool for Remediation of Heavy Metals from Wastewaters and Solid Waste Management

Amita Shakya and Faraz Ahmad

Contents

A. Shakya (✉)
Department of Agriculture and Environmental Sciences, National Institute of Food Technology Entrepreneurship and Management, Sonipat, Haryana, India

F. Ahmad
Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, India

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_4

Abstract Contamination of surface and ground water due to rapid industrial development has become a dangerous problem of high concern to the world, especially to the underdeveloped countries. Heavy metals, organic pollutants, dyes, pesticides, etc. are some of the major pollutants of the water body system. Among them, heavy metals are non-biodegradable entities and persist for prolonged period in water and soil ecosystems, as compared to organic contaminants. Emission of various heavy metals like Pb, Cd, As, Hg, Cu, Cr, and As has become a serious global concern due to their toxic impacts toward living organisms. Moreover, these heavy metals (and metalloids) become carcinogenic to various microorganisms, animals, and plants, when their concentrations exceed the tolerance levels due to bioaccumulation and biomagnification.

The sources of heavy metal pollution in water bodies are primarily anthropogenic, including discharge from industrial, agricultural, or municipal sources. Due to the negative impacts of these heavy metals toward the living world, their removal from wastewaters has become a priority action area. Several conventional methods like ion exchange, chemical precipitation, electrocoagulation, and membrane and ultrafiltration techniques have been applied to achieve effective and rapid remediation of heavy metal-based environmental contaminants. However, high operational cost, requirement of specialized reagents and apparatus, and generation of hazardous sludge are some constraints that limit the use of these techniques. Taking into account the above drawbacks of various technologies for heavy metal ion remediation from polluted water, the adsorption technique has been adopted as an alternative strategy with potential to overcome the limitations of previous techniques. Adsorption-based methods are in use widely for heavy metal remediation from household to industrial wastewater with many advantages including low cost, simplicity, high efficiency, and ease of use. Carbonaceous materials like activated carbon and biochar are among the main adsorbents in vogue in present time for heavy metal remediation from wastewater. Activated carbon requires additional efforts like higher temperature and activation process. Compared with activated carbon, biochar emerged as a new potentially efficient adsorbent with lower production cost and energy requirements.

Biochar is a porous, carbon-rich product obtained from the thermochemical conversion of biomass in a closed container under deoxygenated conditions at moderate to high temperatures (generally ≤700 °C). Various inherent properties of biochar including porous structure and surface functional groups play an important role in heavy metal adsorption. Moreover, the regeneration/reusability potential of biochar makes it more beneficial in terms of economic as well as environmental prospective. Various biochars prepared from different raw materials have been successfully applied for environmental remediation purposes. They have been utilized for their ability to immobilize heavy metals and hence reduced the risk of the pollutant transfer in the environment. The raw material for biochar production generally comprises agricultural biomass and solid waste, which is abundantly available and extremely inexpensive. Hence, conversion of biomass into biochar and utilization of it as a sorbent are synergic sustainable approaches for improvisation of solid waste management and environment protection. Being a renewable resource, biochar is therefore a promising tool of environmental technology to be used for heavy metal contaminated wastewater treatment.

Keywords Biochar · Heavy metals · Water contamination · Wastewater treatment · Regeneration · Reusability

4.1 Introduction

For the last few decades, rapid urbanization and enormous industrial development caused the increasing accumulation of hazardous pollutants in water bodies. With exponentially growing population and increasing urbanization, especially in the developing world, infrastructure for the maintenance of water quality and sanitation facilities did not increase adequately (Reza and Singh 2010). Contaminant discharge into the environment due to various industrial, residential, and commercial anthropogenic activities affects the surrounding ecosystem by infusing hazardous organic and inorganic contaminants into it. Toxic effects of these contaminants become a spiking concern due to the adverse impacts they inflict upon living organisms. Inorganic pollutants like trace and heavy metals, mineral acids, metalloids, cyanides, sulfates, and complex of metal ions with organic compounds are of foremost concern due to their everyday increasing levels in the water bodies. Water contamination due to heavy metals is of serious concern because of their nonbiodegradable nature, widespread occurrence, and bioavailability to the living organisms (Ahmad et al. 2013). Some natural phenomena like weathering, volcanic deposition, and various anthropogenic activities like mining, fertilizer and pesticide application, electronic manufacturing industry, smelting discharge, etc. are the major sources of heavy metal contamination of surface water and groundwater system (Dong et al. 2017). For thousands of years, heavy metals have been in use in different fields for various applications and continue to impose harm on human and other life forms. Reportedly, lead (Pb) is being used for at least 5000 years in building materials, pigments for glazing ceramics, and pipes for transporting water (Järup 2003). Similarly, mercury (Hg) was used to treat syphilis during the late 1800s (Järup 2003). The growing awareness of lethal and adverse effects of exposure to heavy metal contaminants either intentionally or unintentionally warrants developing their efficient remediation measures. For example, arsenic (As) is used for wood preservation, chromium (Cr) for tanning of leather, mercury (Hg) in gold mining, and lead (Pb) in household pipes (Järup 2003). Therefore, strategies for the removal of heavy metals from wastewater discharge are of priority concern to avoid their potentially lethal effects.

To tackle this serious issue of water contamination with heavy metals, different methods have been employed including chemical precipitation, ultrafiltration, reverse osmosis, ion exchange, electrochemical treatments, and use of different membranes (Ma et al. 2014). Among the methods adsorption process has emerged as an environmentally friendly and economically viable technique due its cost-effectiveness, easy operational conditions, and reusability/regeneration capacity (Dong et al. 2017). For adsorption, one of the most popular adsorbents is biochar, which possesses many specific physical and chemical properties for heavy metal remediation from water.

4.1.1 What Biochar Is

The International Biochar Initiative defines biochar as "A solid product obtained by thermochemical conversion of biomass under anoxic conditions, which can be used alone or as an additive to improve soil fertility, increase resource use efficiency, alleviate environmental pollution, and reduce greenhouse gas emissions" (IBI 2014). A wide range of heating temperature (250–900 °C) can be used for biochar production and often relatively low temperature ($\leq$ 700 °C) applied for achieving biochar production. Biochar is considered as a type of black carbon, but differentiated from black carbon in terms of application and purpose of creation. For defining a carbonized material as a biochar, a high organic carbon content consisting of aromatic six-carbon ring is necessary (Lehmann and Joseph 2015). Initially biochar research was motivated by research on Amazonian Dark Earths (also called *Terra Preta de Indio*) found in the Amazon Basin. Due to its multifunctionality including soil fertility enhancement, soil conditioning, bio-energy production, carbon sequestration, and environmental remediation (Inyang et al. 2016), it gained attention as one of the potential drivers of waste management, climate change mitigation, and organic and inorganic contaminant remediation. Biochar properties like high surface area, highly porous structure with meso- and micropores, and the negatively and positively charged surface functional groups (e.g., carboxyl, hydroxyl, and phenolic groups) make it a potential candidate for environmental remediation of heavy metals from contaminated waters and soils as well (Cao et al. 2009; Dong et al. 2017; Inyang et al. 2016). Taking together all the abovementioned properties, biochar is a perfect material to adsorb heavy metals. In recent years, several studies demonstrated the promising capability of biochars for heavy metal removal from water (Agrafioti et al. 2014; Cao et al. 2009; Kołodyńska et al. 2012; Lima et al. 2010, 2015; Mohan et al. 2011; Rajapaksha et al. 2018; Wang et al. 2016). Biochar production from agricultural by-products and food industrial and municipal waste makes it an economical and cost-effective substitute for wastewater treatment technologies. Figure 4.1 summarized the potential of biochar for different applications.

Fig. 4.1 Schematic diagram showing general route of biochar preparation and its potential application

4.1.2 Potential Biomass for Biochar Production (Feedstock)

The raw material used for biochar production is known as feedstock. Biochar can be produced from a wide variety of processed or unprocessed feedstock materials. Generally, the waste or residual biomass is preferred for biochar production. Residual biomasses generated from agro-food industries, municipal waste, invasive plants, and nonconventional waste materials like pipes, bone, feathers, etc. are some of the examples. Figure 4.2 illustrates the potential feedstock materials for biochar production. The type of biomass significantly affects the properties of biochar. Higher lignin content and lower hemicellulose and cellulose content in the feedstock give higher biochar yield, and porosity of biochar increases with the content of lignin in biomass (Nartey and Zhao 2014). When readily available, residual or waste biomass is used for biochar production, and then machinery and heating will be the main elements to which the cost of biochar production is associated, which is estimated to be about only $4 per gigajoule (Lehmann 2007).

4.2 Biochar Production Methods

Use of residual biomass from agriculture and food processing industry for biochar production is an economically viable and an efficient way to utilize these by-products. The thermochemical conversion is the most appropriate technique for

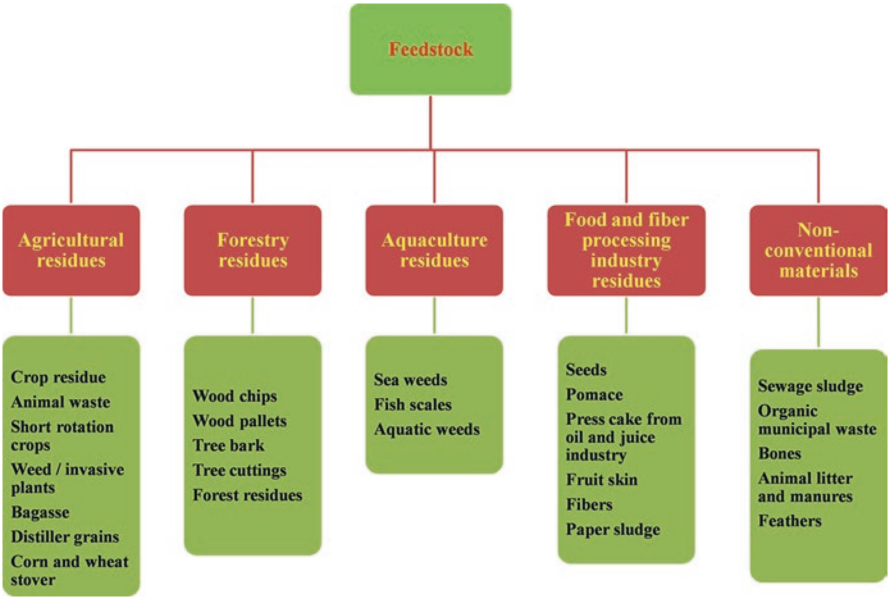


Fig. 4.2 Types of feedstock used for biochar production

biochar production (Fig. 4.3 and Table 4.1). Depending upon the type of the thermochemical technique used, the possible produces are (i) solid material (biochar), (ii) noncondensable gases, and (iii) condensable liquid (bio-oil). The thermochemical conversion of biomass for biochar production includes pyrolysis and gasification when dry biomass is treated and hydrothermal carbonization when biomass is in slurry form.

4.2.1 Pyrolysis

Pyrolysis (dry) is the most common method for biochar production. In dry pyrolysis, biomass is heated at high temperature (>250 °C) in the absence of oxygen. Before thermal treatment the moisture content of biomass is significantly reduced (generally up to <10%) to facilitate the process and reduce the energy consumption. Thermal decomposition of biomass during pyrolysis releases syngas (a mixture of CO, H_2, CH_4, and C_2H_2), bio-oil (condensed high molecular weight compounds), and residual solid as biochar. Depending upon the heating rate and residence time, pyrolysis is categorized as slow and fast pyrolysis. Slow pyrolysis is the conventional carbonization of biomass, producing biochar by heating biomass at a low heating rate with relatively longer residence time (Qian et al. 2015). Fast pyrolysis is conducted over short residence time (in seconds) and very high heating rate. Generally, the primary product for fast pyrolysis is bio-oil, while slow pyrolysis

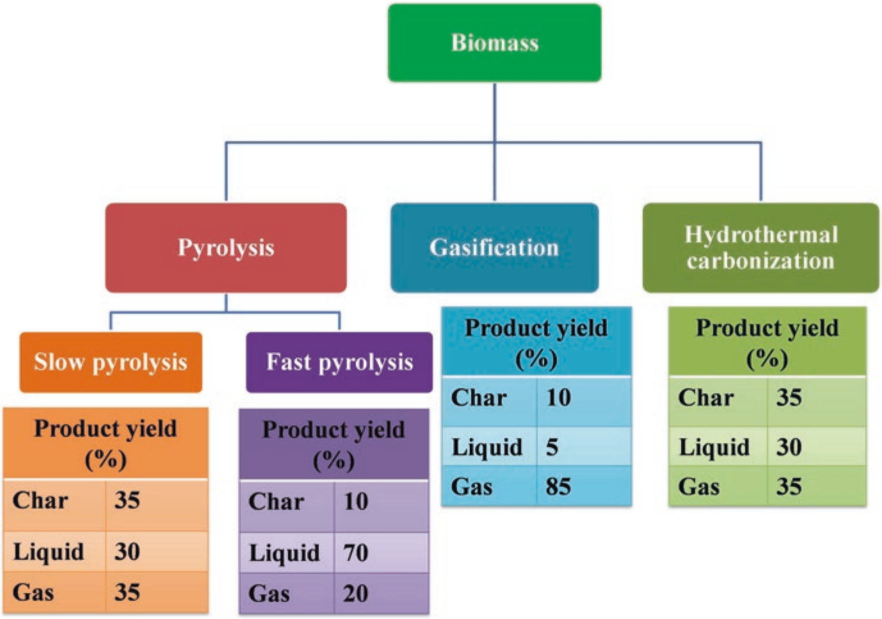


Fig. 4.3 Various thermochemical conversion techniques with their respective primary products

Table 4.1 Thermochemical process of biochar production with their primary products

Process	Temperature range (°C)	Heating rate	Residence time	Pressure	Obtained product	Reference
Slow pyrolysis	300–800	Slow (<10 °C/min, generally 5–7 °C/min)	Hours to days	Atmospheric	Biochar	Libra et al. (2014), Liu et al. (2015), Mohan et al. (2014), Parmar et al. (2014), Qian et al. (2015)
Fast pyrolysis	400–800	Fast (~1000 °C/s)	Seconds	Vacuum – Atmospheric	Bio-oil	
Gasification	700–1500	Moderate to very fast	Seconds to minutes	Elevated atmospheric	Syngas/producer gas	
Hydrothermal carbonization	175–350	Slow	Hours	Elevated atmospheric	Hydrochar	

favors high biochar yield (Liu et al. 2015). The type of reactor, peak temperature, heat transfer rate, vapor solid interactions, and type of biomass are also important factors with heating rate and residence time affecting the pyrolysis process. The mechanism of biochar formation is a complicated multistep process which includes dehydrogenation, decarboxylation, depolymerization, and devolatilization of organic components of biomass (Demirbas 2004):

Step 1 (primary):

$$\text{Biomass} \rightarrow \text{Water} + \text{Unreacted residue}$$

Step 2 (intermediate):

$$\text{Unreacted residue} \rightarrow \left(\text{Volatile} + \text{gases}\right)^1 + \left(char\right)^1$$

Step 3 (final):

$$\left(\text{Char}\right)^1 \rightarrow \left(\text{Volatile} + \text{gases}\right)^2 + \left(char\right)^2$$

At the initial stage of pyrolysis, bound moisture with lower molecular weight volatiles evaporates followed by the formation of primary char in the second step. The reaction of the third step is initially fast followed by slow decomposition and chemical rearrangement of the biochar, which makes the biochar a less reactive, residual carbonaceous solid.

4.2.2 *Gasification*

Conversion of biomass into gaseous fuel which contains CO, H_2, CO_2, and CH_4 (syngas) and smaller quantities of higher hydrocarbons in the presence of controlled amount of oxidizing agent under high temperature is known as gasification (Qian et al. 2015). The obtained gas mixture is used for direct heat or electricity generation. The oxidizing agent used can be oxygen, air, or steam. Gasification favors lower char yield and higher gas yield. During biomass gasification reaction, char gasification forms an important part. The proposed char gasification reactions are as follows (Demirbas 2004):

Reaction 1: Carbon char to methane

$$\text{C} + 2\text{H}_2 \leftrightarrow CH_4$$

Reaction 2: Carbon char to oxide

$$\text{C} + \text{O}_2 \leftrightarrow CO_2$$
$$CO_2 \leftrightarrow 2CO$$

Reaction 3: Carbon char to CO and H_2

$$\text{C} + CO_2 + \text{H}_2\text{O} \leftrightarrow CO + \text{H}_2$$

The produced CO_2 and H_2O (hot combustion products) are further reduced by the char. All the abovementioned reactions are endothermic which generate syngas, CO, and H_2 (reaction 3) which can be utilized directly as gaseous fuel. Reaction between molecules present in biomass (C, H, O) and steam (H, O) generates syngas.

4.2.3 Hydrothermal Carbonization (HTC)

The thermochemical process where biomass is heated at elevated temperature (160–800 °C) under high pressure settings is also called wet pyrolysis. For HTC the key factor is aqueous solution under pressure, which favors decomposition and carbonization of cellulose, hemicelluloses, and lignin at rather lower temperature (Parmar et al. 2014). Based on the reaction temperature, HTC can be categorized as low-temperature HTC (<300 °C) and high-temperature HTC (300–800 °C). The char produced from HTC is better known as hydrochar. The high-temperature HTC process is governed by hydrothermal gasification and gives a high yield of gases (H_2 and CH_4). Low-temperature HTC resembles natural coalification of biomass, with high reaction rate and shorter reaction time as compared to natural coalification (Qian et al. 2015).

4.3 Water Pollution with Heavy Metals and Sources

4.3.1 Heavy Metals

Heavy metals are described as metallic elements with relatively high density (more than 5 g/cm^3) and atomic weight, compared to water (Järup 2003). They are natural elements of earth's crust and also known as trace elements because of their presence in very low concentration (<10 ppm or ppb) in the environment. When the accumulation of such metal (or metalloid) species increases from a threshold limit, it will be considered as a "contaminant," where its increased undesirable concentration causes detrimental and toxic effects to the environment (Singh et al. 2011). Among various metals found naturally in the environment, some are considered as essential nutrients, as their trace concentration is necessary for physiological and biochemical functioning of living organism. In this category, total 17 such metals are identified by WHO including Co, Cu, Cr, Fe, Mg, Mn, Mo, Ni, Se, and Zn as essential nutrients (WHO 1973). However, excess intake or exposure of these metals can also impact adversely and cause cellular and tissue damage leading to pernicious effects and diseases in plants and humans (Tchounwou et al. 2012). Besides essential metals, various other metals are identified as non-essential based on the fact that they do not have any established biological contribution, but can be highly toxic even in trace amount for living organisms. Metals such as Al, As, Ba, Be, Bi, Cd, Ga, Ge, Au, In, Pb, Li, Hg, Ni, Pt, Sr, Sb, Sn, and U come under this category. Metals like As, Cd, Cr, Pb, and Hg are ranked as the most toxic and carcinogenic heavy metals with significant concern for public health. (Tchounwou et al. 2012). These metals have inherent toxic effects, even at lower level, including multiple organ damage.

4.3.2 Sources of Heavy Metal Pollution

Accumulation of various heavy metals and metalloids such as Pb, Hg, Cd, Co, Zn, Cr, As, and Cu in surface water and groundwater is linked to risk of plant, animal, and human poisoning. Despite natural occurrence of heavy metals, major environmental deposition and human exposure often happen from anthropogenic activities. Untreated industrial effluents and domestic and agricultural usage of metal and metal containing products lead to heavy metal contamination of soil and water. Leachate, solid waste and effluents from Various industries such as metal processing in refineries, leather tanning, electroplating, glass, paint, dye and colorant industry, coal burning in power plants, nuclear power stations, mining, high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants, alloy, petroleum combustion, battery industry, agricultural products manufacturing industry are the major cause of environmental deposition and contamination of various heavy metals. Besides anthropogenic activities, natural phenomena of weathering and volcanic eruptions, atmospheric deposition, and metal evaporation from water resources to soil and groundwater have also been reported to contribute significantly to heavy metal pollution (Tchounwou et al. 2012) (Fig. 4.4).

4.3.3 Approaches for Heavy Metal Remediation from Water System

Due to high toxicity and lethal effects, industries are advised to reduce heavy metal content from the effluents and wastewater, before discharging it to the environment. A number of methods and techniques are used to treat or minimize the concentration of heavy metals from discharged wastewater. Chemical precipitation, ion exchange, ultrafiltration, coagulation, membrane processing, electrochemical methods, and adsorption are some of the major techniques that are used for the treatment of heavy metal contaminated wastewater (Farooq et al. 2010). However, majority of the techniques have their own drawbacks like high operational cost, requirement of expensive materials and instrumentations, production of sludge, and high consumption of different chemicals for processing. To overcome all the abovementioned limitations, adsorption process emerged as the more efficient and attractive alternative. Adsorption of contaminants using low-cost adsorbents is the recent trend with many advantages including low cost, regeneracy, and moderate to very high efficiency. Many adsorbents like dolomite, clay, zeolite, and biosorbents derived from organic biomass, activated carbon, biochar, hydrochar, etc. have been applied for

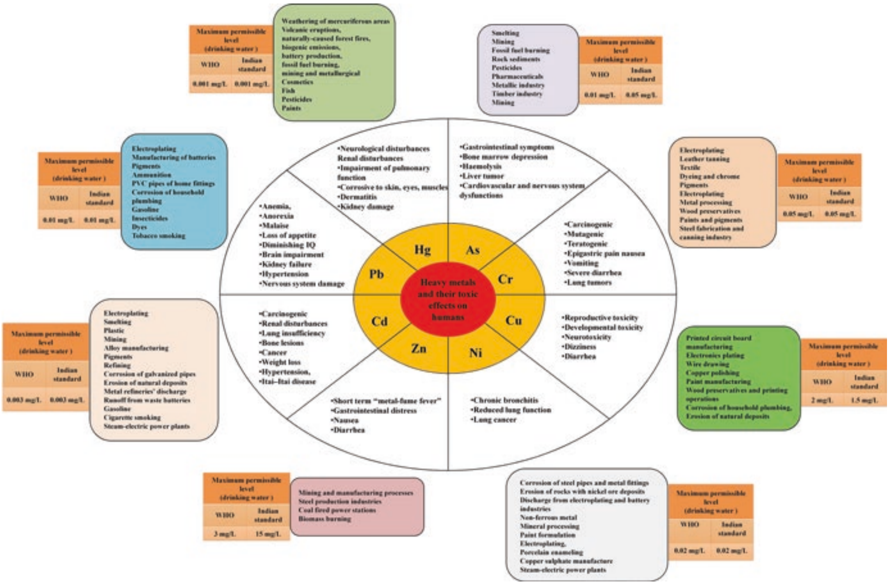


Fig. 4.4 Different heavy metals and their sources with toxic effects on human beings

heavy metal adsorption from wastewater. Among these biochar has gained much attention due to its multifunctional advantages and utility.

4.4 Biochar as a Potential Source for Heavy Metal Remediation: Characteristics Favor the Metal Remediation

Being a renewable resource with low production cost, high efficiency, reusability, environmental stability, and excellent metal binding capacity, biochar is a promising agent of environmental technology for wastewater treatment (Tan et al. 2015). Since biochar is produced from the residual or waste biomass and it does not require any chemical or physical activation process like activated carbon, it becomes a potential substitute for other available adsorbents. Abundance of raw material for biochar production and simple preparation methods are also the positive factors for making it a topmost choice. With porous structure, aromatic as well as mineral content, and various surface functional groups (-COOH, -CH, $C \equiv C$, -CO), biochar shows high affinity toward heavy metal ions (Dong et al. 2017). However, preparation conditions like heating rate, residence time, and end temperature are the key considerations which significantly affect the morphological and physicochemical properties of biochar leading to changes in the metal adsorption capacity. Here are some specific biochar properties discussed which favor metal ion adsorption.

4.4.1 Pyrolysis Conditions and Feedstock

The pyrolysis parameters including high heat temperature, retention time, and the sort of biomass used as feedstock material are the key factors which influence the biochar properties most. High pyrolytic temperature increases the carbonization, fixed carbon content, thermal stability, surface area, porosity, pH, zero point charge, aromatic content, and cation exchange capacity of biochars, while it decreases the surface functional groups (specially oxygenated). However, at high temperature (>800 °C), decrease in surface area and porosity was observed which is due to the destruction of biochar structure or blockage of pores by tar (Tan et al. 2015). The natural composition of feedstock influences the biochar adsorption properties. The same pyrolysis conditions but different feedstocks change the metal binding capacity of the biochars. A comparative analysis was done in between dairy manure and rice husk biochars for Pb, Cu, Zn, and Cd removal, and dairy manure biochar was found to be more efficient (Xu et al. 2013). Feedstock origin (plant or animal) and feedstock type processed or unprocessed have remarkable effect on metal adsorption capacity.

4.4.2 Surface Area

Biochar produced from any pyrolysis possesses high specific surface area compared to raw feedstock. Generally, the biochar prepared at elevated pyrolytic temperature possesses dramatically high surface area and porosity, a key indicator of biochar uptake capacity (Xie et al. 2015). At high temperature, the release of volatiles and decomposition of basic structural compounds of biomass like cellulose, hemicelluloses, and lignin may lead to the formation of porous structure. Biochar does possess highly variable pore size including nano- (<0.9 nm), micro- (<2 nm), and macro-pores (>50 nm). Pore size is an indispensible feature of biochar for metal sorption, as larger metallic ions and molecules cannot be trapped with small pore size, despite their charges or polarity (Dong et al. 2017).

4.4.3 Surface Charge, pH, and Cation Exchange Capacity

In aqueous adsorbate–adsorbent system, the initial solution pH strongly affects the adsorption process. For adsorption process in aqueous media, the critical factor is zero point charge (pHzpc). The pHzpc of the biochar is known as the pH at which the net surface charge of biochar becomes zero. When solution pH > pHzpc, biochar surface is negatively charged and favors the adsorption of metal cations such as Cd^{2+}, Pb^{2+}, and Hg^{2+}. If solution pH < pHzpc, biochar surface becomes positively charged, facilitating metal anion binding such as $HAsO_4^{2-}$ and $HCrO4^{-}$. With increasing pyrolysis temperature, the pH as well as pHzpc was found to be increased (Chen et al. 2014). Increased pyrolysis temperature causes decrease in the amount of negatively charged surface functional groups on biochar (-COO, -COH, and -OH) attributing to lesser negative surface and increased pHzpc. The solution pH significantly affects the behavior of surface functional groups. Lowering the solution pH causes protonation of the surface functional groups present on biochar, and biochar surface gets positive charge. The H^+ and H_3O^+ ions which are present in large number in the aqueous solution also compete with metal cations for active adsorption sites on biochar, leading to electrostatic repulsion between metal cations and positively charged biochar surface resulting in poor adsorption at low pH (Tan et al. 2015). But protonation of functional groups favors metal anion adsorption. The cation exchange capacity (CEC) is defined as the capacity of a solid to hold exchangeable cations. The CEC of biochars is relatively high due to their negative charge leading to high affinity to metal cations. The CEC of biochar is influenced by the type of biomass used as feedstock and the pyrolysis temperature (Suliman et al. 2017). At lower pyrolysis temperature, incomplete charring of biomass may occur which favors biochars to retain polar oxygenated functional groups, while at high pyrolysis temperature, alkaline nature of biochar may cause high CEC of biochar (Chen et al. 2019). This facilitates the metal ion reduction or precipitation onto biochar surface leading to decrease in their availability and mobility.

4.4.4 Non-aromatic Composition

Besides aromatic carbon content, all the biochars contain some inorganic mineral content which is called ash content. The mineral compositions comprising calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), and phosphorus (P) present in biochar are also responsible for metal sorption from aqueous matrix. These minerals react with metal ions present in water by ionic exchange or precipitate heavy metal ions leading to reduction in their availability (Cao et al. 2009; Dong et al. 2017).

4.4.5 Surface Functional Groups

The functional groups present on biochar surface such as –COOH, -OH, C=O, R-CO, etc. play important roles in metal sorption. The surface functionality of a biochar remarkably depends on pyrolysis temperature. Biochars produced at low temperature contain abundant surface functional groups, while high-temperature biochars have lesser. Lower pyrolysis temperature (< 500 °C) leads to incomplete or partial carbonization resulting in biochar with smaller pores, lower surface area, and ample oxygen-containing polar surface functional groups, making biochar more vulnerable for heavy metal removal due to increased ionic interactions between oxygenated functional groups of biochar and metal ions (Oliveira et al. 2017). Atomic ratios (H/C, O/C, and N/C) decrease, with increasing pyrolysis temperature (>500 °C), suggesting lowering of hydroxyl, carboxylic, and amino surface groups in biochar due to carbonization of raw biomass (Dong et al. 2017). The feedstock is also the other key factor which controls the extent of functional groups on biochar surface. To characterize the functional groups on biochar surface, FTIR (Fourier-transform infrared spectroscopy) spectral analysis has been the widely used technique. Reportedly, the FTIR spectra for biochars produced at different temperatures with the same biomass differ with each other (Zhou et al. 2016). Due to pyrolysis process, changes in various characteristic functional groups may takes place which include changes due to dehydration, and moisture loss during pyrolysis (3600–3100 cm^{-1}), changes in O–H, C=C, and C=O stretching of carboxylic, ester, aldehyde, and ketone groups which depicted carbonization, while changes in -CH stretching linked with cellulose and lignin present in feedstock (1560–1480 cm^{-1}), and -C-O-C, -C-O stretching associated with lignin and cellulose (1160–960 cm^{-1}) (Marx et al. 2014). Many studies show significant changes in surface functional groups after adsorption of metals like Cr(VI), Pb, Cu, Cd, Ni, and As (Farooq et al. 2010; Marx et al. 2014; Zhang and Zheng 2015; Zhang et al. 2017; Zhou et al. 2016). Table 4.2 summarizes the studies which used biochar prepared with different feedstocks at different pyrolysis temperatures and applied for different heavy metal remediation from water. Pyrolysis temperature significantly influences the adsorption potential of biochar despite the fact that the same feedstock has been used for the biochar production. At low temperature, biochar retained many polar (O-containing) surface functional groups originally present in feedstock, which bind with the metal ions present in the water. The higher-temperature biochars have high surface area and more porous structure along with some functional moieties which help biochar to retain metal ions. Interestingly, biochars produced with the same feedstock at the same temperature have different adsorption capacities for different metal ions, which leads to the conclusion that for each metal type, the response of biochar is different in the same reaction conditions. Additionally, the type of biomass used for biochar preparation has a great impact on the adsorption potential of biochar. The adsorption capacity of the same metal ion varies with variation in feedstock material. It shows that various factors govern the metal ion–biochar interactions during the adsorption process.

4.5 Heavy Metal Removal Mechanism with Biochar

The biochar interactions with different metal ions are different, and the abovementioned factors influence the process significantly. The possible mechanism of adsorption of metal ions onto biochar involves cumulative interactive effects of

Table 4.2 Summary of studies of heavy metal removal with biochar

Raw material (feedstock) for biochar production	Pyrolysis temperature (°C)	Heavy metal	Maximum adsorption capacity (mg/g)	Reference
Ramie crop residue	300	Cr(VI)	82.23	Zhou et al. (2016)
Ramie crop residue	450	Cr(VI)	72.32	Zhou et al. (2016)
Ramie crop residue	600	Cr(VI)	61.18	Zhou et al. (2016)
Wheat straw	350	Cr(VI)	24.6	Tytlak et al. (2015)
Wheat wicker	650	Cr(VI)	23.6	Tytlak et al. (2015)
Oak wood (mixed char)	400 and 450	Cr(VI)	3.031	Mohan et al. (2011)
Oak wood (mixed char)	400 and 450	Cr(VI)	4.076	Mohan et al. (2011)
Oak wood (mixed char)	400 and 450	Cr(VI)	4.930	Mohan et al. (2011)
Hard wood	450	Cu,	6.79	Chen et al. (2011)
Hard wood	450	Zn	4.54	Chen et al. (2011)
Corn straw	600	Cu	12.52	Chen et al. (2011)
Corn straw	600	Zn	11.00	Chen et al. (2011)
Spartina alterniflora	400	Cu(II)	48.49	Li et al. (2013)
Microalgae *Scenedesmus dimorphus*	500	Co(II)	0.672	Bordoloi et al. (2017)
Wood shaving	650	Cd	5.46	Qi et al. (2017)
Chicken litter	550	Cd	48.5	Qi et al. (2017)
Softwood pine	700	Cu	1.47	Jiang et al. (2016)
Softwood pine	700	Zn	1.00	Jiang et al. (2016)
Jarrah hardwood	700	Cu	4.39	Jiang et al. (2016)
Jarrah hardwood	700	Zn	2.31	Jiang et al. (2016)
S. hermaphrodita	700	Cd	35.71	Bogusz et al. (2015)
S. hermaphrodita	700	Cu	33.33	Bogusz et al. (2015)
S. hermaphrodita	700	Zn	48.08	Bogusz et al. (2015)
Pine wood (magnetic biochar)	600	As	428.2	Wang et al. (2015c)
Pine wood	600	As	265.2	Wang et al. (2015c)
Pineapple peel	350	Cr(VI)	2.49	Wang et al. (2016)
Pineapple peel	500	Cr(VI)	2.36	Wang et al. (2016)
Pineapple peel	750	Cr(VI)	7.44	Wang et al. (2016)
Celery biomass	350	Pb	288	Zhang et al. (2017)
Celery biomass	500	Pb	304	Zhang et al. (2017)
Plum kernel	500	Pb (II)	38.77	Pap et al. (2018)
Apricot kernel	500	Pb (II)	23.89	Pap et al. (2018)
Apricot kernel	500	Cr (III)	12.68	Pap et al. (2018)
Corn bran	300	Cr (VI)	37.35	Zhang and Zheng (2015)
Corn bran	400	Cr (VI)	39.75	Zhang and Zheng (2015)
Corn bran	500	Cr (VI)	41.48	Zhang and Zheng (2015)

(continued)

Table 4.2 (continued)

Raw material (feedstock) for biochar production	Pyrolysis temperature (°C)	Heavy metal	Maximum adsorption capacity (mg/g)	Reference
Corn bran	600	Cr (VI)	42.83	Zhang and Zheng (2015)
Anaerobic digestion sludge	600	Pb	53.96	Ho et al. (2017)
Coconut coir	250	Cr(VI)	31.1	Shen et al. (2012)
Coconut coir	350	Cr(VI)	10.9	Shen et al. (2012)
Coconut coir	500	Cr(VI)	7.9	Shen et al. (2012)
Coconut coir	600	Cr(VI)	4.1	Shen et al. (2012)
Melia azedarach wood (magnetic biochar)	400	Cr(VI)	25.27	Zhang et al. (2018)
Municipal sewage sludge	900	Cr(III)	25.27	Chen et al. (2015)
Corn cob	350	Cd	30.76	Luo et al. (2018)
Corn cob	450	Cd	23.53	Luo et al. (2018)
Corn cob	550	Cd	33.36	Luo et al. (2018)
Corn cob (modified biochar with acrylonitrile)	350	Cd	144.09	Luo et al. (2018)
Corn cob (modified biochar with acrylonitrile)	450	Cd	39.83	Luo et al. (2018)
Corn cob (modified biochar with acrylonitrile)	550	Cd	26.99	Luo et al. (2018)
Municipal solid waste	400	As	24.20	Jin et al. (2014)
Municipal solid waste	500	As	24.49	Jin et al. (2014)
Municipal solid waste	600	As	18.06	Jin et al. (2014)
Corn straw	500	Hg	1.94	Tan et al. (2016)
Corn straw (Na_2S modified biochar)	500	Hg	5.71	Tan et al. (2016)
Corn straw (KOH modified biochar)	500	Hg	4.27	Tan et al. (2016)
Hickory wood ($KMnO_4$ treated)	600	Pb	153.1	Wang et al. (2015a)
Hickory wood ($KMnO_4$ treated)	600	Cu	34.2	Wang et al. (2015a)
Hickory wood ($KMnO_4$ treated)	600	Cd	28.1	Wang et al. (2015a)

several phenomena. Physical adsorption and various chemical reactions like ion exchange, electrostatic attraction, reduction-coupled sorption, surface complexation, and surface perception are the proposed interactions (Ahmad et al. 2013; Dong et al. 2017; Inyang et al. 2016; Tan et al. 2015). During adsorption process, physical as well as chemical interactions can simultaneously take place including various complex reactions between metal ions and biochar. Figure 4.5 and Table 4.3 summarize the possible metal–biochar interactions and mechanism.

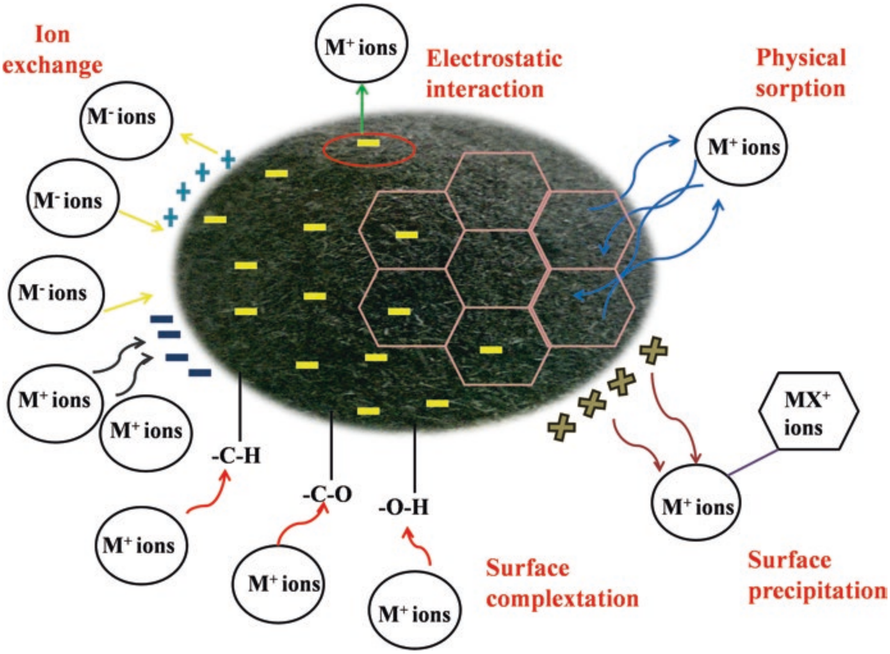


Fig. 4.5 Proposed mechanism of heavy metal adsorption with biochar

Table 4.3 Mechanism of biochar and metal ion interactions

Mechanism	Type of interactions of ions and biochar	Factors affecting the process
Physical sorption	Diffusion of metal ions into biochar pores without any chemical interactions. Diffusion can be of either pore or film diffusion or combination of both	Surface pore size is the important factor
Ion exchange	Exchange of dissolved metal ions with ionizable cations present on biochar surface	Cation exchange capacity and pH of the solution play important roles in this kind of interaction
	The metal ion size and surface functional group chemistry (specially –COO⁻ and OH⁻) of the biochar are the key factors in this mechanism for efficient retaining of the metal	Selective replacement of cations present on biochar surface with targeted metal species (Inyang et al. 2016)
Complexation	Formation of multi-atom metal–ligand interaction. More favorable in the case of transition metals having partially filled d-orbital with high affinity for ligands	Surface functional groups with soft basic nature and substitution reactions with strong interaction between acid-base components play role

(continued)

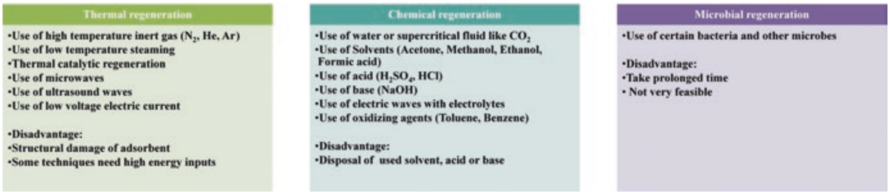
Table 4.3 (continued)

Mechanism	Type of interactions of ions and biochar	Factors affecting the process
Electrostatic interactions	Protonation and deprotonation of surface functional group (–COO, -CH, and -OH). If solution pH > pHzpc, biochar surface is negatively charged and favors the cationic adsorption. When solution pH < pHzpc, biochar surface is positively charged facilitating metal anion binding	Solution pH and pHzpc play the key role
Precipitation	Mineral (ash) content present in biochar reacts with metal ions which may cause precipitation of the metal ions. Generally, high-temperature biochars contain more residual matters (ash/minerals), which help to bind with the metal ions and immobilize them	High biochar pH (alkaline), alkaline pH of the solution
Reduction-coupled sorption	In the case of Cr(VI) adsorption, Cr(VI) reduced to Cr(III) when it comes in contact with organic matter. The functional groups of biochar surface like –OH, -CHO, and -COOH work as electron donors	Presence of oxidizing functional groups

4.6 Regeneration or Reusability of Biochar

For metal remediation, use of biochar/activated carbon is a feasible process in terms of production cost and handing. To be environmentally friendly and economically feasible in view of cost and recycling, an adsorbent should have repetitive usability for industrial applications (Ifthikar et al. 2018). The continuous accumulation of adsorbate on the surface of adsorbent causes the gradual decrease in adsorption capacity of the material. The concept of regeneration process is based on "removal" and "recovery": removal of adsorbate from exhausted adsorbent and recovery of the original sorption capacity of adsorbent without compromising its properties (Salvador et al. 2015). However, reuse of adsorbent with the same adsorption capacity and retrieval of metals from the exhausted adsorbent are challenging tasks and a matter of further exploration at present. Reusability of exhausted adsorbents especially in the case of magnetic, engineered, designer, composite, or activated carbon/biochar is much more important in terms of the extra inputs and production cost. Desorption and decomposition can be the possible two ways to regenerate the exhausted adsorbents once used for contaminant adsorption (Dai et al. 2019). Desorption or decomposition can be achieved by using various thermal, chemical, and microbiological methods (Fig. 4.6).

The regeneration potential can be measured with the following parameters: desorption efficiency or extraction efficiency and regeneration efficiency. Desorption efficiency (Eq. 4.1) generally depicted the efficiency of the method and is calculated



Thermal regeneration

- Use of high temperature inert gas (N_2, He, Ar)
- Use of low temperature steaming
- Thermal catalytic regeneration
- Use of microwaves
- Use of ultrasound waves
- Use of low voltage electric current

- Disadvantage:
- Structural damage of adsorbent
- Some techniques need high energy inputs

Chemical regeneration

- Use of water or supercritical fluid like CO_2
- Use of Solvents (Acetone, Methanol, Ethanol, Formic acid)
- Use of acid (H_2SO_4, HCl)
- Use of base (NaOH)
- Use of electric waves with electrolytes
- Use of oxidizing agents (Toluene, Benzene)

- Disadvantage:
- Disposal of used solvent, acid or base

Microbial regeneration

- Use of certain bacteria and other microbes

- Disadvantage:
- Take prolonged time
- Not very feasible

Fig. 4.6 Regeneration/reusability methods (summerized from Salvador et al. 2015)

as the relationship between the amounts of adsorbate removed (desorb) and adsorbate retained on the exhausted adsorbate (Salvador et al. 2015). Regeneration efficiency (Eq. 4.2) is calculated as the ratio of amount of adsorbate retained by the regenerated adsorbent and the original adsorbent in the same conditions (Salvador et al. 2015).

$$\text{Desorption efficiency}\left(\%\right)=\left(Q_d / Q_a\right)\times 100 \tag{4.1}$$

$$\text{Regeneration efficiency}\left(\%\right)=\left(Q_R / Q_a\right)\times 100 \tag{4.2}$$

where Q_d is the amount of desorbed metal in mg, Q_R is the adsorption capacity of regenerated adsorbent, and Q_a is the amount of adsorbed metal on original adsorbent in per unit mass.

Generally, adsorbents exhausted with heavy metal are treated with various inorganic solvents, acid (HCl, H_2SO_4, H_3PO_4) or base (NaOH), for the desorption of metal from adsorbent (acid–base method) with 0.5–1 mol/L concentration. However, some organic solvents like acetone, ethanol, and benzene as well as tap water and distilled water were also used for the regeneration of biochar/activated carbon. Several studies on the regeneration of exhausted biochar/activated carbon with heavy metal employed different eluents. Wang et al. (2015b), used 0.1 mol/L HCL, acetic acid (HAc), EDTA-Na_2, and distilled water for the regeneration of magnetic biochar and found reduction in Pb(II) adsorption capacity only in the first regeneration cycle with EDTA-Na_2 and that further cycles do not affect the Pb(II) removal efficiency (Wang et al. 2015b). Fan et al. (2019) modified sewage sludge biochar with nanozero-valent (nZVI) iron and used it for Cr(VI) remediation from water. They further repyrolyzed the exhausted nZVI biochar at 600 °C for 0.5 h. The authors reported slight increase in total Cr in biochar; however, significant decrease in toxic Cr(VI) was reported (Fan et al. 2019). In most of the studies in general trend, the re-adsorption capacity of biochar/AC gradually decreases with the subsequent cycles of regenerations. However, regenerated biochar showed notable re-adsorption capacity of metal with more than 50% re-adsorption (Banerjee et al. 2016; Cherdchoo et al. 2019).

On lab scale, use of desorbing agent is a widely tried application, but for industrial scale, thermal regeneration process is the most used. Heating of exhausted activated carbon to provide the amount of energy necessary to remove the retained adsorbate is known as thermal regeneration (Salvador et al. 2015). Use of electric current, applying steam and purging with high-temperature inert gas (800–900 °C) to remove pollutants from exhausted adsorbent, use of (metal) catalysts, and application of microwaves and ultrasound waves are parts of the thermal process. However, these methods certainly cause structural destruction and blockage of the pores which lead to the lowering of the potential of adsorbent. Use of microbes for the degradation of adsorbate and recovery of adsorption potential of biochar/AC come under microbial degradation, generally applied for the adsorbent used for organic pollutant remediation.

4.7 Biochar as a Potential Precursor for Activated Carbon and Designer Adsorbents

Activated carbon (AC) is produced from any carbon source which was intentionally engineered for sorption of contaminants. With high surface area and enhanced adsorption capacity, activated carbon is extensively applied for adsorption purposes (Guo et al. 2003; Zbair et al. 2018). Besides sorption, AC is used as a catalyst and as an electrode in microbial fuel cells. Many activation processes like chemical activation (treatment with H_3PO_4, HCl, NaOH, KOH, K_2CO_3), physical activation (steam, CO_2, or plasma treatment), and activation with other agents such as $ZnCl_2$ can be applied for making the biochar as activated carbon. Activation process will enhance the adsorption capacity of biochar by increasing its surface area manyfold (>500 m^2/g compared to 10 m^2/g of precursor biochar with steam activation) and pore size (Azargohar and Dalai 2005). Generally, preparation of activated carbon with biochar is a two-step process: (i) carbonization of biomass under deoxygenated conditions and (ii) activation of the prepared biochar with physical or chemical method (Qian et al. 2015). However, a one-step chemical activation process can also be used for AC synthesis. For one-step chemical activation, the raw material was activated by the chemical agent and then pyrolyzed, although, due to environmental concerns, physical activation is advantageous as it does not require any chemical agent. Also, as can be fetched from Table 4.2, adsorption capacity of biochar increases manyfold when it is used as a precursor material for activated carbon or engineered biochar. Thus, various methods can be applied to biochar to modify its physicochemical properties to enhance its metal adsorption capacity. Such engineered or designer biochars would result in cost-effective and efficient alternatives to commercial activated carbons.

4.8 Biochar as a Strategy for Solid Waste Management

Biochar can be produced with various enormously available by-products and biomass which is considered as bio-waste. Majority of the raw materials are from agro-industrial waste products, sewage sludge, and household garbage which will be dumped to landfill sites, ultimately increasing the greenhouse gas (GHG) emissions. Biochar production with such kind of material is an economical and beneficial environmental management strategy with added advantage of carbon sequestration. High carbon content biochar prepared with waste feedstock can also be used as soil conditioning agent. Eventually, this strategy would also help to cut down the disposal cost by providing a low-cost, environmentally friendly alternative to the existing commercial and synthetic process and activated carbon (Niazi et al. 2016). During pyrolysis, microbes present in biomass particularly like animal manure and sewage sludge get killed due to high heat treatment which reduces the associated environmental health risks (Ahmad et al. 2013). In addition to activation, nanoparticle incorporation also increases the biochar surface area, resulting in better metal ion sorption capacity.

4.9 Conclusion and Future Scope

Biochar obtained from the residual and waste biomass of agro-food processing sector, municipal sewage waste, forestry residues, etc. could be an economical and sustainable stratagem for wastewater treatment. For the preparation of an efficient biochar, certain factors should always be taken into consideration including the purpose of biochar production, end use of the biochar, heat treatment conditions, and feedstock material. For efficient heavy metal remediation from water by employing biochar, a group of factors works together including solution pH, biochar's CEC, pHzpc, surface functional groups, surface area, and porosity.

On pilot scale, high efficiency for the removal of various metals from simulated water/wastewater has been achieved, and further efforts are ongoing. The next necessity is to conduct the large-scale field studies to establish feasibility of the use of the biochar for heavy metal contaminated wastewater treatment. Also, many heavy metals react and combine with other organic moieties in water which is also a matter of great concern. Studies directed toward remediation of such metal–organic combination using biochar are sorely needed to be explored. The final disposal of exhausted biochar also is an important issue to be addressed. Wastewater from industrial discharge mostly contains a combination of different kinds of heavy metals, and understanding their interaction with biochar opens new vistas to be explored in the future.

References

Agrafioti E, Kalderis D, Diamadopoulos E (2014) Arsenic and chromium removal from water using biochars derived from rice husk, organic solid wastes and sewage sludge. J Environ Manag 133:309–314

Ahmad M, Rajapaksha AU, Lim JE, Zhang M, Bolan N, Mohan D, Vithanage M, Lee SS, Ok YS (2013) Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere 99:19–33

Azargohar R, Dalai A (2005) Production of activated carbon from Luscar char: experimental and modeling studies. Microporous Mesoporous Mater 85(3):219–225

Banerjee S, Mukherjee S, LaminKa-ot A, Joshi S, Mandal T, Halder G (2016) Biosorptive uptake of Fe2+, Cu2+ and As5+ by activated biochar derived from Colocasia esculenta: isotherm, kinetics, thermodynamics, and cost estimation. J Adv Res 7(5):597–610

Bogusz A, Oleszczuk P, Dobrowolski R (2015) Application of laboratory prepared and commercially available biochars to adsorption of cadmium, copper and zinc ions from water. Bioresour Technol 196:540–549

Bordoloi N, Goswami R, Kumar M, Kataki R (2017) Biosorption of co (II) from aqueous solution using algal biochar: kinetics and isotherm studies. Bioresour Technol 244:1465–1469

Cao X, Ma L, Gao B, Harris W (2009) Dairy-manure derived biochar effectively sorbs lead and atrazine. Environ Sci Technol 49(9):3285–3291

Chen X, Chen G, Chen L, Chen Y, Lehmann J, McBride MB, Hay AG (2011) Adsorption of copper and zinc by biochars produced from pyrolysis of hardwood and corn straw in aqueous solution. Bioresour Technol 102(19):8877–8884

Chen T, Zhang Y, Wang H, Lu W, Zhou Z, Zhang Y, Ren L (2014) Influence of pyrolysis temperature on characteristics and heavy metal adsorptive performance of biochar derived from municipal sewage sludge. Bioresour Technol 164:47–54

Chen T, Zhou Z, Xu S, Wang H, Lu W (2015) Adsorption behavior comparison of trivalent and hexavalent chromium on biochar derived from municipal sludge. Bioresour Technol 190:388–394

Chen W, Meng J, Han X, Lan Y, Zhang W (2019) Past, present, and future of biochar. Biochar 1:75–87. https://link.springer.com/article/10.1007/s42773-019-00008-3

Cherdchoo W, Nithettham S, Charoenpanich J (2019) Removal of Cr(VI) from synthetic wastewater by adsorption onto coffee ground and mixed waste tea. Chemosphere 221:758–767

Dai Y, Zhang N, Xing C, Cui Q, Sun Q (2019) The adsorption, regeneration and engineering applications of biochar for removal organic pollutants: a review. Chemosphere 223:12–27. https://www.sciencedirect.com/science/article/pii/S0045653519301778

Demirbas A (2004) Effects of temperature and particle size on bio-char yield from pyrolysis of agricultural residues. J Anal Appl Pyrolysis 72(2):243–248

Dong X, da Silva EB, de Oliveira LM, Chen Y, Ma LQ (2017) Mechanisms of metal sorption by biochars: biochar characteristics and modifications. Chemosphere 178:466–478

Fan Z, Zhang Q, Gao B, Li M, Liu C, Qiu Y (2019) Removal of hexavalent chromium by biochar supported nZVI composite: batch and fixed-bed column evaluations, mechanisms, and secondary contamination prevention. Chemosphere 217:85–94

Farooq U, Kozinski JA, Khan MA, Athar M (2010) Biosorption of heavy metal ions using wheat based biosorbents–a review of the recent literature. Bioresour Technol 101(14):5043–5053

Guo Y, Qi J, Yang S, Yu K, Wang Z, Xu H (2003) Adsorption of Cr (VI) on micro-and mesoporous rice husk-based active carbon. Mater Chem Phys 78(1):132–137

Ho SH, Chen YD, Yang ZK, Nagarajan D, Chang JS, Ren NQ (2017) High-efficiency removal of lead from wastewater by biochar derived from anaerobic digestion sludge. Bioresour Technol 246:142–149

IBI (2014) IBI_Biochar Standards. 2

Ifthikar J, Jiao X, Ngambia A, Wang T, Khan A, Jawad A, Xue Q, Liu L, Chen Z (2018) Facile one-pot synthesis of sustainable carboxymethyl chitosan–sewage sludge biochar for effective heavy metal chelation and regeneration. Bioresour Technol 262:22–31

Inyang MI, Gao B, Yao Y, Xue Y, Zimmerman A, Mosa A, Pullammanappallil P, Ok YS, Cao X (2016) A review of biochar as a low-cost adsorbent for aqueous heavy metal removal. Crit Rev Environ Sci Technol 46(4):406–433

Järup L (2003) Hazards of heavy metal contamination. Br Med Bull 68(1):167–182

Jiang S, Huang L, Nguyen TAH, Ok YS, Rudolph V, Yang H, Zhang D (2016) Copper and zinc adsorption by softwood and hardwood biochars under elevated sulphate-induced salinity and acidic pH conditions. Chemosphere 142:64–71

Jin H, Capareda S, Chang Z, Gao J, Xu Y, Zhang J (2014) Biochar pyrolytically produced from municipal solid wastes for aqueous As(V) removal: adsorption property and its improvement with KOH activation. Bioresour Technol 169:622–629

Kołodyńska D, Wnętrzak R, Leahy JJ, Hayes MHB, Kwapiński W, Hubicki Z (2012) Kinetic and adsorptive characterization of biochar in metal ions removal. Chem Eng J 197:295–305

Lehmann J (2007) A handful of carbon. Nature 447:143–144

Lehmann J, Joseph S (2015) Biochar for environmental management: science, technology and implementation. Routledge, Oxon

Li M, Liu Q, Guo L, Zhang Y, Lou Z, Wang Y, Qian G (2013) Cu(II) removal from aqueous solution by Spartina alterniflora derived biochar. Bioresour Technol 141:83–88

Libra JA, Ro KS, Kammann C, Funke A, Berge ND, Neubauer Y, Titirici M-M, Fühner C, Bens O, Kern J, Emmerich K-H (2014) Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis. Biofuels 2(1):71–106

Lima IM, Boateng AA, Klasson KT (2010) Physicochemical and adsorptive properties of fast-pyrolysis bio-chars and their steam activated counterparts. J Chem Technol Biotechnol:n/a–n/a

Lima I, Ro K, Reddy G, Boykin D, Klasson K (2015) Efficacy of chicken litter and wood biochars and their activated counterparts in heavy metal clean up from wastewater. Agriculture 5(3):806–825

Liu W-J, Jiang H, Yu H-Q (2015) Development of biochar-based functional materials: toward a sustainable platform carbon material. Chem Rev 115(22):12251–12285

Luo M, Lin H, Li B, Dong Y, He Y, Wang L (2018) A novel modification of lignin on corncob-based biochar to enhance removal of cadmium from water. Bioresour Technol 259:312–318

Ma Y, Liu WJ, Zhang N, Li YS, Jiang H, Sheng GP (2014) Polyethylenimine modified biochar adsorbent for hexavalent chromium removal from the aqueous solution. Bioresour Technol 169:403–408

Marx S, Chiyanzu I, Piyo N (2014) Influence of reaction atmosphere and solvent on biochar yield and characteristics. Bioresour Technol 164:177–183

Mohan D, Rajput S, Singh VK, Steele PH, Pittman CU Jr (2011) Modeling and evaluation of chromium remediation from water using low cost bio-char, a green adsorbent. J Hazard Mater 188(1–3):319–333

Mohan D, Sarswat A, Ok YS, Pittman CU (2014) Organic and inorganic contaminants removal from water with biochar, a renewable, low cost and sustainable adsorbent–a critical review. Bioresour Technol 160:191–202

Nartey OD, Zhao B (2014) Biochar preparation, characterization, and adsorptive capacity and its effect on bioavailability of contaminants: an overview. Adv Mater Sci Eng 2014:1–12

Niazi NK, Murtaza B, Bibi I, Shahid M, White J, Nawaz M, Bashir S, Shakoor M, Choppala G, Murtaza G (2016) Removal and recovery of metals by biosorbents and biochars derived from biowastes. In: Environmental materials and waste. Elsevier, pp 149–177

Oliveira FR, Patel AK, Jaisi DP, Adhikari S, Lu H, Khanal SK (2017) Environmental application of biochar: current status and perspectives. Bioresour Technol 246:110–122

Pap S, Bezanovic V, Radonic J, Babic A, Saric S, Adamovic D, Sekulic MT (2018) Synthesis of highly-efficient functionalized biochars from fruit industry waste biomass for the removal of chromium and lead. J Mol Liq 268:315–325

Parmar A, Nema PK, Agarwal T (2014) Biochar production from agro-food industry residues: a sustainable approach for soil and environmental management. Curr Sci 107(10)

Qi F, Yan Y, Lamb D, Naidu R, Bolan NS, Liu Y, Ok YS, Donne SW, Semple KT (2017) Thermal stability of biochar and its effects on cadmium sorption capacity. Bioresour Technol 246:48–56

Qian K, Kumar A, Zhang H, Bellmer D, Huhnke R (2015) Recent advances in utilization of biochar. Renew Sust Energ Rev 42:1055–1064

Rajapaksha AU, Alam MS, Chen N, Alessi DS, Igalavithana AD, Tsang DCW, Ok YS (2018) Removal of hexavalent chromium in aqueous solutions using biochar: chemical and spectroscopic investigations. Sci Total Environ 625:1567–1573

Reza R, Singh G (2010) Heavy metal contamination and its indexing approach for river water. Int J Environ Sci Technol 7(4):785–792

Salvador F, Martin-Sanchez N, Sanchez-Hernandez R, Sanchez-Montero MJ, Izquierdo C (2015) Regeneration of carbonaceous adsorbents. Part I: thermal regeneration. Microporous Mesoporous Mater 202:259–276

Shen YS, Wang SL, Tzou YM, Yan YY, Kuan WH (2012) Removal of hexavalent Cr by coconut coir and derived chars – the effect of surface functionality. Bioresour Technol 104:165–172

Singh R, Gautam N, Mishra A, Gupta R (2011) Heavy metals and living systems: an overview. Indian J Pharmacol 43(3):246

Suliman W, Harsh JB, Fortuna A-M, Garcia-Pérez M, Abu-Lail NI (2017) Quantitative effects of biochar oxidation and pyrolysis temperature on the transport of pathogenic and nonpathogenic Escherichia coli in biochar-amended sand columns. Environ Sci Technol 51(9):5071–5081

Tan X, Liu Y, Zeng G, Wang X, Hu X, Gu Y, Yang Z (2015) Application of biochar for the removal of pollutants from aqueous solutions. Chemosphere 125:70–85

Tan G, Sun W, Xu Y, Wang H, Xu N (2016) Sorption of mercury (II) and atrazine by biochar, modified biochars and biochar based activated carbon in aqueous solution. Bioresour Technol 211:727–735

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy metal toxicity and the environment. In: Molecular, clinical and environmental toxicology. Springer, Basel, pp 133–164

Tytlak A, Oleszczuk P, Dobrowolski R (2015) Sorption and desorption of Cr(VI) ions from water by biochars in different environmental conditions. Environ Sci Pollut Res Int 22(8):5985–5994

Wang H, Gao B, Wang S, Fang J, Xue Y, Yang K (2015a) Removal of Pb(II), Cu(II), and Cd(II) from aqueous solutions by biochar derived from KMnO4 treated hickory wood. Bioresour Technol 197:356–362

Wang S-y, Tang Y-k, Chen C, Wu J-t, Huang Z, Mo Y-y, Zhang K-x, Chen J-b (2015b) Regeneration of magnetic biochar derived from eucalyptus leaf residue for lead (II) removal. Bioresour Technol 186:360–364

Wang S, Gao B, Zimmerman AR, Li Y, Ma L, Harris WG, Migliaccio KW (2015c) Removal of arsenic by magnetic biochar prepared from pinewood and natural hematite. Bioresour Technol 175:391–395

Wang C, Gu L, Liu X, Zhang X, Cao L, Hu X (2016) Sorption behavior of Cr(VI) on pineapple-peel-derived biochar and the influence of coexisting pyrene. Int Biodeterior Biodegradation 111:78–84

WHO (1973) Trace elements in human nutrition: report of a WHO expert committee [Meeting held in Geneva from 9 to 17 April 1973]

Xie T, Reddy KR, Wang C, Yargicoglu E, Spokas K (2015) Characteristics and applications of biochar for environmental remediation: a review. Crit Rev Environ Sci Technol 45(9):939–969

Xu X, Cao X, Zhao L (2013) Comparison of rice husk-and dairy manure-derived biochars for simultaneously removing heavy metals from aqueous solutions: role of mineral components in biochars. Chemosphere 92(8):955–961

Zbair M, Anfar Z, Ahsaine HA, El Alem N, Ezahri M (2018) Acridine orange adsorption by zinc oxide/almond shell activated carbon composite: operational factors, mechanism and performance optimization using central composite design and surface modeling. J Environ Manag 206:383–397

Zhang J, Zheng P (2015) A preliminary investigation of the mechanism of hexavalent chromium removal by corn-bran residue and derived chars. RSC Adv 5(23):17768–17774

Zhang T, Zhu X, Shi L, Li J, Li S, Lu J, Li Y (2017) Efficient removal of lead from solution by celery-derived biochars rich in alkaline minerals. Bioresour Technol 235:185–192

Zhang X, Lv L, Qin Y, Xu M, Jia X, Chen Z (2018) Removal of aqueous Cr(VI) by a magnetic biochar derived from Melia azedarach wood. Bioresour Technol 256:1–10

Zhou L, Liu Y, Liu S, Yin Y, Zeng G, Tan X, Hu X, Hu X, Jiang L, Ding Y (2016) Investigation of the adsorption-reduction mechanisms of hexavalent chromium by ramie biochars of different pyrolytic temperatures. Bioresour Technol 218:351–359

Chapter 5
Microplastics: An Emerging Threat to the Aquatic Ecosystem

Arti Srivastava and Ashutosh Srivastava

Contents

Abstract Plastic pollution in the aquatic ecosystem has been a growing environmental problem worldwide where it has pervaded everywhere even in the most remote areas. Plastic particles (smaller than 5 mm; microplastics) have been accumulated at higher level (i.e., 100,000 particles per m^3) in the environment. They are transferring from low trophic fauna to higher level organisms via food chain resulting in adverse consequences for the health of aquatic biota. Various countries have implemented different policies to overwhelm the effect of plastic contamination. However in some countries, it needs to be effectively implemented. Present chapter

---

A. Srivastava
Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Noida, India
e-mail: asrivastava@amity.edu

A. Srivastava (✉)
Amity Institute of Biotechnology, Amity University, Uttar Pradesh, Noida, India

Amity Institute of Marine Science and Technology, Amity University,
Uttar Pradesh, Noida, India
e-mail: asrivastava4@amity.edu

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_5

focuses on various aspects related with the microplastics. Some main points that have been discussed in this chapter are (1) types of microplastics, major sources, and the way of transportation through which they are reaching into the aquatic ecosystem; (2) occurrence of microplastics in water bodies and sediments; (3) ingestion and the adverse impact caused by microplastics in the marine organisms; and (4) different policies executed by various countries to overwhelm its effects. This chapter has given an overview on different facets of microplastics that might help in the future research and proper management of microplastics.

Keywords Microplastics · Aquatic ecosystem · Plastic litters · Polymers · Bioaccumulation

5.1 Introduction

Plastic is a common term that states the family of organic polymers including polyethylene (PE), nylon, polystyrene (PS), polyvinylchloride (PVC), and polypropylene (PP) (Anderson et al. 2016; Andrady 2011). Their size might be from a few micrometers to several millimeters and shapes varied from spherical to elongated fibers (Thompson 2015). Global plastic production (i.e., synthetic organic polymers) has been grown exponentially during the last few decades. Production of plastics has reached from 225 million tons to 322 million tons in between 2004 and 2015 (Plastics Europe 2016), and its usage patterns suggest that the production and quantity of plastics will have to be increase globally (Wright et al. 2013). Owing to a myriad of applications, it is not surprising that human activities continuously increase the pressure of plastic and pose a threat to the health of world's sea and ocean (Hammer et al. 2012).

Plastics are dispersing over a long distance due to their buoyancy and finally got settled in the sediments where they present for a long duration (Goldberg 1997). Presence of plastic in aquatic system is considered to be most problematic as plastic polymers show minimal biodegradation and are a potential vector for transferring the persistent bioaccumulatives and toxic substances (Neves et al. 2015; Engler 2012; Cole et al. 2011; Sivan 2011). It remains in the environment for thousands of years, where they do not ever fully go away but rather break down into the smaller pieces by various physical factors such as ultraviolet radiations, ocean currents, wave actions, and hydrolysis (Moore 2008). These small fragments of plastics are known as microplastics (0.1 μm to 5 mm in size) and nanoplastics (0.001–0.01 μm) (GESAMP 2015; Galloway et al. 2017). Ingestion of microplastics and nanoplastics caused potential changes in the integrity of the habitats and therefore becomes an emerging area of research (Auta et al. 2017; Horton et al. 2017b; Sruthy and Ramasamy 2017; Wright et al. 2013).

Microplastics/nanoplastics are generated by numerous domestic and industrial activities; enter into marine ecosystem via wastewater, river, or water current; and hence can be considered as a prime source for these litters in the marine habitat (Thompson et al. 2005; Moore 2008; Haegerbaeumer et al. 2018). Effluents of sewage treatment plants are also responsible for the occurrence of great amount of polyester and acrylic fibers into marine water ecosystem (Gatidou et al. 2019). Cheung and Fok (2017) assessed that 306.9 tons of microbeads per year are released into the environment from Mainland China, where 80% are contributed from the effluents of sewage treatment plants (STPs). The microplastics in the form of bio-beads are primarily present in STPs as a part of filtration process. These bio-beads provide a high surface area substrate for the attachment of a biofilm of bacteria used to digest compounds such as ammonia (Turner et al. 2019). Other vital sources might be the disintegration of larger plastics into smaller microsize fragments due to the culmination of various physical, chemical, and biological processes (Cole et al. 2011). Indeed further work indicates a wide range of habitats (Law et al. 2010; Van Cauwenberghe et al. 2013; Gall and Thompson 2015; de Souza Machado et al. 2018) and organisms (Graham and Thompson 2009; Lusher et al. 2013; Tanaka and Takada 2016; Caron et al. 2018; Choi et al. 2018) that are affected by the accumulation of microplastics in the environment. Figure 5.1 has shown an overview on the types and fate of microplastics along with their ingestion by various aquatic organisms. Another concern has been rising recently at the scientific community that is

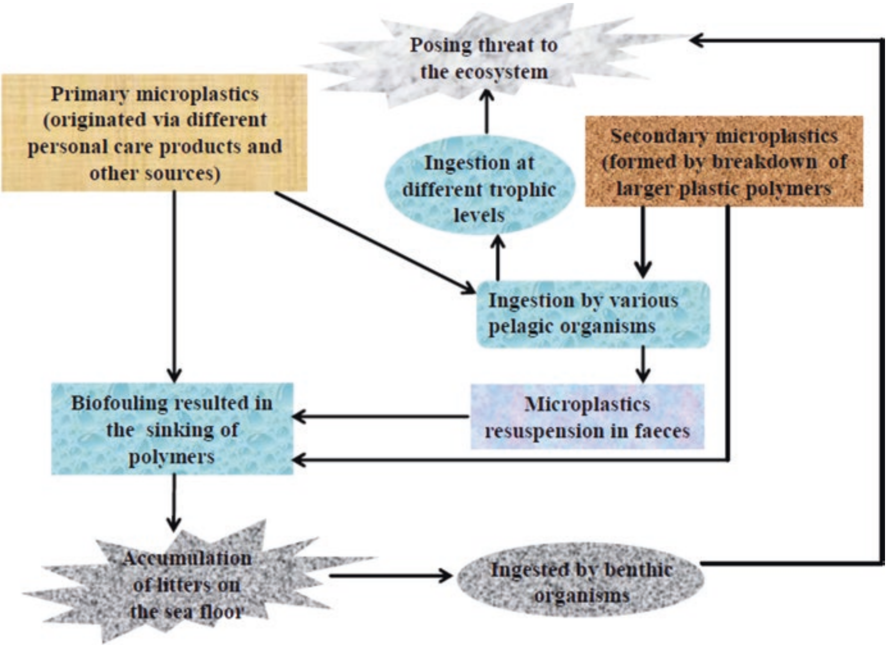


Fig. 5.1 An overall representation of types of microplastics, fate, and their ingestion by various aquatic fauna

the presence of hydrophobic organic pollutants and metals on the microplastics fragments adsorbed from the surrounding seawater (Engler 2012; Andrady 2011; Endo et al. 2005). Other issue is the presence of chemical additives in most of the plastics that are toxic to various marine organisms because it might be released into organisms upon ingestion (Hermabessiere et al. 2017).

Microplastics/nanoplastics are dispersing throughout the aquatic ecosystem whether it is shoreline, benthic sediments, beaches, surface water, or all over the water column (Auta et al. 2017; Gallagher et al. 2016; Wright et al. 2013). Moreover, they have pervaded even in Arctic and Antarctic environment and transported by water current and wind (Auta et al. 2017; Barnes et al. 2010; Lusher et al. 2015). Non biodegradable nature and size of the microplastic makes them bioavailable to wide range of aquatic organisms (Sharma and Chatterjee 2017; Shim and Thompson 2015). Zooplanktons, copepods, corals, bivalves, worms, shrimp, lobsters, sea urchin, sea birds, turtle, fish, and whales have been reported to ingest the microplastics (Auta et al. 2017; Sharma and Chatterjee 2017; Van Cauwenberghe et al. 2015b). Consequently, these persistent microplastics may bioaccumulate and bio-magnify through the food chain whereby poses a risk of physical injury to the intestinal tract along with its translocation to other tissues or organs (Auta et al. 2017; Sharma and Chatterjee 2017; Van Cauwenberghe et al. 2015b; Wright et al. 2013).

Unremitting increasing loads of these tiny debris in the water bodies resulted in growing efforts to understand their toxic effects on the aquatic biota. Available literatures reveal the potential of microplastics to cause various toxic effect, viz., oxidative stress, reduction of feeding activity, impairment in reproductive performance, genotoxicity, and general decrease in fitness (e.g., reduced survival and fecundity) (Anbumani and Kakkar 2018; Gatidou et al. 2019; Guzzetti et al. 2018; Prata et al. 2019; Strungaru et al. 2019). Yangtze River in China is considered as the largest plastic export river to the ocean due to its high population density (Xiong et al. 2019). Other author also reported high abundance of microplastics in Yangtze River (Wang et al. 2017). It is well-known that a huge amount of plastics are transported to ocean through the rivers. Various authors reported the presence of microplastics in marine water worldwide (Brach et al. 2018; Eriksen et al. 2013, 2018; Goldstein et al. 2013; Kanhai et al. 2017; Lusher et al. 2015; Poulain et al. 2018). Hence, based on the previous literatures, the aim of the present chapter is to compile the available information that is relevant to understand the term microplastics and nanoplastics. We have defined the distribution, the accumulation, and the consequences of microplastics in the aquatic organisms. Finally, we discuss the policies related with the use of the plastics. Present information will definitely help to give an insight on the future research as well as the proper management of microplastics/nanoplastics pollution.

5.2 Microplastics: Background

Plastic debris in the aquatic environment is considered as one of the most serious concerns worldwide. They are broadly classified into following sizes: mega-plastic (> 100 mm), macroplastics (> 20 mm), meso-plastics (20–5 mm), microplastics (0.1 μm to 5 mm), and nanoplastics (0.001–0.1 μm) (Horton et al. 2017b; Haegerbaeumer et al. 2018). Among these the small-sized micro- and nanoplastic particles have emerged as a "novel" topic for research in the scientific community over the last few years due to its potential threat to human health and biota. As a term, they cover a broad definition that includes a wide range of polymers, sizes of particles, and the polymer densities. Total nine types of polymers of microplastics were identified (Thompson et al. 2004) that differ in size, shape, color, density, and charges (Duis and Coors 2016; Van Cauwenberghe et al. 2015b). Microplastics/nanoplastics can be either primary, directly released in the environment in the form of small plastic pellets, or, secondary, formed by the breakdown of the larger plastic fragments in the coastal and/or oceanic environments (Thompson et al. 2004, 2005; Alimi et al. 2018).

Primary microplastics are intentionally manufactured for particular industrial or domestic applications having the pellet size in the range of 1 nm to 5 mm (Ogunola and Palanisami 2016). Primary microplastics have the applications in the personal care products such as toothpaste, facial cleanser, scrubs, shower gels, shaving cream, deodorant, and cosmetics that include eyeshadow, blush powder, nail polish, mascara, foundations, sunscreen, and baby products (Auta et al. 2017; Cole et al. 2011; Costa et al. 2010; Fendall and Sewell 2009; Zitko and Hanlon 1991).

Other sources of microplastics in the environment include the synthetic fibers spread from the domestic washings (Cesa et al. 2017). They are also used in various other areas like in medical and used as a dentist tooth polish and as a carrier to deliver the pharmaceutical agents (Duis and Coors 2016). Microplastics also have the applications in the abrasive industries (shot blasting) and can be used for drilling fluids (Gregory 1996; Derraik 2002). These small pellets directly released into the aquatic ecosystem either after accidental loss during transportation or from processing facilities because of improper handling (Duis and Coors 2016).

Secondary microplastics can be described as the small plastic particles formed by breakdown of the larger plastic litters both in aquatic system and on land (Cole et al. 2011; Ryan et al. 2009). Photodegradation or oxidation of plastic debris can be caused by the prolong exposure to sunlight. Cleavage of the bond reduces the average molecular weight of polymers that will make it weak and brittle (Andrady 2011). Any mechanical forces such as abrasion, wave action, or turbulence can break the highly degraded and embrittled plastic fragments into the smaller fragments (Horton et al. 2017b; Ivar do Sul et al. 2013). These smaller plastic fragments are further susceptible to many small organisms for biodegradation (i.e., microbial degradation) (Skariyachan et al. 2017; Singh et al. 2016; Shim and Thompson 2015).

Previous studies showed that most of the microplastics and nanoplastics in the marine environment are due to the breakage of larger plastic polymers (Waller et al. 2017; Jiang 2018; Alimi et al. 2018). These smaller fragments enhance the potential threat to the marine ecosystem by increasing their susceptibly to various toxicants (hydrophobic organic chemicals and heavy metals) and bioavailability for numerous microbial communities (Chua et al. 2014; Avio et al. 2015; Brennecke et al. 2016; Reisser et al. 2014).

5.3 Sources and Transport of Microplastics in the Aquatic Ecosystem

There are various sources for the pollution of microplastics into the aquatic ecosystems; they are reaching either directly or indirectly (Ryan et al. 2009). Effluents from the sewage treatment are also a critical source for the microplastics contamination into the aquatic ecosystems. It has been estimated that about 8 trillion pieces of MP (including microfibers) enters in the aquatic environment via wastewater treatment plant (Rochman et al. 2015). Plastic litters that do not degrade during the initial filtering steps will remain in the sludge or effluents. These effluents are eventually released into the rivers, whereas sludge may be applied to the agricultural land (DEFRA 2012). They are ultimately entering the ocean from inland waterways, wastewater outflows, and the wind (Cieślik et al. 2015; Tibbetts 2015; Browne et al. 2011; Zubris and Richards 2005). It has been observed that rivers are contributing approximately 70–80% of plastics in the oceans due to mishandled debris during manufacture and use, from agriculture, land, and WWTP effluent (Alimi et al. 2018).

Another significant source for secondary microplastics comprises the fragmented macroplastic litters derived from low-density plastic like bags, milk/juice jugs, rope, netting, packaging and high-density particles such as food containers, beverage bottles and films (Medrano et al. 2015), particle runoff from roads (small pieces of tier), or parts of vehicles (Kole et al. 2017; Hidalgo-Ruz et al. 2012). There is positive relationship between the level of commercial fishing and the number of ocean-based plastic items (Li et al. 2016a). They float at the specific depth within the ocean from “ghost fishing” and ultimately ingested by the aquatic organisms (Lozano and Mouat 2009). Paints used for the road-marking or applying to the boat hulls for antifouling formulations, once abraded from the surface, may also enter into the aquatic environment (Imhof et al. 2016; Song et al. 2014; Takahashi et al. 2012).

All ecosystems whether it is freshwater, terrestrial, or marine water are polluted with synthetic microfibers (Horton et al. 2017a). Agricultural waste originated from the plastic mulches (used to protect the crops from unfavorable growing conditions) and polytunnels (used to control the temperature and moisture) after fragmentation adds a sources for the microplastics in the aquatic ecosystem (Steinmetz et al. 2016;

Kasirajan and Ngouajio 2012; Kyrikou and Briassoulis 2007). Furthermore, other plastic products used in the agriculture including packaging, storage, irrigation tapes and pipes, agrochemical containers, rain shelters, vineyard covering, netting, and pots for the ornamental plants also have the potential for their dispersal within the environment (Scarascia-Mugnozza et al. 2012). Overall the sources of microplastics litters into the aquatic environment are numerous and varied.

5.4 Prevalence of Microplastics in Different Ecosystems

From the last few decades, several studies have been performed to investigate the presence of microplastics around the world (Esiukova 2017; Wessel et al. 2016; Bråte et al. 2016; Reisser et al. 2015). They might float on the water, be present in the mid-water column, or occur on the sediments (Andrady 2011). Microplastics are present everywhere whether it is aquatic or terrestrial habitats (Barnes et al. 2009; Thompson et al. 2009); even it can also pervade the world's most remote zones (Van Cauwenberghe et al. 2013; Woodall et al. 2014). They can be also present in the Arctic and the Antarctic regions of the world (Bergmann et al. 2017; Peeken et al. 2018). The buoyant plastic fragments can be deposited in the pelagic habitat, whereas the non-floating fragments accumulate on the floor of deep sea and sediments of the beach to pose the threat for the respective populations (Imhof et al. 2013).

5.4.1 Presence of Microplastics in Aquatic Ecosystem

Presence of microplastics in the aquatic ecosystem has been well documented. Several studies have been performed to evaluate the presence of microplastics into the aquatic ecosystems (Barrows et al. 2018; Sighicelli et al. 2018; Waller et al. 2017). As compared to marine water, less data is available on the presence of microplastics in the freshwater ecosystem (Kalčikova et al. 2017). However, the attention might be turning toward freshwater environment due to its significant role in microplastics pollution in the coastal and marine environment (Costa and Barletta 2015).

Table 5.1 has summarized the presence of the microplastics in both fresh- and marine water column. Some of the previous studies have focused on the rivers such as Danube, Rhine-Main, and Thames and showed that the transport of microplastics also is influenced by the changes in the flow of water due to bends, particularly in deep or shallow sections (Klein et al. 2015; Morritt et al. 2014; Lechner et al. 2014).

Study of microplastics in the surface water samples of Italian Subalpine Lakes was performed, which provides the reference for better understanding of microplastics levels in the inland freshwaters (Sighicelli et al. 2018). Polyethylene (45%), polystyrene (18%), and polypropylene (15%) were the most prevalent polymers of microplastics among the studied waters samples. Pyrolysis–gas chromatography/

Table 5.1 Sampling locations, compartment, concentration, or quantity present in aquatic system, polymer types, mesh size, and reference of corresponding studies

Location	Compartment	Concentration or quantity	Polymer type	Mesh size	References
North Atlantic subtropical gyre	Surface water	18,000 particles/km^2	–	300 µm	Brach et al. (2018)
Northeast Atlantic Ocean	Subsurface water	185.6 particles/m^3	–	250 µm	Lusher et al. (2014)
North Atlantic Ocean	Water column	70.8 particles/m^3	PET, acrylic, acrylic+ cellulose, polyester, PA,		Courtene-Jones et al. (2017)
North Atlantic gyre	Surface water	1.69 particles/m^3	–	150 µm	Reisser et al. (2015)
Northeastern section of Qatar’s	Surface water	~0.71 particles/m^3	LDPE, PE, PS, PA, ABS, PMMA, cellophane	120 µm	Castillo et al. (2016)
North Atlantic subtropical gyre	Water column	29,850 particles	PE, PP	330 µm	Kooi et al. (2016)
North Atlantic subtropical gyre	Surface water	13 to 501 particles/m^3	PE, PP, PS, PA, PMMA, PU, PVC, PES	50 µm	Enders et al. (2015)
Northeast Pacific Ocean	Surface water	0.832 particles/m^2	–	333 µm	Goldstein et al. (2013)
South Pacific subtropical gyre		26,898 particles/km^2	PS, PP	333 lm	Eriksen et al. (2013)
Mediterranean Sea	Surface water	1.25 particles/m^2	LDP, HDP	200 µm	Suaria et al. (2016)
Arabian gulf	Surface water	4.04×10^5 particles/km^2	–	300 µm	Abayomi et al. (2017)
Bay of Biscay to Cape Town	Subsurface water	499 particles	Polyester, blends, PA, PP, acrylic, PVC, PS, PU	250 µm	Kanhai et al. (2017)
South Pacific	Surface water	100,000 particles/km^2	–	335 µm	Eriksen et al. (2018)
Bay of Bengal	Surface water	10,000 particles/km^2	–	335 µm	Eriksen et al. (2018)
Arctic waters	Surface water	0.34 particles/m^3	Polyester, PA, PE, PVC, acrylic, cellulose	250 µm	Lusher et al. (2015)
Arctic waters	Subsurface water	2.68 particles/m^3	Polyester, PA, PE, PVC, acrylic, cellulose	250 µm	Lusher et al. (2015)

(continued)

Table 5.1 (continued)

Location	Compartment	Concentration or quantity	Polymer type	Mesh size	References
NW Spanish Atlantic coast	Surface water	0.285 particles/m^2	–	333 μm	Gago et al. (2015)
East China Sea	Surface water	0.167 particles/m^3	–	32 μm	Zhao et al. (2014)
Yangtze Estuary, China	Surface water	4137.3 particles/m^3	–	32 μm	Zhao et al. (2014)
Western Lake	Surface water	91–3538 particles/km^2	PVC, PP, PE, PET	333 μm	Hendrickson et al. (2018)
Lake Michigan	Surface water	~1400 to 100,000 particles/km^2	PE, HDPE, PP, LDPE	333 μm	Mason et al. (2016)

mass spectrometry (Pyr-GC/MS) and Fourier-transform infrared spectroscopy (FTIR) were used to quantify and identify the microplastics particles from the surface waters of western Lake Superior (Hendrickson et al. 2018). Polyvinyl chloride was the most frequently observed polymer, while most common polymer identified by FTIR was polyethylene. Wang et al. (2017) investigated microplastics level in the urban waters of Wuhan, China, and reported microplastics concentrations ranged from 1660 ± 639.1 n/m^3 to 8925 ± 1591 n/m^3 in 22 surface water samples. They identified two dominant types of polymers i.e., polyethylene terephthalate and polypropylene with more than 80% of microplastics in size less than 2 mm. Urbanization is one of the key factors that influenced the presence of microplastics in the freshwater environment.

Indeed, with the widespread contamination of microplastics in the freshwater ecosystem, marine water also polluted with these tiny particles (Barrows et al. 2018; Waller et al. 2017; Lusher et al. 2015). Microplastics concentration and distribution were evaluated in Bohai Sea (Zhang et al. 2017), to provide an understanding upon their potential effects on the environment. Authors reported the average concentration of microplastics, i.e., 0.33 ± 0.34 particles/m^3 with polyethylene, polypropylene, and polystyrene the most prevalent polymers by using μ-FTIR spectroscopy. Fragments, lines, and films were mostly present in the collected samples.

In the previous studies, presence of microplastics in the seawater was mostly studied in the Northern hemisphere (85%) where the north coast of Europe was the most prevalent area (Gago et al. 2018). Eriksen et al. (2014) found 26,898 particles/km^2 plastic fragments ranged from 0.355 mm up to 4.750 mm in the South Pacific subtropical gyre. However in the contrast region, i.e., the North Pacific subtropical gyre, Moore et al. (2002) found more than 33,000 particles/km^2 plastic fragments ranged in size from 0.355 mm up to 4.760 mm. Density and distribution of plastic accumulation in the oceanic environment are affected by both natural activities and anthropogenic activities (Kanehiro et al. 1995). Abundance and distribution of plastic debris in the marine environment are also attributable by some of human activities such as tourism as well as presence of large metropolitan areas (Galgani et al. 2000). Bryant et al. (2016) observed the microplastics ranged from 0.35 to 3.7 particles m^{-3} in the surface seawater of North Pacific Gyre.

Fibers, blue nodules, orange chips, and various other shapes of microplastics ranging from 38 to 234 particles/m^3 were present in the Arctic Ocean (Obbard et al. 2014). Increased level of floating plastics have been observed in Antarctic and Arctic water (Barnes et al. 2010; Lusher et al. 2015); hence they can be considered as a microplastics accumulative zones. Similarly, Courtene-Jones et al. (2017) reported the high level of microplastics (70.8 particles m^{-3}) in the deep-sea water of Northeast Atlantic Ocean. Along with water microplastics are also present on sediments of beaches and seafloor. Most of the anthropogenic activities are responsible for the abundance of plastic debris on the coastal areas. Presence of various hydrophobic organic pollutants and microbial communities can increase the density of plastic fragment that eventually sink down at the sea floor and poses the adverse effect to the deep-sea marine biota.

5.4.2 Presence of Microplastics in the Sediments

Tidal waves and the oscillations of the waterline play a major role for the distribution of microplastics along the beach of marine ecosystems (Hengstmann et al. 2018; Moreira et al. 2016). At river sediments of Shanghai, the average microplastics concentration was measured (802 items per kilogram of dry weight) by using density separation, microscopic inspection, and μFTIR. Total seven different types of polymers were identified, of which polypropylene was the most prevailing polymer (Peng et al. 2018). Near the Pearl river of China (Fok and Cheung 2015), plastic fragments and pellets were reported on 25 beaches of Hong Kong. Expanded PS (92%), fragments (5%), and pellets (3%) were present in more than 90% of microplastics (0.315–5 mm). Authors reported the mean microplastics abundance of 5595 items/m^2, since it is higher as compared to the international averages that make the Hong Kong as a hotspot for marine plastic pollution.

At the lake of Bolsena and Chiusi, Central Italy (Fischer et al. 2016), both fragments and fibers were identified that measured about 0.3 to 0.5 mm at the Lake Chiusi and < 0.3 mm at the Lake Bolsena sediments. Furthermore, they also reported 112 particles/kg dry weight and 234 particles/kg dry weights of microplastics from the Lake Bolsena and Lake Chiusi, respectively. This difference may be attributed by higher organic content and a shift in the grain size distribution.

By analyzing the seven locations in the northern Gulf of Mexico estuary, Wessel et al. (2016) most often observed the polymers in the form of the polypropylene and polyethylene. Their study also provides a new insight into the distribution and accumulation of the microplastics. They found significant difference between the two locations since the values of microplastics abundance ranged from a minimum of 5 pieces per square at site 2 to a maximum of 117 pieces per square meter at site 7. This vast diversity in the presence of microplastics at different locations may be due to the exposure from marine currents and tides (Wessel et al. 2016).

On the Baltic coast, two high-density polymers, viz., PET and PVC, are analyzed by using density separation via a glass elutriation column (Hengstmann et al. 2018). Authors found 93.8 particles kg^{-1} dry sediment (most of them smaller than 1 mm) at plateau of the beaches on the Baltic Sea. One of the most important factors that might affect the concentration of microplastics on beach sediments is the degree of urbanization near the area used for its assessment (Hengstmann et al. 2018). Bergmann et al. (2017) analyzed nine sediment samples taken from the Hausgarten observatory and found the microplastics contamination in the range of 42–6595 particles kg^{-1} dry sediment, where PE, PA, and PP were the most prevalent polymers.

Sandy beaches of Mumbai have been recognized as one of the most polluted beaches due to various recreational, religious, and fishing activities. There was high abundance of the microplastics, i.e., 7.49 g and 68.83 items m^{-2}, where the small particles (1–20 mm) were predominant with 41.85% of microplastics, having the size in the range of 1–5 mm. These small particles have high potential for the ingestion by the marine organisms that pose a threat for their transfer within the food web (Jayasiri et al. 2013). Heavy fouling of microplastics might be a possible mechanism for their sinking at the pristine environments of the deep sea. Presence of microplastics particles in the beach sediments from subalpine Lake Garda, Italy, was examined by Imhof et al. (2013); five different polymers were identified in the range of 9–500 μm by using Raman microspectroscopy. Polystyrene (45.6%) and polyethylene (43.1%) were prevalent polymers, originated mainly from rope, fishing gear, and carpets (Claessens et al. 2011).

5.5 Uptake and Potential Impacts Cause by Contamination of Microplastics in Aquatic Organisms

Bioavailability of microplastics entirely depends upon their quantity present in the environment. The bioavailability of microplastics for different organisms is continuously increasing due to increase in plastic production day by day. The physical properties such as color, size, shape, density, and abundance of these small particles affect their ingestion by the marine organisms (Auta et al. 2017; Wright et al. 2013). Table 5.2 has summarized the ingestion of microplastics by various marine organisms.

Mostly the microplastics are ingested by the organisms because it looks like their prey (Boerger et al. 2010). Several studies have been conducted on the ingestion of microplastics by marine fauna; however most of the studies restricted to controlled laboratory experiments. Ingestion of microplastics has many physical impacts to organisms like blockage of intestinal tract, starvation, gastric rapture, and loss of fitness with decreased in secretion of gastric enzymes, reduction of steroid hormone secretion, delays in ovulation, and failure of reproduction (Auta et al. 2017; Li et al. 2016a; de Stephanis et al. 2013; Tanaka et al. 2013; Wright et al. 2013; McCauley and Bjorndal 1999).

Table 5.2 Sampling location, microplastics ingestion by marine organisms, presence of microplastics (percentage), and reference of corresponding studies

Location	Marine organism	Occurrence of microplastics (%)	Polymer type	References
Mussel				
Culture farms along the coastline of China	*Crassostrea gigas, Crassostrea angulate*, *Crassostrea hongkongensis*, and *Crassostrea sikamea*	84	CP, PE, PET, PP, PA, PS, PC and PVC	Teng et al. (2019)
Southeast coast of India	*Perna viridis*	–	Polystyrene	Naidu (2019)
South Korea	*Crassostrea gigas*, *Mytilus edulis, Tapes philippinarum, Patinopecten yessoensis*	95	PE, PP, PS, polyester, PET, PEVA, PTFE,PVC,PPS,PBT,SAN,SEBS	Cho et al. (2019)
Fishery farm area of East China Sea	*Mytilus edulis*	42–59 (through adherence)	**–**	Kolandhasamy et al. (2018)
Coastal waters of China	*Mytilus edulis*	0.9–4.6 items/g	CP, PET, PES	Li et al. (2016b)
Fish				
Adriatic Sea	*Solea solea*	95	PP, PE and PA	Pellini et al. (2018)
Western Mediterranean Sea	*Sardina pilchardus* and *Engraulis encrasicolus*	14.28–15.24	PET	Compa et al. (2018)
Mallorca Island	*Mullus surmuletus*	27.3	PET	Alomar et al. (2017)
Mediterranean Sea	*Xiphias gladius*, *Thunnus thynnus*, and *Thunnus alalunga*	18.2	–	Romeo et al. (2015)
Northeastern Pacific Ocean	*Ammodytes personatus* and *Clupea pallasii*	1.5 and 2	–	Hipfner et al. (2018)
Seabird				
Oregon and Washington beaches	Northern fulmar and sooty shearwater	8.5 and 33	–	Terepocki et al. (2017)
Labrador Sea	Northern fulmars	79	–	Avery-Gomma et al. (2018)

Sable Island	Northern fulmars, sooty shearwaters, and great shearwaters	> 72	–	Bond et al. (2014)
Eastern North Pacific	Northern fulmars	92.5	–	Avery-Gomm et al. (2012)
Turtle				
Brazilian coast	Sea turtles	39	PET	de Carvalho et al. (2015)
North Atlantic subtropical gyre	Sea turtles	83	PE, PP	Pham et al. (2017)
Marine mammal				
Irish waters	Baleen whales, fin whales, humpback whale, True's beaked whale	8840.00%	–	Lusher et al. (2018)
Southwest Atlantic Ocean	*Arctocephalus australis*	90	–	Denuncio et al. (2017)
North Sea coast	*Physeter macrocephalus*	40.9	–	Unger et al. (2016)
North and west coasts of Ireland	*Mesoplodon mirus*	85%	PP, PE, rayon, polyester, acrylic	Lusher et al. (2015)
Sympatric area of Atlantic Ocean	*Pontoporia blainvillei* and *Sotalia guianensis*	15.7 and 1.3	–	Di Beneditto and Ramos (2014)

Microplastics are consumed through the foraging by numerous organisms including zooplanktons, invertebrates, fishes, amphibians, turtles, seabirds, and mammals. In particular, fish and seabirds are the most widely studied organisms across the marine ecosystem (Phuong et al. 2016). Existing studies have shown the ingestion of microplastics debris in a wide range of species at numerous organizational levels and with different feeding practices, including detritivores, filter feeders, and predators (Horton et al. 2017b).

5.5.1 Bioavailability and Effects of Microplastics on Invertebrates

Marine biota is explored widely for studying the impact of microplastics as compared to freshwater organisms, especially for benthic organisms (Ziajahromi et al. 2018). Microplastics are ingested by both wild and cultured invertebrates such as phytoplanktons (Nerland et al. 2014), zooplanktons (Ziajahromi et al. 2017; Gago et al. 2018), bivalves (Kolandhasamy et al. 2018; Naidu et al. 2018; Bour et al. 2018; Green 2016; Rochman et al. 2015), lobster (Murray and Cowie 2011), crabs (Watts et al. 2014), shrimps (Devriese et al. 2015), and corals (Reichert et al. 2018; Hall et al. 2015). Most of the studies on microplastics ingestion by marine invertebrates have been carried out in controlled laboratory condition. Thompson et al. (2004) studied the contamination of microplastics in detritivores, deposit feeders, and filter feeders. They found that marine invertebrates like amphipods, lugworms, and barnacles consumed microplastics within the few days of exposure. *Mytilus edulis* is one of the most established model organisms for the study of ingestion of microplastics by the invertebrates. Recently, Vandermeersch et al. (2015) investigated the presence of microplastics particles in caged mussels, *Mytilus edulis*. Microplastics (20–30 μm) with average concentration of 0.2 particles per gram tissue were observed in *Mytilus edulis* (Van Cauwenberghe et al. 2015a). Likewise, Van Cauwenberghe and Janssen (2014) detected the presence of microplastics from the soft tissues of *Mytilus edulis* (0.36 ± 0.07 particles per gram wet weight) and *Crassostrea gigas* (0.47 ± 0.16 particles per gram wet weight). Presence of high-density polyethylene particles (0–80 μm) was also observed in the gills and digestive gland of *Mytilus edulis* (von Moos et al. 2012). Authors found the presence of the microplastics in the gill tissues that were transferred into the digestive glands via stomach. Bråte et al. (2018) studied the behavior of tiny PE particles once they entered into the marine ecosystem. They investigated that the ingestion of weathered particles was significantly higher than the virgin; however there was no significant difference in the degree of damage caused by weathered and virgin PE particles. Moreover, the uptake of 30 nm polystyrene (PS) beads by *Mytilus edulis* showed its ability to ingest the nanoparticles along with microplastics (Wegner et al. 2012).

Researchers have also studied the ingestion of the microplastics on other mollusk such as brown mussel (*Perna perna*), Mediterranean mussel (*Mytilus galloprovin-*

cialis), oyster (*Crassostrea gigas*), clam (*Venerupis philippinarum*), and snail (*Assiminea grayana*) (Bråte et al. 2018; Santana et al. 2018; Watermann et al. 2017; Gandara et al. 2016; Davidson and Dudas 2016; Cole and Galloway 2015). Microplastics once ingested by the lower trophic organisms can pass at higher level by the food chain. Therefore the contaminated mussels might have a great risk for the humans as the transfer of microplastics from *Mytilus edulis* to higher trophic levels has been already known (Farrell and Nelson 2013). Microplastics accumulation at tissue and cellular level can result in an inflammatory response in the tissues and decreased membrane stability in the cells of digestive tract (Medrano et al. 2015).

Microplastics ingestion was also studied in some other phyla of marine invertebrates such as Echinodermata, Arthropoda, and Cnidarian (Taylor et al. 2016). Authors observed the presence of microfibers in various deep-sea organisms having different feeding mechanisms ranges from zoanthids, sea pen, armored sea cucumbers (suspension feeders), sea cucumber (deposit feeders) and hermit crabs, and squat lobsters (detritivores and predators). These microfibers primarily present either in oral areas, feeding apparatus, tentacles, gills, or stomach area of different organisms. Presence of microplastics was also reported in some of the benthic holothurians including *Thyonella gemmata, Holothuria grisea, Holothuria floridana, and Cucumaria frondosa* (Wright et al. 2013). Similarly Graham and Thompson (2009) also demonstrated the ingestion of PVC and nylon fragments (0.25–15 mm) by four species of deposit and suspension feeder sea cucumbers (Echinodermata, Holothuroidea) over sediment grains.

The entanglement of irregular-shaped microplastics form the filament balls inside the stomach and resulted in the gut blockage of predatory crustacean *Nephrops norvegicus* (Murray and Cowie 2011). Most of the plastic fragments were the tangled nylon strand balls. Watts et al. (2014) found the uptake of microspheres via both food source and water column in the shore crab (*Carcinus maenas*). Microplastics in sediment samples were equivalent to the fecal pellets of two polychaete species (*Alitta virens* and *Clymenella torquata)*, suggesting the ingestion and egestion of microplastics are equal in these organisms (Mathalon and Hill 2014). Size-based acceptance of microplastics was found in marine polychaete, *Arenicola marina* (Zebe and Schiedek 1996). Microplastics accumulation in the digestive tract can result in reduction of feeding or false sanitation in *Arenicola marina* (Medrano et al. 2015). Benthic detritivores are also dependent on the fecal pellets as their food source; hence the presence of microplastics in the fecal casts can play a potential role for its transfer within the benthic food web.

In pelagic habitat, microplastics are consumed by various zooplanktonic taxa (Ziajahromi et al. 2017; Frydkjær et al. 2017; Setälä et al. 2014). Ingestion of regular- and irregular-shaped polyethylene microplastics was studied in the freshwater flea, *Daphnia magna* (Frydkjær et al. 2017). Presence of microplastics (size 20–1000 nm) in the oil storage droplets has been observed in the freshwater flea *Daphnia magna* (Rosenkranz et al. 2009). Presence of microplastics in the copepods resulted in reduced feeding suggesting the adverse impact of microplastics on the zooplanktons (Cole et al. 2013). Transfer of ingested microplastics via meso-zooplanktons to higher trophic level was observed during the food web transfer experiments (Setälä et al. 2014). Since zooplanktons have the ability to pass the energy from lower trophic level to higher trophic level, the bioaccumulation of these tiny plastics in these organisms might pose a serious threat for the higher organisms.

5.5.2 Bioavailability and Effects of Microplastics on Vertebrates

Most of the literatures have focused on the ingestion of microplastics by fishes and seabirds (Compa et al. 2018; Neves et al. 2015; McGoran et al. 2017; Floren and Shugart 2017). Uptake of both macro- and microplastics debris have been observed in the gastrointestinal tract of many species of fish from different locations, including western Mediterranean Sea (Compa et al. 2018), Northeastern Pacific Ocean (Hipfner et al. 2018), South Pacific subtropical gyres (Ory et al. 2017), and North Sea (Smith 2018; Hermsen et al. 2017).

Ingestion of the microplastics by teleost fish has been known from the beginning of the 1970s (Carpenter et al. 1972). They reported the ingestion of polystyrene spherules (0.5 mm in diameter) by the larvae and juvenile of *Pseudopleuronectes flounder* and adult of *Morone America* and *Prionotus evolans* from the coastal waters of southern New England. Hoss and Settle (1990) observed the ingestion of plastic pellets (100–500 μm) by the early stage of fish in a controlled laboratory experiments. Early stages of the fish larvae are more vulnerable to the ingestion of microplastics (Steer et al. 2017). Bessa et al. (2018) investigated the presence of microplastics in three commercial fish species; in which 38% of the total fishes ingested the microplastics, where the fibers (96%) were the most prevalent. To reveal the presence of anthropogenic contaminant on the seafloor habitats, Alomar and Deudero (2017) investigated the presence of microplastics in the shark *Galeus melastomus*. Authors found the ingestion of microplastics in 16.80% of total *Galeus melastomus* from the western Mediterranean Sea.

It is well established that whether it is benthic or pelagic fishes, both are contaminating with the presence of microplastics. Neves et al. (2015) observed the microplastics ingestion in 63.5% of the benthic and 36.5% of the pelagic fish species. Fibers (65.8%) were the most prevalent among all types of plastics, and particles were mostly ingested by the pelagic fish, while the benthic fish mostly ingested the fibers. Natural fibers (83%) were the most common type of microplastics observed from the gastrointestinal tract of two pelagic fish species (14.28–15.24%), *Sardina pilchardus* and *Engraulis encrasicolus*, at the western Mediterranean Sea (Compa et al. 2018). Plastic fragment (size, 0.04–4.88 mm) contamination was reported in 2.6% of common fish from the North Sea (Foekema et al. 2013). In this study, the physical effects were insignificant because there was no intestinal blockage due to the low amount and small size of the microplastics. While in another study, fish contained high amount of synthetic polymers (36.5%) in their gastrointestinal tract (Lusher et al. 2013). Blockage of the digestive tract is more common in fish due to the ingestion of high amount of plastic debris (Hoss and Settle 1990). Jovanović et al. (2018) observed low retention of virgin microplastics in the gastrointestinal tract, suggesting a nonsignificant accumulation and effective elimination of virgin microplastics from the body of *Sparus aurata*. Large plastic items can cause suffocation and entanglement and disrupt digestion in the higher organisms (Thompson et al. 2004).

At higher trophic level, seabirds are probably one of the most vulnerable species due to surface foraging that increases the probability of more microplastics ingestion (Li et al. 2015). They ingest the microplastics both directly and indirectly (via fish that have already consumed microplastics) (Medrano et al. 2015). It increases the chance for the accumulation of plastics in the gastrointestinal tract that leads to the blockage of tract eventually resulting in problem with the feeding stimuli (Li et al. 2016a). Susceptibility of plastic ingestion is more in seabirds due to the foraging at the surface of water. Seabirds consumed the small neutonic plastic debris in the confusion with their prey (Avery-Gomm et al. 2013). Occurrence of plastics in the Northern fulmars was higher as compared to other seabird species (Acampora et al. 2016; Poon et al. 2017). Presence of plastics in 79% of the northern fulmars was observed from the Labrador Sea (Avery-Gomma et al. 2018). Trevail et al. (2015) observed the plastics in the stomach of 87.5% of fulmars. Another study done in between 2009 and 2010 showed 92.5% of the northern fulmars consumed the plastics particles (Avery-Gomm et al. 2012).

Other birds such as fledgling Cory's shearwaters (*Calonectris diomedea*) from the eastern North Atlantic Sea and Laysan Albatrosses *(Phoebastria immutabilis*) and black-footed Albatrosses (*Phoebastria nigripes)* near the Hawaiian Islands were also contaminated with the plastics litters (Gray et al. 2012). Previous studies also showed the occurrence of plastics litter in the remote areas such as Arctic region. More than 80% of northern fulmars (*Fulmarus glacialis*) consumed microplastics from the Canadian Arctic (Provencher et al. 2009). Amélineau et al. (2016) studied the presence of microplastics in the zooplanktivorous bird species little auks (*Alle alle*) from the Arctic region. They revealed the presence of high abundances of filaments (97.2%) in the sea birds as compared to other plastics. This zooplanktivorous species could not distinguish between zooplanktons and microplastics that increase the susceptibility of plastic ingestion in these species (Avery-Gomm et al. 2012).

Studies on the occurrence of microplastics in the marine mammals are restricted. Presence of microplastics in baleen whales, deep-diving whales, and delphinids was analyzed by Lusher et al. (2018), who identified that 88.4% of cetaceans were contaminated with the plastics debris. Fibers (83.6%) and fragments (16.4%) were the most prevalent type of microplastics. Abundance of microplastics in the neustonic samples of the Mediterranean Sea showed a potential risk for the large filter feeders like baleen whale who have the foraging on small particles by their skimming action (Frydkjær et al. 2017). Presence of microplastics in the pelagic environment resulted in the occurrence of these litters in large filter feeding marine organisms such as Mediterranean fin whale and basking shark (Fossi et al. 2014, 2016). Gastric rapture is the most common incidence in marine mammals by ingestion of plastic debris (de Stephanis et al. 2013). Baulch and Perry (2014) reviewed the presence of plastic debris in 56% of cetacean species that demonstrated its occurrence at different levels of the water column. Some reserach had been reported on the impact of microplastics at different trophic levels, but more work is needed to explain the consequences of this contamination in detail.

5.6 Policies Related to Plastics by Different Organizations

Nowadays people are more concern about the adverse impacts caused by presence of plastics in the environment. Different policies have been implemented worldwide to overwhelm the effect results from plastic litters. California implemented the ban on even a single use of the plastics shopping bags by passing the Prop 67 bill (Plastic Pollution Coalition 2016a). Similarly, Scotland and Ireland have also introduced a Plastic Pollution Coalition (2016b) and Lowenthal's bill, respectively, to ban on the use of plastics. Ireland charges a minimum fine of 10 cent on the plastic shopping bags provided by retailers during the purchasing of groceries and other items. Implementation of fines by the USA and other developing countries has reduced the use of plastics bags by 60–90% (Plastic Pollution Coalition 2016a).

Face washes and toothpastes of different brands contain the microbeads of PE and PP that play a significant role in the marine pollution. To reduce the marine pollution caused by microbeads in personal cosmetic products, the US President, Barrack Obama, had signed a law known as "Microbead-Free Waters Act of 2015," that was approved by the House of Representatives (Pallone 2015). To stop the use of microbeads in cosmetic products, the federal administration of different countries such as the Netherlands, Austria, Sweden, Belgium, Germany, Australia, Luxembourg, and Canada have also imposed different laws (Perschbacher 2016). Different international companies like LivOn, Johnson & Johnson, and L'Oreal have promised to exclude the use of microbeads and microplastics from their products used for personal care (Copeland 2015).

5.7 Conclusion

Plastic litters are present at different levels of the water bodies, beach sediments, and on the sea floor. They originated either from the land-based or ocean-based sources, with different industrial, domestic, and fishing actions. After origin, they are ultimately released into the aquatic ecosystem that makes them difficult to biodegrade. Thus it persists in the environment for a long time and available for a wide range of organisms. From the last few decades, a lot of research is going on to know the adverse effects of microplastics on the aquatic biota; however major consequences are yet to be established. Different policies have been made regarding use of plastics; however in some of the countries, the way of implementations needs to be more effective. On the basis of available literatures, it can be recommended that more work is needed for the establishment of the harmful consequences due to the ingestion of these tiny particles by the aquatic biota.

Acknowledgments The authors are grateful to Amity Institute of Biotechnology and Amity Institute of Marine Science and Technology, Amity University Uttar Pradesh, for providing the research and laboratory facilities. We did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Abayomi OA, Range P, Al-Ghouti MA, Obbard JP, Almeer SH, Ben-Hamadou R (2017) Microplastics in coastal environments of the Arabian gulf. Mar Pollut Bull 124:181–188. https://doi.org/10.1016/j.marpolbul.2017.07.011

Acampora H, Lyashevska O, Van Franeker JA, O'Connor I (2016) The use of beached bird surveys for marine plastic litter monitoring in Ireland. Mar Environ Res 120:122–129. https://doi.org/10.1016/j.marenvres.2016.08.002

Alimi OS, Farner Budarz J, Hernandez LM, Tufenkji N (2018) Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. Environ Sci Technol 52:1704–1724. https://doi.org/10.1021/acs.est.7b05559

Alomar C, Deudero S (2017) Evidence of microplastic ingestion in the shark *Galeus melastomus* Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environ Pollut 223:223–229. https://doi.org/10.1016/j.envpol.2017.01.015

Alomar C, Sureda A, Capó X, Guijarro B, Tejada S, Deudero S (2017) Microplastic ingestion by *Mullus surmuletus* Linnaeus, 1758 fish and its potential for causing oxidative stress. Environ Res 159:135–142. https://doi.org/10.1016/j.envres.2017.07.043

Amélineau F, Bonnet D, Heitz O, Mortreux V, Harding AMA, Karnovsky N, Walkusz W, Fort J, Grémillet D (2016) Microplastic pollution in the Greenland Sea: background levels and selective contamination of planktivorous diving seabirds. Environ Pollut 219:1131–1139. https://doi.org/10.1016/j.envpol.2016.09.017

Anbumani S, Kakkar P (2018) Ecotoxicological effects of microplastics on biota: a review. Environ Sci Pollut Res Int 25:14373–14396. https://doi.org/10.1007/s11356-018-1999-x

Anderson JC, Park BJ, Palace VP (2016) Microplastics in aquatic environments: implications for Canadian ecosystems. Environ Pollut 218:269–280. https://doi.org/10.1016/j.envpol.2016.06.074

Andrady AL (2011) Microplastics in the marine environment. Mar Pollut Bull 62:1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Auta HS, Emenike CU, Fauziah SH (2017) Distribution and importance of microplastics in the marine environment: a review of the sources, fate, effects, and potential solutions. Environ Int 102:165–176. https://doi.org/10.1016/j.envint.2017.02.013

Avery-Gomm S, O'Hara PD, Kleine L, Bowes V, Wilson LK, Barry KL (2012) Northern fulmars as biological monitors of trends of plastic pollution in the eastern North Pacific. Mar Pollut Bull 64:1776–1781. https://doi.org/10.1016/j.marpolbul.2012.04.017

Avery-Gomm S, Provencher JF, Morgan KH, Bertram DF (2013) Plastic ingestion in marine-associated bird species from the eastern North Pacific. Mar Pollut Bull 72:257–259. https://doi.org/10.1016/j.marpolbul.2013.04.021

Avery-Gomma S, Provencher JF, Liboiron M, Poon FE, Smith PA (2018) Plastic pollution in the Labrador Sea: an assessment using the seabird northern fulmar *Fulmarus glacialis* as a biological monitoring species. Mar Pollut Bull 127:817–822. https://doi.org/10.1016/j.marpolbul.2017.10.001

Avio CG, Gorbi S, Milan M, Benedetti M, Fattorini D, d'Errico G, Pauletto M, Bargelloni L, Regoli F (2015) Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ Pollut 198:211–222. https://doi.org/10.1016/j.envpol.2014.12.021

Barnes DK, Galgani F, Thompson RC, Barlaz M (2009) Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc Lond Ser B Biol Sci 364:1985–1998. https://doi.org/10.1098/rstb.2008.0205

Barnes DKA, Walters A, Gonçalves L (2010) Macroplastics at sea around Antarctica. Mar Environ Res 70:250–252. https://doi.org/10.1016/j.marenvres.2010.05.006

Barrows APW, Cathey SE, Petersen CW (2018) Marine environment microfiber contamination: global patterns and the diversity of microparticle origins. Environ Pollut 237:275–284. https://doi.org/10.1016/j.envpol.2018.02.062

Baulch S, Perry C (2014) Evaluating the impacts of marine debris on cetaceans. Mar Pollut Bull 80:210–221. https://doi.org/10.1016/j.marpolbul.2013.12.050

Bergmann M, Wirzberger V, Krumpen T, Lorenz C, Primpke S, Tekman MB, Gerdts G (2017) High quantities of microplastic in Arctic deep-sea sediments from the Hausgarten Observatory. Environ Sci Technol 51:11000–11010. https://doi.org/10.1021/acs.est.7b03331

Bessa F, Barría P, Neto JM, Frias JPGL, Otero V, Sobral P, Marques JC (2018) Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar Pollut Bull 128:575–584. https://doi.org/10.1016/j.marpolbul.2018.01.044

Boerger CM, Lattin GL, Moore SL, Moore CJ (2010) Plastic ingestion by planktivorous fishes in the North Pacific central gyre. Mar Pollut Bull 60:2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007

Bond AL, Provencher JF, Daoust PY, Lucas ZN (2014) Plastic ingestion by fulmars and shearwaters at Sable Island, Nova Scotia, Canada. Mar Pollut Bull 87:68–75. https://doi.org/10.1016/j.marpolbul.2014.08.010

Bour A, Haarr A, Keiter S, Hylland K (2018) Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environ Pollut 236:652–660. https://doi.org/10.1016/j.envpol.2018.02.006

Brach L, Deixonne P, Bernard MF, Durand E, Desjean MC, Perez E, van Sebille E, Ter Halle A (2018) Anticyclonic eddies increase accumulation of microplastic in the North Atlantic subtropical gyre. Mar Pollut Bull 126:191–196. https://doi.org/10.1016/j.marpolbul.2017.10.077

Bråte ILN, Eidsvoll DP, Steindal CC, Thomas KV (2016) Plastic ingestion by Atlantic cod (*Gadus morhua*) from the Norwegian coast. Mar Pollut Bull 112:105–110. https://doi.org/10.1016/j.marpolbul.2016.08.034

Bråte ILN, Blázquez M, Brooks SJ, Thomas KV (2018) Weathering impacts the uptake of polyethylene microparticles from toothpaste in Mediterranean mussels (*M. galloprovincialis*). Sci Total Environ 626:1310–1318. https://doi.org/10.1016/j.scitotenv.2018.01.141

Brennecke D, Duarte B, Paiva F, Caçador I, Canning-Clode J (2016) Microplastics as vector for heavy metal contamination from the marine environment. Estuar Coast Shelf Sci 178:189–195. https://doi.org/10.1016/j.ecss.2015.12.003

Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, Thompson R (2011) Accumulation of microplastic on shorelines worldwide: sources and sinks. Environ Sci Technol 45:9175–9179. https://doi.org/10.1021/es201811s

Bryant JA, Clemente TM, Viviani DA, Fong AA, Thomas KA, Kemp P, Karl DM, White AE, DeLong EF (2016) Diversity and activity of communities inhabiting plastic debris in the North Pacific gyre. mSystems 1:e00024–e00016. https://doi.org/10.1128/mSystems.00024-16.

Caron AGM, Thomas CR, Berry KLE, Motti CA, Ariel E, Brodie JE (2018) Ingestion of microplastic debris by green sea turtles (*Chelonia mydas*) in the Great Barrier Reef: validation of a sequential extraction protocol. Mar Pollut Bull 127:743–751. https://doi.org/10.1016/j.marpolbul.2017.12.062

Carpenter EJ, Anderson SJ, Harvey GR, Miklas HP, Peck BB (1972) Polystyrene spherules in coastal waters. Science 178:749–750. https://doi.org/10.1126/science.178.4062.749

Castillo AB, Al-Maslamani I, Obbard JP (2016) Prevalence of microplastics in the marine waters of Qatar. Mar Pollut Bull 111:260–267. https://doi.org/10.1016/j.marpolbul.2016.06.108

Cesa FS, Turra A, Baruque-Ramos J (2017) Synthetic fibers as microplastics in the marine environment: a review. Sci Total Environ 598:1116–1129. https://doi.org/10.1016/j.scitotenv.2017.04.172.

Cheung PK, Fok L (2017) Characterisation of plastic microbeads in facial scrubs and their estimated emissions in Mainland China. Water Res 122:53–61. https://doi.org/10.1016/j.watres.2017.05.053

Cho Y, Shim WJ, Jang M, Han GM, Hong SH (2019) Abundance and characteristics of microplastics in market bivalves from South Korea. Environ Pollut 245:1107–1116. https://doi.org/10.1016/j.envpol.2018.11.091

Choi JS, Jung YJ, Hong NH, Hong SH, Park JW (2018) Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*). Mar Pollut Bull 129:231–240. https://doi.org/10.1016/j.marpolbul.2018.02.039

Chua EM, Shimeta J, Nugegoda D, Morrison PD, Clarke BO (2014) Assimilation of polybrominated diphenyl ethers from microplastics by the marine amphipod, *Allorchestes compressa*. Environ Sci Technol 48:8127–8134. https://doi.org/10.1021/es405717z

Cieślik BM, Namieśnik J, Konieczka P (2015) Review of sewage sludge management: standards, regulations and analytical methods. J Clean Prod 90:1–15. https://doi.org/10.1016/j.jclepro.2014.11.031

Claessens M, De Meester S, Van Landuyt L, De Clerck K, Janssen CR (2011) Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar Pollut Bull 62:2199–2204. https://doi.org/10.1016/j.marpolbul.2011.06.030

Cole M, Galloway TS (2015) Ingestion of nanoplastics and microplastics by pacific oyster larvae. Environ Sci Technol 49:14625–14632. https://doi.org/10.1021/acs.est.5b04099

Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: a review. Mar Pollut Bull 62:2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J, Galloway TS (2013) Microplastic ingestion by zooplankton. Environ Sci Technol 47:6646–6655. https://doi.org/10.1021/es400663f

Compa M, Ventero A, Iglesias M, Deudero S (2018) Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. Mar Pollut Bull 128:89–96. https://doi.org/10.1016/j.marpolbul.2018.01.009

Copeland C (2015) Microbeads: an emerging water quality issue. Retrieved from http://www.fas.org/sgp/crs/misc/IN10319

Costa MF, Barletta M (2015) Microplastics in coastal and marine environments of the western tropical and sub-tropical Atlantic Ocean. Environ Sci Process Impacts 17:1868–1879. https://doi.org/10.1039/c5em00158g

Costa M, Ivar do Sul J, Silva-Cavalcanti J, Araújo M, Spengler Â, Tourinho P (2010) On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. Environ Monit Assess 168:299–304. https://doi.org/10.1007/s10661-009-1113-4

Courtene-Jones W, Quinn B, Gary SF, Mogg AOM, Narayanaswamy BE (2017) Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall trough, North Atlantic Ocean. Environ Pollut 231:271–280. https://doi.org/10.1016/j.envpol.2017.08.026

Davidson K, Dudas SE (2016) Microplastic ingestion by wild and cultured manila clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. Arch Environ Contam Toxicol 71:147–156. https://doi.org/10.1007/s00244-016-0286-4 de Carvalho RH, Lacerda PD, da Silva Mendes S, Barbosa BC, Paschoalini M, Prezoto F, de Sousa BM (2015) Marine debris ingestion by sea turtles (Testudines) on the Brazilian coast: an underestimated threat? Mar Pollut Bull 101:746–749. https://doi.org/10.1016/j.marpolbul.2015.10.002

de Souza Machado AA, Kloas W, Zarfl C, Hempel S, Rillig MC (2018) Microplastics as an emerging threat to terrestrial ecosystems. Glob Chang Biol 24:1405–1416. https://doi.org/10.1111/gcb.14020

de Stephanis R, Gimenez J, Carpinelli E, Gutierrez E, Canadas A (2013) As main meal for sperm whales: plastics debris. Mar Pollut Bull 69:206–214. https://doi.org/10.1016/j.marpolbul.2013.01.033

DEFRA (2012) Waste water treatment in the United Kingdom-2012, implementation of the European Union urban waste water treatment directive-91/271/EEC. Department for Environment, Food and Rural Affairs, London

Denuncio P, Mandiola MA, Pérez Salles SB, Machado R, Ott PH, De Oliveira LR, Rodriguez D (2017) Marine debris ingestion by the South American fur seal from the Southwest Atlantic Ocean. Mar Pollut Bull 122:420–425. https://doi.org/10.1016/j.marpolbul.2017.07.013

Derraik JG (2002) The pollution of the marine environment by plastic debris: a review. Mar Pollut Bull 44:842–852. https://doi.org/10.1016/S0025-326X(02)00220-5

Devriese LI, van der Meulen MD, Maes T, Bekaert K, Paul-Pont I, Frère L, Robbens J, Vethaak AD (2015) Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. Mar Pollut Bull 98:179–187. https://doi.org/10.1016/j.marpolbul.2015.06.051

Di Beneditto AP, Ramos RM (2014) Marine debris ingestion by coastal dolphins: what drives differences between sympatric species? Mar Pollut Bull 83:98–301. https://doi.org/10.1016/j.marpolbul.2014.03.057; https://doi.org/10.1016/j.envpol.2017.08.058; https://doi.org/10.1038/srep33882

Duis K, Coors A (2016) Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ Sci Eur 28:2. https://doi.org/10.1186/s12302-015-0069-y

Enders K, Lenz R, Stedmon CA, Nielsen TG (2015) Abundance, size and polymer composition of marine microplastics ≥10µm in the Atlantic Ocean and their modelled vertical distribution. Mar Pollut Bull 100:70–81. https://doi.org/10.1016/j.marpolbul.2015.09.027

Endo S, Takizawa R, Okuda K, Takada H, Chiba K, Kanehiro H, Ogi H, Yamashita R, Date T (2005) Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: variability among individual particles and regional differences. Mar Pollut Bull 50:1103–1114. https://doi.org/10.1016/j.marpolbul.2005.04.030

Engler RE (2012) The complex interaction between marine debris and toxic chemicals in the ocean. Environ Sci Technol 46:12302–12315. https://doi.org/10.1021/es3027105

Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Farley H, Amato S (2013) Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar Pollut Bull 77:177–182. https://doi.org/10.1016/j.marpolbul.2013.10.007

Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, Galgani F, Ryan PG, Reisser J (2014) Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9:e111913. https://doi.org/10.1371/journal.pone.0111913

Eriksen M, Liboiron M, Kiessling T, Charron L, Alling A, Lebreton L, Richards H, Roth B, Ory NC, Hidalgo-Ruz V, Meerhoff E, Box C, Cummins A, Thiel M (2018) Microplastic sampling with the AVANI trawl compared to two neuston trawls in the Bay of Bengal and South Pacific. Environ Pollut 232:430–439. https://doi.org/10.1016/j.envpol.2017.09.058

Esiukova E (2017) Plastic pollution on the Baltic beaches of Kaliningrad region, Russia. Mar Pollut Bull 114:1072–1080. https://doi.org/10.1016/j.marpolbul.2016.10.001

Farrell P, Nelson K (2013) Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ Pollut 177:1–3. https://doi.org/10.1016/j.envpol.2013.01.046

Fendall LS, Sewell MA (2009) Contributing to marine pollution by washing your face: microplastics in facial cleansers. Mar Pollut Bull 58:1225–1228. https://doi.org/10.1016/j.marpolbul.2009.04.025

Fischer EK, Paglialonga L, Czech E, Tamminga M (2016) Microplastic pollution in lakes and lake shoreline sediments – a case study on Lake Bolsena and Lake Chiusi (Central Italy). Environ Pollut 213:648–657. https://doi.org/10.1016/j.envpol.2016.03.012

Floren HP, Shugart GW (2017) Plastic in Cassin's auklets (*Ptychoramphus aleuticus)* from the 2014 stranding on the Northeast Pacific coast. Mar Pollut Bull 117:496–498. https://doi.org/10.1016/j.marpolbul.2017.01.076

Foekema EM, De Gruijter C, Mergia MT, van Franeker JA, Murk AJ, Koelmans AA (2013) Plastic in north sea fish. Environ Sci Technol 47:8818–8824. https://doi.org/10.1021/es400931b

Fok L, Cheung PK (2015) Hong Kong at the Pearl River estuary: a hotspot of microplastic pollution. Mar Pollut Bull 99:112–118. https://doi.org/10.1016/j.marpolbul.2015.07.050

Fossi MC, Coppola D, Baini M, Giannetti M, Guerranti C, Marsili L, Panti C, de Sabata E, Clò S (2014) Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). Mar Environ Res 100:17–24. https://doi.org/10.1016/j.marenvres.2014.02.002

Fossi MC, Marsili L, Baini M, Giannetti M, Coppola D, Guerranti C, Caliani I, Minutoli R, Lauriano G, Finoia MG, Rubegni F, Panigada S, Bérubé M, Urbán Ramírez J, Panti C (2016) Fin whales and microplastics: the Mediterranean Sea and the sea of Cortez scenarios. Environ Pollut 209:68–78. https://doi.org/10.1016/j.envpol.2015.11.022

Frydkjær CK, Iversen N, Roslev P (2017) Ingestion and egestion of microplastics by the Cladoceran *Daphnia magna*: effects of regular and irregular shaped plastic and sorbed phenanthrene. Bull Environ Contam Toxicol 99:655–661. https://doi.org/10.1007/s00128-017-2186-3

Gago J, Henry M, Galgani F (2015) First observation on neustonic plastics in waters off NW Spain (spring 2013 and 2014). Mar Environ Res 111:27–33. https://doi.org/10.1016/j.marenvres.2015.07.009

Gago J, Carretero O, Filgueiras AV, Viñas L (2018) Synthetic microfibers in the marine environment: a review on their occurrence in seawater and sediments. Mar Pollut Bull 127:365–376. https://doi.org/10.1016/j.marpolbul.2017.11.070

Galgani F, Leaute JP, Moguedet P, Souplet A, Verin Y, Carpentier A, Goraguer H, Latrouite D, Andral B, Cadiou Y, Mahe JC, Poulard C, Nerisson P (2000) Litter on the sea floor along European coasts. Mar Pollut Bull 40:516–527. https://doi.org/10.1016/S0025-326X(99)00234-9

Gall SC, Thompson RC (2015) The impact of debris on marine life. Mar Pollut Bull 92:170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041.

Gallagher A, Rees A, Rowe R, Stevens J, Wright P (2016) Microplastics in the Solent estuarine complex, UK: an initial assessment. Mar Pollut Bull 102:243–249. https://doi.org/10.1016/j.marpolbul.2015.04.002

Galloway TS, Cole M, Lewis C, Atkinson A, Allen JI (2017) Interactions of microplastic debris throughout the marine ecosystem. Nat Ecol Evol 1:116. https://doi.org/10.1038/s41559-017-0116

Gandara E, Silva PP, Nobre CR, Resaffe P, Pereira CDS, Gusmão F (2016) Leachate from microplastics impairs larval development in brown mussels. Water Res 106:364–370. https://doi.org/10.1016/j.watres.2016.10.016

Gatidou G, Arvaniti OS, Stasinakis AS (2019) Review on the occurrence and fate of microplastics in sewage treatment plants. J Hazard Mater 367:504–512. https://doi.org/10.1016/j.jhazmat.2018.12.081

GESAMP (2015) Sources, fate and effects of microplastics in the marine environment: a global assessment. Kershaw PJ (ed.) Joint Group Experts on the Scientific Aspects of Marine Environmental Protection 96.

Goldberg ED (1997) Plasticizing the seafloor: an overview. Environ Technol 18:195–202. https://doi.org/10.1080/09593331808616527

Goldstein MC, Titmus AJ, Ford M (2013) Scales of spatial heterogeneity of plastic marine debris in the Northeast Pacific Ocean. PLoS One 8:e80020. https://doi.org/10.1371/journal.pone.0080020

Graham ER, Thompson JT (2009) Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J Exp Mar Biol Ecol 368:22–29. https://doi.org/10.1016/j.jembe.2008.09.007

Gray H, Lattin GL, Moore CJ (2012) Incidence, mass and variety of plastics ingested by laysan (*Phoebastria immutabilis*) and black-footed albatrosses (*P. nigripes*) recovered as by-catch in the North Pacific Ocean. Mar Pollut Bull 64:2190–2192. https://doi.org/10.1016/j.marpolbul.2012.07.053

Green DS (2016) Effects of microplastics on European flat oysters, *Ostrea edulis* and their associated benthic communities. Environ Pollut 216:95–103. https://doi.org/10.1016/j.envpol.2016.05.043

Gregory MR (1996) Plastic 'scrubbers' in hand cleansers: a further (and minor) source for marine pollution identified. Mar Pollut Bull 32:867–871. https://doi.org/10.1016/s0025-326x(96)00047-1

Guzzetti E, Sureda A, Tejada S, Faggio C (2018) Microplastics in marine organisms: environmental and toxicological effects. Environ Toxicol Pharmacol 64:164–171. https://doi.org/10.1016/j.etap.2018.10.009

Haegerbaeumer A, Mueller M, Fueser H, Traunspurger W (2018) Impacts of micro- and nano-sized plastic particles on benthic invertebrates: a literature review and gap analysis. Front Environ Sci 7:17. https://doi.org/10.3389/fenvs.2019.00017.

Hall NM, Berry KLE, Rintoul L, Hoogenboom MO (2015) Microplastic ingestion by scleractinian corals. Mar Biol 162:725–732. https://doi.org/10.1007/s00227-015-2619-7

Hammer J, Kraak MH, Parsons JR (2012) Plastics in the marine environment: the dark side of a modern gift. Rev Environ Contam Toxicol 220:1–44. https://doi.org/10.1007/978-1-4614-3414-6_1.

Hendrickson E, Minor EC, Schreiner K (2018) Microplastic abundance and composition in Western Lake Superior as determined via microscopy, Pyr-GC/MS, and FTIR. Environ Sci Technol 52:1787–1796. https://doi.org/10.1021/acs.est.7b05829

Hengstmann E, Tamminga M, vom Bruch C, Fischer EK (2018) Microplastic in beach sediments of the isle of Rügen (Baltic Sea) – implementing a novel glass elutriation column. Mar Pollut Bull 126:263–274. https://doi.org/10.1016/j.marpolbul.2017.11.010

Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, Duflos G (2017) Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere 182:781–793. https://doi.org/10.1016/j.chemosphere.2017.05.096

Hermsen E, Pompe R, Besseling E, Koelmans AA (2017) Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. Mar Pollut Bull 122:253–258. https://doi.org/10.1016/j.marpolbul.2017.06.051

Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M (2012) Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ Sci Technol 46:3060–3075. https://doi.org/10.1021/es2031505

Hipfner JM, Galbraith M, Tucker S, Studholme KR, Domalik AD, Pearson SF, Good TP, Ross PS, Hodum P (2018) Two forage fishes as potential conduits for the vertical transfer of microfibres in Northeastern Pacific Ocean food webs. Environ Pollut 239:215–222. https://doi.org/10.1016/j.envpol.2018.04.009

Horton AA, Svendsen C, Williams RJ, Spurgeon DJ, Lahive E (2017a) Large microplastic particles in sediments of tributaries of the River Thames, UK-Abundance, sources and methods for effective quantification. Mar Pollut Bull 114:218–226. https://doi.org/10.1016/j.marpolbul.2016.09.004

Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C (2017b) Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci Total Environ 586:127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190

Hoss DE, Settle LR (1990) Ingestion of plastics by teleost fishes. In: RS Shomura, ML Godrey (eds) Proceedings of the second international conference on marine debris 2–7 April 1989, Honolulu, Hawaii. U.S. Department of Commerce, pp 693–709. http://www.congress.gov/bill/114th-congress/housebill/1321. Accessed on 14 Aug 2017

Imhof HK, Ivleva NP, Schmid J, Niessner R, Laforsch C (2013) Contamination of beach sediments of a Subalpine Lake with microplastic particles. Curr Biol 23:R867–R868. https://doi.org/10.1016/j.cub.2013.09.001

Imhof HK, Laforsch C, Wiesheu AC, Schmid J, Anger PM, Niessner R, Ivleva NP (2016) Pigments and plastic in limnetic ecosystems: a qualitative and quantitative study on microparticles of different size classes. Water Res 98:64–74. https://doi.org/10.1016/j.watres.2016.03.015

Ivar do Sul JA, Costa MF, Barletta M, Cysneiros FJA (2013) Pelagic microplastics around an archipelago of the Equatorial Atlantic. Mar Pollut Bull 75:305–309. https://doi.org/10.1016/j.marpolbul.2013.07.040

Jayasiri HB, Purushothaman CS, Vennila A (2013) Quantitative analysis of plastic debris on recreational beaches in Mumbai, India. Mar Pollut Bull 77:107–112. https://doi.org/10.1016/j.marpolbul.2013.10.024

Jiang JQ (2018) Occurrence of microplastics and its pollution in the environment: a review. Sust Prod Consumption 13:16–23. https://doi.org/10.1016/j.spc.2017.11.003

Jovanović B, Gökdağ K, Güven O, Emre Y, Whitley EM, Kideys AE (2018) Virgin microplastics are not causing imminent harm to fish after dietary exposure. Mar Pollut Bull 130:123–131. https://doi.org/10.1016/j.marpolbul.2018.03.016

Kalčikova G, Alič B, Skalar T, Bundschuh M, Gotvajn AŽ (2017) Wastewater treatment plant effluents as source of cosmetic polyethylene microbeads to freshwater. Chemoshere 188:25–31. https://doi.org/10.1016/j.chemosphere.2017.08.131

Kanehiro H, Tokai T, Matuda K (1995) Marine litter composition and distribution on the sea-bed of Tokyo Bay. J Fish Eng 31:195–199. https://doi.org/10.18903/fisheng.31.3_195

Kanhai DK, Officer R, Lyashevska O, Thompson RC, O'Connor I (2017) Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. Mar Pollut Bull 115:307–314. https://doi.org/10.1016/j.marpolbul.2016.12.025

Kasirajan S, Ngouajio M (2012) Polyethylene and bio degradable mulches for agricultural applications: a review. Agron Sustain Dev 32:501–529. https://doi.org/10.1007/s13593-011-0068-3

Klein S, Worch E, Knepper TP (2015) Occurrence and spatial distribution of microplastics in river shore sediments of the Rhine-main area in Germany. Environ Sci Technol 49:6070–6076. https://doi.org/10.1021/acs.est.5b00492

Kolandhasamy P, Su L, Li J, Qu X, Jabeen K, Shi H (2018) Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. Sci Total Environ 610-611:635–640. https://doi.org/10.1016/j.scitotenv.2017.08.053

Kole PJ, Löhr AJ, Van Belleghem FGAJ, Ragas AMJ (2017) Wear and tear of tyres: a stealthy source of microplastics in the environment. Int J Environ Res Public Health 14:E1265. https://doi.org/10.3390/ijerph14101265

Kooi M, Reisser J, Slat B, Ferrari FF, Schmid MS, Cunsolo S, Brambini R, Noble K, Sirks LA, TEW L, Schoeneich-Argent RI, Koelmans AA (2016) The effect of particle properties on the depth profile of buoyant plastics in the ocean. Sci Rep 6:33882

Kyrikou I, Briassoulis D (2007) Biodegradation of agricultural plastic films: a critical review. J Polym Environ 15:125–150. https://doi.org/10.1007/s10924-007-0053-8

Law KL, Morét-Ferguson S, Maximenko NA, Proskurowski G, Peacock EE, Hafner J, Reddy CM (2010) Plastic accumulation in the North Atlantic subtropical gyre. Science 329:1185–1188. https://doi.org/10.1126/science.1192321

Lechner A, Keckeis H, Lumesberger-Loisl F, Zens B, Krusch R, Tritthart Glas M, Schludermann E (2014) The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ Pollut 188:177–181. https://doi.org/10.1016/j.envpol.2014.02.006

Li J, Yang D, Li L, Jabeen K, Shi H (2015) Microplastics in commercial bivalves from China. Environ Pollut 207:190–195. https://doi.org/10.1016/j.envpol.2015.09.018

Li WC, Tse HF, Fok L (2016a) Plastic waste in the marine environment: a review of sources, occurrence and effects. Sci Total Environ 566-567:333–349. https://doi.org/10.1016/j.scitotenv.2016.05.084

Li J, Qu X, Su L, Zhang W, Yang D, Kolandhasamy P, Li D, Shi H (2016b) Microplastics in mussels along the coastal waters of China. Environ Pollut 214:177–184. https://doi.org/10.1016/j.envpol.2016.04.012

Lozano RL, Mouat J (2009) Marine litter in the north-East Atlantic region: assessment and priorities for response. KIMO International. http://hdl.handle.net/20.500.11822/2431.

Lusher A, McHugh M, Thompson RC (2013) Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar Pollut Bull 67:94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028

Lusher AL, Burke A, O'Connor I, Officer R (2014) Microplastic pollution in the Northeast Atlantic Ocean: validated and opportunistic sampling. Mar Pollut Bull 88:325–333. https://doi.org/10.1016/j.marpolbul.2014.08.023

Lusher A, Milian GH, O'Brien J, Berrow S, O'Connor I, Officer R (2015) Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale *Mesoplodon mirus*. Environ Pollut 199:185–191. https://doi.org/10.1016/j.envpol.2015.01.023

Lusher AL, Hernandez-Milian G, Berrow S, Rogan E, O'Connor I (2018) Incidence of marine debris in cetaceans stranded and bycaught in Ireland: recent findings and a review of historical knowledge. Environ Pollut 232:467–476. https://doi.org/10.1016/j.envpol.2017.09.070

Mason S, Kammin L, Eriksen M, Aleid G, Wilson S, Box C, Williamson A, Riley A (2016) Pelagic plastic pollution within the surface waters of Lake Michigan, USA. J Great Lakes Res 42:753–759. https://doi.org/10.1016/j.jglr.2016.05.009

Mathalon A, Hill P (2014) Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. Mar Pollut Bull 81:69–79. https://doi.org/10.1016/j.marpolbul.2014.02.018

McCauley SJ, Bjorndal KA (1999) Conservation implications of dietary dilution from debris ingestion: sublethal effects in post-hatchling loggerhead sea turtles. Conserv Biol 13:925–929. https://doi.org/10.1046/j.1523-1739.1999.98264.x

McGoran AR, Clark PF, Morritt D (2017) Presence of microplastic in the digestive tracts of European flounder, *Platichthys flesus*, and European smelt, *Osmerus eperlanus*, from the River Thames. Environ Pollut 220:744–751. https://doi.org/10.1016/j.envpol.2016.09.078.

Medrano ED, Thompson RC, Aldridge DC (2015) Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res 75:63–82. https://doi.org/10.1016/j.watres.2015.02.012

Moore CJ (2008) Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ Res 108:131–139. https://doi.org/10.1016/j.envres.2008.07.025

Moore CJ, Moore SL, Leecaster MK, Weisberg SB (2002) A comparison of plastic and plankton in the North Pacific central gyre. Mar Pollut Bull 42:1297–1300. https://doi.org/10.1016/S0025-326X(01)00114-x

Moreira FT, Prantoni AL, Martini B, de Abreu MA, Stoiev SB, Turra A (2016) Small-scale temporal and spatial variability in the abundance of plastic pellets on sandy beaches: methodological considerations for estimating the input of microplastics. Mar Pollut Bull 101:114–121. https://doi.org/10.1016/j.marpolbul.2015.11.051

Morritt D, Stefanoudis PV, Pearce D, Crimmen OA, Clark PF (2014) Plastic in the Thames: a river runs through it. Mar Pollut Bull 78:196–200. https://doi.org/10.1016/j.marpolbul.2013.10.035

Murray F, Cowie PR (2011) Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar Pollut Bull 62:1207–1217. https://doi.org/10.1016/j.marpolbul.2011.03.032

Naidu SA (2019) Preliminary study and first evidence of presence of microplastics and colorants in green mussel, *Perna viridis* (Linnaeus, 1758), from southeast coast of India. Mar Pollut Bull 140:416–422

Naidu SA, Rao VR, Ramu K (2018) Microplastics in the benthic invertebrates from the coastal waters of Kochi, Southeastern Arabian Sea. Environ Geochem Health 4:1377–1383. https://doi.org/10.1007/s10653-017-0062-z

Nerland IL, Halsband C, Allan I, Thomas KV (2014) Microplastics in marine environment: occurrence, distribution and effects project no. 14338 report no. 6754–2014 Oslo.

Neves D, Sobral P, Ferreira JL, Pereira T (2015) Ingestion of microplastics by commercial fish off the Portuguese coast. Mar Pollut Bull 101:119–126. https://doi.org/10.1016/j.marpolbul.2015.11.008

Obbard RW, Sadri S, Wong YQ, Khitun AA, Baker I, Thompson RC (2014) Global warming releases microplastic legacy frozen in Arctic Sea ice. Earth's Future 2:315–320. https://doi.org/10.1002/2014EF000240

Ogunola OS, Palanisami T (2016) Microplastics in the marine environment: current status, assessment methodologies, impacts and solutions. J Pollut Eff Control 4:161. https://doi.org/10.4172/2375-4397.1000161

Ory NC, Sobral P, Ferreira JL, Thiel M (2017) Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci Total Environ 586:430–437. https://doi.org/10.1016/j.scitotenv.2017.01.175

Pallone FHR (2015) Microbead-Free Waters Act of 2015, Public Law No. 114-114.

Peeken I, Primpke S, Beyer B, Gütermann J, Katlein C, Krumpen T, Bergmann M, Hehemann L, Gerdts G (2018) Arctic Sea ice is an important temporal sink and means of transport for microplastic. Nat Commun 9:1505. https://doi.org/10.1038/s41467-018-03825-5

Pellini G, Gomiero A, Fortibuoni T, Ferrà C, Grati F, Tassetti AN, Polidori P, Fabi G, Scarcella G (2018) Characterization of microplastic litter in the gastrointestinal tract of *Solea solea* from the Adriatic Sea. Environ Pollut 234:943–952. https://doi.org/10.1016/j.envpol.2017.12.038

Peng G, Xu P, Zhu B, Bai M, Li D (2018) Microplastics in freshwater river sediments in Shanghai, China: a case study of risk assessment in mega-cities. Environ Pollut 234:448–456. https://doi.org/10.1016/j.envpol.2017.11.034

Perschbacher E (2016) Microbeads-legislative update. Retrieved from. http://www.ijc.org/en_/blog/2016/03/01/microbeads_legislative_update_story4

Pham CK, Rodríguez Y, Dauphin A, Carriço R, Frias JPGL, Vandeperre F, Otero V, Santos MR, Martins HR, Bolten AB, Bjorndal KA (2017) Plastic ingestion in oceanic-stage loggerhead sea turtles (*Caretta caretta*) off the North Atlantic subtropical gyre. Mar Pollut Bull 121:222–229. https://doi.org/10.1016/j.marpolbul.2017.06.008

Phuong NN, Zalouk-Vergnoux A, Poirier L, Kamari A, Châtel A, Mouneyrac C, Lagarde F (2016) Is there any consistency between the microplastics found in the field and those used in laboratory experiments? Environ Pollut 211:111–123. https://doi.org/10.1016/j.envpol.2015.12.035

Plastic Pollution Coalition (2016a) California defeats big plastic http://www.plasticpollutioncoalition.org/pft/2016/11/9/california-defeatsbig-plastic. Accessed on 20 Jan 2017

Plastic Pollution Coalition (2016b) California introduces National Trash Reduction Act. http://www.plasticpollutioncoalition.org/pft/2015/11/6/california-introduces-national-trash-reduction-act. Accessed on 20 Jan 2017

Plastics Europe (2016) Plastics – the facts 2016. An analysis of European plastics production, demand and waste data

Poon FE, Provencher JF, Mallory ML, Braune BM, Smith PA (2017) Levels of ingested debris vary across species in Canadian Arctic seabirds. Mar Pollut Bull 116:517–520. https://doi.org/10.1016/j.marpolbul.2016.11.051

Poulain M, Mercier MJ, Brach L, Martignac M, Routaboul C, Perez E, Desjean MC, Halle A (2018) Small microplastics as a main contributor to plastic mass balance in the North Atlantic subtropical gyre. Environ Sci Technol 53:1157–1164. https://doi.org/10.1021/acs.est.8b05458

Prata JC, da Costa JP, Lopes I, Duarte AC, Rocha-Santos T (2019) Effects of microplastics on microalgae populations: a critical review. Sci Total Environ 665:400–405. https://doi.org/10.1016/j.scitotenv.2019.02.132

Provencher JF, Gaston AJ, Mallory ML (2009) Evidence for increased ingestion of plastics by northern fulmars (*Fulmarus glacialis*) in the Canadian Arctic. Mar Pollut Bull 58:1092–1095. https://doi.org/10.1016/j.marpolbul.2009.04.002

Reichert J, Schellenberg J, Schubert P, Wilke T (2018) Responses of reef building corals to microplastic exposure. Environ Pollut 237:955–960. https://doi.org/10.1016/j.envpol.2017.11.006

Reisser J, Shaw J, Hallegraeff G, Proietti M, Barnes DKA, Thums M, Wilcox C, Hardesty BD, Pattiaratchi C (2014) Millimeter-sized marine plastics: a new pelagic habitat for microorganisms and invertebrates. PLoS One 9:e100289. https://doi.org/10.1371/journal.pone.0100289

Reisser J, Slat B, Noble K, du Plessis K, Epp M, Proietti M, de Sonneville J, Becker T, Pattiaratchi C (2015) The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic gyre. Biogeosciences 12:1249–1256. https://doi.org/10.5194/bg-12-1249-2015.

Rochman CM, Kross SM, Armstrong JB, Bogan MT, Darling ES, Green SJ, Smyth AR, Veŕissimo D (2015) Scientific evidence supports a ban on microbeads. Environ Sci Technol 49:10759–10761. https://doi.org/10.1021/acs.est.5b03909

Romeo T, Pietro B, Pedà C, Consoli P, Andaloro F, Fossi MC (2015) First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar Pollut Bull 95:358–361. https://doi.org/10.1016/j.marpolbul.2015.04.048

Rosenkranz P, Chaudhry Q, Stone V, Fernandes TF (2009) A comparison of nanoparticle and fine particle uptake by *Daphnia magna*. Environ Toxicol Chem 28:2142–2149. https://doi.org/10.1016/j.marpolbul.2015.04.048.

Ryan PG, Moore CJ, van Franeker JA, Moloney CL (2009) Monitoring the abundance of plastic debris in the marine environment. Philos Trans R Soc Lond Ser B Biol Sci 364:1999–2012. https://doi.org/10.1098/rstb.2008.0207

Santana MFM, Moreira FT, Pereira CDS, Abessa DMS, Turra A (2018) Continuous exposure to microplastics does not cause physiological effects in the cultivated mussel *Perna perna*. Arch Environ Contam Toxicol 74:594–604. https://doi.org/10.1007/s00244-018-0504-3

Scarascia-Mugnozza G, Sica C, Russo G (2012) Plastic materials in European agriculture: actual use and perspectives. J Agric Eng Res 42:15–28. https://doi.org/10.4081/jae.2011.3.15

Setälä O, Fleming-Lehtinen V, Lehtiniemi M (2014) Ingestion and transfer of microplastics in the planktonic food web. Environ Pollut 185:77–83. https://doi.org/10.1016/j.envpol.2013.10.013

Sharma S, Chatterjee S (2017) Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environ Sci Pollut Res 24:21530–21547. https://doi.org/10.1007/s11356-017-9910-8

Shim WJ, Thompson RC (2015) Microplastics in the ocean. Arch Environ Contam Toxicol 69:265–268. https://doi.org/10.1007/s00244-015-0216-x

Sighicelli M, Pietrelli L, Lecce F, Iannilli V, Falconieri M, Coscia L, Di Vito S, Nuglio S, Zampetti G (2018) Microplastic pollution in the surface waters of Italian Subalpine Lakes. Environ Pollut 236:645–651. https://doi.org/10.1016/j.envpol.2018.02.008

Singh G, Singh AK, Bhatt K (2016) Biodegradation of polyethylene by bacteria isolated from soil. Int J Res Dev Pharm Life Sci 5:2056–2062

Sivan A (2011) New perspectives in plastic biodegradation. Curr Opin Biotechnol 22:422–426. https://doi.org/10.1016/j.copbio.2011.01.013.

Skariyachan S, Setlur AS, Naik SY, Naik AA, Usharani M, Vasist KS (2017) Enhanced biodegradation of low and high-density polyethylene by novel bacterial consortia formulated from plastic-contaminated cow dung under thermophilic conditions. Environ Sci Pollut Res Int 24:8443–8457. https://doi.org/10.1007/s11356-017-8537-0

Smith LE (2018) Plastic ingestion by *Scyliorhinus canicula* trawl captured in the North Sea. Mar Pollut Bull 130:6–7. https://doi.org/10.1016/j.marpolbul.2018.03.001

Song YK, Hong SH, Jang M, Kang JH, Kwon OY, Han GM, Shim WJ (2014) Large accumulation of micro-sized synthetic polymer particles in the sea surface microlayer. Environ Sci Technol 48:9014–9021. https://doi.org/10.1021/es501757s

Sruthy S, Ramasamy EV (2017) Microplastic pollution in Vembanad Lake, Kerala, India: the first report of microplastics in lake and estuarine sediments in India. Environ Pollut 222:315–322. https://doi.org/10.1016/j.envpol.2016.12.038

Steer M, Cole M, Thompson RC, Lindeque PK (2017) Microplastics ingestion in fish larvae in the western English Channel. Environ Pollut 226:250–259. https://doi.org/10.1016/j.envpol.2017.03.062

Steinmetz Z, Wollmann C, Schaefer M, Buchmann C, David J, Troger J, Munoz K, Fror O, Schaumann GE (2016) Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? Sci Total Environ 550:690–705. https://doi.org/10.1016/j.scitotenv.2016.01.153

Strungaru SA, Jijie R, Nicoara M, Plavan G, Faggio C (2019) Micro- (nano) plastics in freshwater ecosystems: abundance, toxicological impact and quantification methodology. Trends Anal Chem 110:116–128. https://doi.org/10.1016/j.trac.2018.10.025

Suaria G, Avio CG, Mineo A, Lattin GL, Magaldi MG, Belmonte G, Moore CJ, Regoli F, Aliani S (2016) The Mediterranean plastic soup: synthetic polymers in Mediterranean surface waters. Sci Rep 6:37551. https://doi.org/10.1038/srep37551

Takahashi CK, Turner A, Millward GE, Glegg GA (2012) Persistence and metallic composition of paint particles in sediments from a tidal inlet. Mar Pollut Bull 64:133–137. https://doi.org/10.1016/j.marpolbul.2011.10.010

Tanaka K, Takada H (2016) Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Sci Rep 6:34351. https://doi.org/10.1038/srep34351

Tanaka K, Takada H, Yamashita R, Mizukawa K, Fukuwaka MA, Watanuki Y (2013) Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Mar Pollut Bull 69:219–222. https://doi.org/10.1016/j.marpolbul.2012.12.010

Taylor ML, Gwinnett C, Robinson LF, Woodall LC (2016) Plastic microfibre ingestion by deep-sea organisms. Sci Rep 6:33997. https://doi.org/10.1038/srep33997

Teng J, Wang Q, Ran W, Wu D, Liu Y, Sun S, Liu H, Cao R, Zhao J (2019) Microplastic in cultured oysters from different coastal areas of China. Sci Total Environ 653:1282–1292. https://doi.org/10.1016/j.scitotenv.2018.11.057

Terepocki AK, Brush AT, Kleine LU, Shugart GW, Hodum P (2017) Size and dynamics of microplastic in gastrointestinal tracts of northern fulmars (*Fulmarus glacialis*) and sooty shearwaters (*Ardenna grisea*). Mar Pollut Bull 116:143–150. https://doi.org/10.1016/j.marpolbul.2016.12.064.

Thompson RC (2015) Microplastics in the marine environment: sources, consequences and solution. In: Bergmann M, Gutow L, Klages M (eds) Marine anthropogenic litter. Springer, Cham

Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, McGonigle D, Russell AE (2004) Lost at sea: where is all the plastic? Science 304:838. https://doi.org/10.1126/science.1094559.

Thompson R, Moore C, Andrady A, Gregory M, Takada H, Weisberg S (2005) New directions in plastic debris. Science 310:1117. https://doi.org/10.1126/science.310.5751.1117b.

Thompson RC, Swan SH, Moore CJ, vom Saal FS (2009) Our plastic age. Philos Trans R Lond Soc B 364:1973–1976. https://doi.org/10.1098/rstb.2009.0054

Tibbetts JH (2015) Managing marine plastic pollution: policy initiatives to address wayward waste. Environ Health Perspect 123:A90–A93. https://doi.org/10.1289/ehp.123-A90.

Trevail AM, Gabrielsen GW, Kühn S, Van Franeker JA (2015) Elevated levels of ingested plastic in a high Arctic seabird, the northern fulmar (*Fulmarus glacialis*). Polar Biol 38:975–981. https://doi.org/10.1007/s00300-015-1657-4

Turner A, Wallerstein C, Arnold R (2019) Identification, origin and characteristics of bio-bead microplastics from beaches in western Europe. Sci Total Environ 664:938–947. https://doi.org/10.1016/j.scitotenv.2019.01.281

Unger B, Rebolledo ELB, Deaville R, Gröne A, IJsseldijk LL, Leopold MF, Siebert U, Spitz J, Wohlsein P, Herr H (2016) Large amounts of marine debris found in sperm whales stranded along the North Sea coast in early 2016. Mar Pollut Bull 112:134–141. https://doi.org/10.1016/j.marpolbul.2016.08.027

Van Cauwenberghe L, Janssen CR (2014) Microplastics in bivalves cultured for human consumption. Environ Pollut 193:65–70. https://doi.org/10.1016/j.envpol.2014.06.010

Van Cauwenberghe L, Vanreusel A, Mees J, Janssen CR (2013) Microplastic pollution in deep-sea sediments. Environ Pollut 182:495–499. https://doi.org/10.1016/j.envpol.2013.08.013

Van Cauwenberghe L, Claessens M, Vandegehuchte MB, Janssen CR (2015a) Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ Pollut 199:10–17. https://doi.org/10.1016/j.envpol.2015.01.008

Van Cauwenberghe L, Devriese L, Galgani F, Robbens J, Janssen CR (2015b) Microplastics in sediments: a review of techniques, occurrence and effects. Mar Environ Res 111:5–17. https://doi.org/10.1016/j.marenvres.2015.06.007

Vandermeersch G, Van Cauwenberghe L, Janssen CR, Marques A, Granby K, Fait G, Kotterman MJ, Diogène J, Bekaert K, Robbens J, Devriese L (2015) A critical view on microplastic quantification in aquatic organisms. Environ Res 143:46–55. https://doi.org/10.1016/j.envres.2015.07.016.

von Moos N, Burkhardt-Holm P, Köhler A (2012) Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ Sci Technol 46:11327–11335. https://doi.org/10.1021/es302332w

Waller CL, Griffiths HJ, Waluda CM, Thorpe SE, Loaiza I, Moreno B, Pacherres CO, Hughes KA (2017) Microplastics in the Antarctic marine system: an emerging area of research. Sci Total Environ 598:220–227. https://doi.org/10.1016/j.scitotenv.2017.03.283

Wang W, Ndungu AW, Li Z, Wang J (2017) Microplastics pollution in inland freshwaters of China: a case study in urban surface waters of Wuhan, China. Sci Total Environ 575:1369–1374. https://doi.org/10.1016/j.scitotenv.2016.09.213

Watermann BT, Löder M, Herlyn M, Daehne B, Thomsen A, Gall K (2017) Long-term 2007-2013 monitoring of reproductive disturbance in the dun sentinel *Assiminea grayana* with regard to polymeric materials pollution at the coast of Lower Saxony, North Sea, Germany. Environ Sci Pollut Res Int 24:3352–3362. https://doi.org/10.1007/s11356-016-8058-2

Watts AJ, Lewis C, Goodhead RM, Beckett SJ, Moger J, Tyler CR, Galloway TS (2014) Uptake and retention of microplastics by the shore crab *Carcinus maenas*. Environ Sci Technol 48:8823–8830. https://doi.org/10.1021/es501090e

Wegner A, Besseling E, Foekema EM, Kamermans P, Koelmans AA (2012) Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ Toxicol Chem 31:2490–2497. https://doi.org/10.1002/etc.1984

Wessel CC, Lockridge GR, Battiste D, Cebrian J (2016) Abundance and characteristics of microplastics in beach sediments: insights into microplastic accumulation in northern Gulf of Mexico estuaries. Mar Pollut Bull 109:178–183. https://doi.org/10.1016/j.marpolbul.2016.06.002

Woodall LC, Sanchez-Vidal A, Canals M, Paterson GLJ, Coppock R, Sleight V, Calafat A, Rogers AD, Narayanaswamy BE, Thompson RC (2014) The deep sea is a major sink for microplastic debris. R Soc Open Sci 1:140317. https://doi.org/10.1098/rsos.140317

Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine organisms: a review. Envion Pollut 178:483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Xiong X, Wu C, Elser JJ, Mei Z, Hao Y (2019) Occurrence and fate of microplastic debris in middle and lower reaches of the Yangtze River – from inland to the sea. Sci Total Environ 659:66–73. https://doi.org/10.1016/j.scitotenv.2018.12.313

Zebe E, Schiedek D (1996) The lugworm *Arenicola marina*: a model of physiological adaptation to life in intertidal sediments. Helgol Mar Res 50:37–68. https://doi.org/10.1007/BF02367136

Zhang W, Zhang S, Wang J, Wang Y, Mu J, Wang P, Lin X, Ma D (2017) Microplastic pollution in the surface waters of the Bohai Sea, China. Environ Pollut 231:541–548

Zhao S, Zhu L, Wang T, Li D (2014) Suspended microplastics in the surface water of the Yangtze estuary system, China: first observations on occurrence, distribution. Mar Pollut Bull 86:562–568. https://doi.org/10.1016/j.marpolbul.2014.06.032

Ziajahromi S, Kumar A, Neale PA, Leusch FDL (2017) Impact of microplastic beads and fibers on waterflea *(Ceriodaphnia dubia)* survival, growth and reproduction: implications of single and mixture exposures. Environ Sci Technol 51:13397–13406. https://doi.org/10.1021/acs.est.7b03574

Ziajahromi S, Kumar A, Neale PA, Leusch FDL (2018) Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. Environ Pollut 236:425–431. https://doi.org/10.1016/j.envpol.2018.01.094

Zitko V, Hanlon M (1991) Another source of pollution by plastics: skin cleaners with plastic scrubbers. Mar Pollut Bull 22:41–42. https://doi.org/10.1016/0025-326x(91)90444-w

Zubris KA, Richards BK (2005) Synthetic fibers as an indicator of land application of sludge. Environ Pollut 138:201–211. https://doi.org/10.1016/j.envpol.2005.04.013

Chapter 6
Impacts of Sustainable Energy Resource Use on the Health of the Environment

Rajesh K. Srivastava

Contents

Abstract Utilization of different types of energy has critically influenced the health of both the environment and living organisms. Further, the quantity and variety of energy resources utilized pose very serious issues for the survival of living organisms such as plants, animals, and human beings. People worldwide have developed the habit of using unsustainable or nonrenewable energy sources, creating environmental issues at the global level, such as release of greater quantities of

---

R. K. Srivastava (✉)
Department of Biotechnology, Gitam Institute of Technology and Management (GITAM) (Deemed to be a University), Visakhatpatnam, Andhra Pradesh, India

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_6

greenhouse gases and other air pollutants, harming biological life and the health of the environment. To resolve the current issues and to maintain the health of all types of organisms, first we need to refine our current practices to utilize renewable energy sources (e.g., cellulosic biofuels, biodiesel, and hydrogen gas) obtained from sustainable resources such as lignocellulosic biomass or from use of bacteria or marine algae. Worldwide, lignocellulosic biomass is available in plentiful quantities, and its burning by farmers creates a lot of problems through air pollution (primary or secondary). Thus, we should develop biofuel plants and other types of renewable energy plants on a commercial scale for production of energy in a cost-effective way, as it is important to promote utilization of clean energy to maintain the health of organisms and the environment.

Keywords Energy · Renewable · Pollution · Greenhouse gases · Microorganisms · Lignocellulosic biomass

Abbreviations

AC	Air conditioning
AD	Anaerobic digestion
CCRD	Central composite rotatable design
CE	Cost efficiency
CFC	Chlorofluorocarbon
Chl	Chlorophyll
CNG	Compressed natural gas
CORE	Cornwall Ontario River Energy
CPT	Columbia Power Technologies
CSTBR	Continuous stirred-tank bioreactor
CSTR	Continuous stirred-tank reactor
EE	Energy efficiency
GHG	Greenhouse gas
HRT	Hydraulic retention time
IEQ	Indoor environmental quality
MEC	Microbial electrolysis cell
NIMBY	Not-in-my-backyard
NOx	Nitrous oxide
ORPC	Ocean Renewable Power Company
PB	Power buoy
PBS	Phycobilisome
PM	Particulate matter
P_{max}	Maximum photosynthetic O_2 evolution
PV	Photovoltaic
RITE	Roosevelt Island Tidal Energy

RPM	Revolutions per minute
RSM	Response surface methodology
RZT	Root zone temperature
RZTa	Ambient root zone temperature
SHP	Small hydropower
SM2	Synthetic medium
SPM	Suspended particulate matter
TGU	Turbine generator unit
VOC	Volatile organic compound

6.1 Introduction

Energy has the ability to heat an object or transform one type of object into another in order to perform work. There are basically two types of energy available, which are known as potential or kinetic in nature. Various forms of energy (i.e., light, heat, sound, and motion in nature) are utilized by humans for various purposes to meet their needs. These forms of energy are used to drive automobiles on roads and to drive boats through water. Furthermore, energy is used to cook food on stoves (using gas or petroleum fuel), to make ice in freezers, and to light our homes. The world's population is currently heavily dependent on nonrenewable energy sources such as coal, petroleum oil, and natural gas to fulfill our needs for energy. Coal, oil, and natural gas are the best examples of fossil fuels with the nature of nonrenewable energy, as these are finite resources on our planet. These resources will eventually dwindle and are expensive, and their utilization for energy damages the environment greatly.

Only sustainable energy sources can fulfill our current energy demands without causing dangerous effects or the threat of future depletion of energy sources. Sustainable energy sources can be used multiple times and for a long time. There are many resources that can provide sustainable energy (e.g., solar power, wind power, geothermal power, hydropower, and ocean energy), and use of these resources can be widely encouraged without causing harm to the health of the environment or its components, because greater quantities of sustainable energy are available at no additional cost (Sovacool 2013). Storage of energy in various materials is a potential source of energy and other form of energy is found in wave motion, electrons, atoms, substances, and other moving objects in the form of kinetic energy. Chemical energy is also a type of potential energy, stored in the bonds of atoms or molecules, and its best sources are batteries, biomass, petroleum, natural gas, and coal. Its sources can be converted into thermal energy via burning or combustion processes. Recent advances in technology have helped a lot in the production of *n*-butanol, an advanced second-generation biofuel obtained from utilization of hydrolyzed agricultural-source biomass or its residue, and it represents a good attempt at creating a sustainable means of energy production (Qureshi et al. 2012).

Other objects such as compressed springs and stretched rubber bands also contain mechanical energy (a type of potential energy) as they store energy by tension. Nuclear energy is another type of potential energy, which is stored in the nuclei of atoms (The NEED Project 2017). This energy is released via splitting or combining of nuclei. However, our current processes for this type of energy production usually entail splitting unstable nuclei of uranium atoms. Recently, there has been increasing research interest in use of radiant energy sources (i.e., radio waves, visible light, x-rays, or gamma rays) with the capability to travel as transverse waves. The radiant energy of sunshine provides fuel and warmth for us and makes life possible on Earth (Vezmar et al. 2014).

Many plants have a good capability to photosynthetically utilize radiant energy, and this has been studied in temperate climate plants such as lettuce (*Lactuca sativa* L.), cultivated under natural tropical and fluctuating temperatures for its chlorophyll (Chl) fluorescence and maximum photosynthetic O_2 evolution (P_{max}). In these studies the plant roots were exposed to two different root zone temperatures (RZTs), such as a constant 20 °C (RTZ-20) and a fluctuating ambient RZT (RZTa) of approximately 23–40 °C (He and Lee 2004).

Many reports published recently have shown that utilization of wind energy is greater in some countries such as India, China, the USA, Germany, and Spain, which have good wind flow in all seasons throughout the year. This energy is dependent on good wind flow in the relevant geographical location. Now wind energy generation in India is actively taking place by use of new wind turbine technologies, and this type of progress is also gradually achieving remarkable improvements (in comparison with past efforts) in many other countries too. From 2009 to 2019, wind energy capacity has generated about 21.264 GW of energy, and it was previously estimated that an additional 15–16 GW of new-generation capacity would be installed by 2020 (Sitharthan and Geethanjali 2014). Extending the solar and wind energy power system in India has shown more opportunities and possibilities for use of these renewable energy resources via application of grid parity, providing coherent and integrated ways of resolving major constraints and obstacles in the development of renewable energy sources in India (Khare et al. 2013).

In efforts to utilize lignocellulosic material for energy generation, development of a conceptual framework for waste-to-biomethane synthesis is being applied in an urban energy system and has provided bioprocesses for a technoeconomic evaluation of biogas production and consumption, with a particular interest in, and enthusiasm for, biomethane utilization in the transport sector (Dzene et al. 2016). Energy from algal biofuel has also been shown to be a viable alternative option to fossil fuel energy and can be exploited via use of innovative technology to overcome a number of hurdles so it can be broadly deployed and compete in the fuel market. Many challenges in algal biofuel synthesis have been reported, including identification of suitable algal strains and their improvement in terms of oil content productivity and crop protection, nutrient and resource allocation and use, and production of coproducts that can improve the economics of the entire system (Hannon et al. 2010). *Botryococcus braunii* has been identified as being suitable and can be exploited to store biofuel, with long-chain hydrocarbons constituting up to 50% of their dry mass. Identification of algal species with the desired traits in low-cost media (i.e., a high content of lipids, a high growth rate of algal cells, greater growth density, and/

or the presence of valuable coproducts) could be of great importance for bioprospecting on a commercial scale (Kojima and Zhang 1999).

As noted earlier, fossil fuel utilization has resulted in substantially greater generation of harmful gases (i.e., air pollutants and greenhouse gases (GHGs)) than renewable energy sources, and it affects the air and water quality by causing pollution. It also damages the health of humans and wildlife, and it entails habitat loss, water or land use, and emission of GHGs, causing global warming (Radziemska 2014). These impacts of nonrenewable energy sources force us to utilize sustainable/renewable energy resources to meet our energy needs while maintaining healthy climatic conditions. This chapter discusses current issues and challenges involved in implementation of sustainable energy practices and recent developments in sustainable energy worldwide.

6.2 Sustainable Energy Resources and Their Applications

The energy demand at the global level is estimated to more than double by 2050. If this trend continues, the energy demand will more than triple by the end of the century. Nonrenewable energy resources such as coal, oil, and natural gas cannot fulfill the total demand for energy. Our future total demand for energy can be fulfilled only by a greater contribution from renewable energy resources. Sources of renewable energy include solar power, wind power, geothermal power, hydropower, and ocean energy, which are sustainable, stable, and plentiful (Fig. 6.1). At present, around

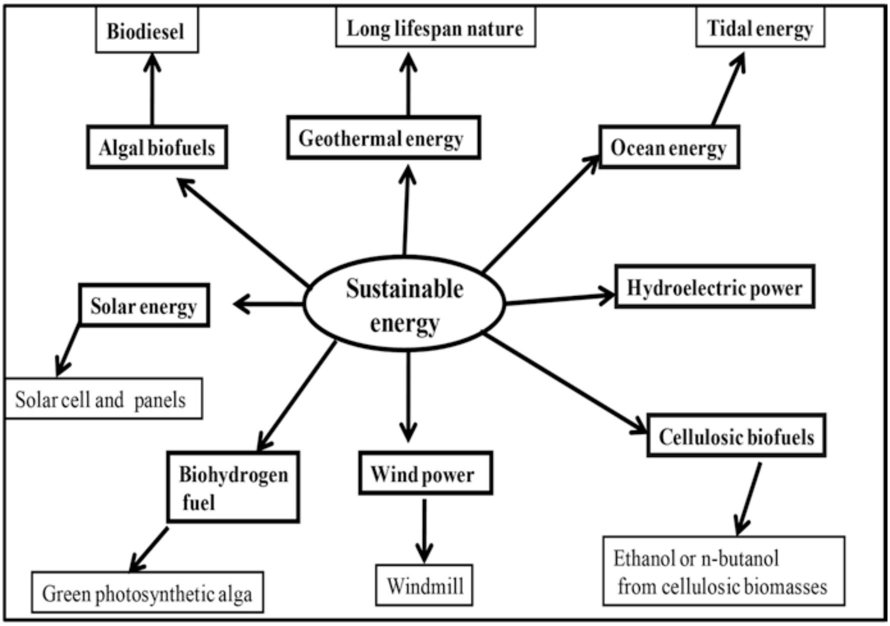


Fig. 6.1 Forms of sustainable energy available worldwide

20% of the total global energy requirements come from renewable energy sources. These sources of energy are replenishable, with the capability to mitigate GHG emissions without causing damage to the health of the environment or its components. They also have the capacity to continue providing energy to the next and subsequent generations.

Since time immemorial, the Sun has provided radiant energy, sunlight, and heat to all living and inanimate objects on Earth. It is the source of energy for the wind, and the wind is kinetic in nature, being able to produce energy to be utilized for various purposes. The Earth also produces heat from its interior, which is due to the presence of radioactive elements, and it will not cool down anytime soon. The motions of the Earth, Sun, and Moon do not stop and will keep on generating tides in the oceans. Heat energy in the oceans causes the process of evaporation and vaporization of water; then, in the atmosphere, it cools down and falls in the forms of rainwater or ice, which again goes into rivers, ponds, or streams and merges into the oceans, and this can be used in the production of energy through hydropower techniques and schemes.

Hydroelectric power, geothermal energy, and wind power are the main renewable energy resources, and these resources are also sustainable. The Sun has the capacity to shine upon the Earth for more than 4 billion more years; thus, solar energy is considered renewable. Hydroelectric power is based on the Sun's energy, which also provides power for the Earth's water cycle and the flow of rivers. Hydropower plants and distribution systems are connected to them to exploit this energy source, which is also seen as a renewable resource. In 2015, hydropower systems generated 16.6% of the total electric power generation and 70% of all forms of renewable electric power generation (Mastny 2016).

Geothermal power is continually being renewed although the rate of renewal varies in different geothermal areas. Geothermal power comes from deep within the Earth and is not dependent on solar energy; instead, it depends on decay of radioactive materials in the Earth's mantle. According to the literature, individual geothermal systems with a short life-span are considered nonrenewable sources of energy, whereas those with a long life-span (of more than 100 years) are considered renewable energy sources relative to the human scale. Geothermal energy is utilized for both industrial and domestic energy requirements in many parts of the world. It is used as an energy source for electric power generation mainly at active tectonic plate boundaries (Sharma and Trikha 2013).

The ocean has the capability for production of two types of energy. The first is thermal energy, which comes from the Sun's heat, and the second is mechanical energy, which comes from the tides and wave generation. A wide range of companies involved in the marine sector utilize this energy as renewable energy. This form of energy exploitation is dependent on insurance and finance, resource assessments, environmental surveys, design, manufacturing, offshore construction, operation, and decommissioning (Kydd and Brinckerhoff 2014).

India is surrounded by the ocean on three sides, with potential to harness the capacity for tidal energy. Tidal power is produced through combined forces exerted by the gravitational pull of the Sun and the Moon and the rotation of the Earth. The relative motions of these three bodies generate the different tidal cycles. Tidal power ranges can be enhanced substantially by local effects (i.e., shelving, funneling, or resonance phenomena). Energy can be extracted from tidal generation via creation of a reservoir or a basin behind a barrage, and tidal waters pass through turbines in the barrage to produce electricity.

Exploitation of tidal energy requires a specific type of ocean site with a mean tidal size difference of more than 4 m. Favorable topographical conditions (i.e., an estuary or a certain type of bay) are also preferable to help reduce the costs of dams, etc. (Yadav 2015).

The next section focuses on various devices that utilize renewable energy. Application of these devices can fulfill our daily energy needs.

6.3 Solar Energy as a Renewable Resource

More radiant energy comes from the sunlight that strikes the Earth's surface in 1 h (4.3×10^{20} J) than is consumed on the planet in a year (4.1×10^{20} J). This energy can be exploited as solar electricity and solar-derived fuel from plant biomass. As reported in the literature, systems for converting solar radiation energy are classified into three main categories: (1) solar electricity, (2) solar fuel, and (3) solar thermal systems. In 2001, solar electricity provided less than 0.1% of the world's electricity and solar fuel from sustainable agricultural biomass (obtained by modern means) contributed less than 1.5% of the world's energy supply. There are huge gaps between our current use of solar energy and our total energy needs, and there is enormous undeveloped potential for use of this source of energy, which provides a big challenge for research on systems for capturing solar energy. Figure 6.2 depicts a number of solar energy devices that consume solar energy by utilizing solar radiation for lighting, heating, cooking, and large-scale electric energy conversion.

Silicon solar array/ panel power on roof

Photovoltaic

Electric car heater

Solar cooker

Sieges 60 LED Solar lights 5 model

Fig. 6.2 Harnessing of solar power as a renewable energy resource

6.3.1 Solar Energy–Utilizing Devices

Solar Electricity

Solar electric or photovoltaic (PV) systems are used to produce electricity for lighting of homes and offices. A 25-kilowatt Sterling dish system has the capability to catch even the last rays of the setting Sun at the end of the day. An advanced generation of solar power plants that concentrate solar radiation energy are utilized for obtaining energy from the Sun, which is applied as a heat source or steam generator to boil water. This can be used for rotation of a larger turbine for active electricity power generation on a small scale (Mishra and Tripathy 2012). At the worldwide level, parabolic-trough, dish/engine, and power tower–type designs are the three main types of solar power systems with the capacity to concentrate solar energy.

A parabolic-trough system with a long, rectangular, curved (U-shaped) mirror has the capability for concentrating or storing solar energy by tilting toward the Sun and focusing sunlight energy onto a pipe that runs down the center of the trough. Oil flowing through the pipe is heated by this arrangement. The hot oil is utilized for boiling water. This arrangement is used in a conventional or traditional mode of steam generation for production of electric energy.

In another arrangement, a mirrored dish (similar to a very large satellite dish) is used in a dish/engine system. Its surface collects and concentrates the heat from the Sun's energy onto a receiver system. The engine contains a fluid that absorbs the heat from the receiver and transfers it to the next process. This heat causes expansion of fluid against a piston or turbine for production of mechanical power for electricity generation via generator usage.

In the third design, a large field of mirrors is used in a power tower system, which concentrates sunlight energy onto the top of a tower consisting of receiver sites and heats molten salt flowing through the receivers. The heat energy in the salt is used for generation of electricity with help from a conventional steam generator. Molten salt has the capability to store heat for few days before it is converted into electric energy (Garud and Purohit 2007).

Solar Fuel

A solar fuel system is a synthetic chemical fuel system in which the fuel is produced directly or indirectly by utilizing solar radiation in combination with photochemical or photobiological processes. In this type of system, light energy or heat is utilized as an energy source and transduced into chemical energy through proton reduction to hydrogen or carbon dioxide reduction to organic molecules. In photochemical processes, solar energy is used indirectly for conversion of biomass into biofuels by use of photosynthesis. Solar fuel systems produce clean fuel and offer highly efficient ways of producing hydrogen. An effective way of storing solar fuel is use of hydrogen peroxide (H_2O_2) in a one-compartment fuel cell (Fukuzumi and Yamad 2016).

Most of the energy harvested by photosynthesis processes in green plants is used in life-sustaining processes with never loosing system for energy utilization. Hydrogen is produced by electrolysis in photoelectrochemical cells using photosensitive electrodes with conversion of light energy into an electric current via water-splitting to produce electricity for use in production of hydrogen. Hydrogen is also produced by some species of photosynthetic microalgae and cyanobacteria, which can be used in photobioreactors. The overall energy efficiencies of these systems have been calculated to be between 0.88% and 9.7%, and the minimum and maximum overall exergy efficiencies are between and 0.77% and 9.3%, based on selection of various energy paths (Yilanci et al. 2009). Insights from computational modeling have provided new photocatalysts for solar fuel synthesis by describing relevant material properties (i.e., the nature of the materials, such as polymeric materials and nanoparticles), and these approaches to calculation can be utilized for screening of promising new materials for photocatalytic water splitting and reduction of carbon dioxide molecules (Guiglion et al. 2016).

Solar Thermal Plants

According to the literature, it was aimed to increase the global capacity of solar thermal power plants to 12 GW by 2020. We still face a lot of challenges to utilize climate-neutral energy in an efficient and cost-effective manner (Pitz-Paal et al. 2013). Solar thermal systems can convert sunlight into heat. Heat from the ground is also used via low-temperature extraction (in the range of 70–80 °F (21–27 °C)) to heat water for home and business needs in the range of 90–120 °F (32–49 °C), whereas high-temperature-process heating of water is applied for industrial applications in the range of 200–400 °F (93–204 °C). Solar thermal power plants can produce heat energy in the range of 1000 °F (538 °C) or higher. A solar wall collector uses perforated black sheet metal fastened directly to the exterior of a building (Kalogirou 2004). It contains small computerized fans, which pull outside air into slots and heat it; the air is then pushed through a pipe into the building.

In connection to a solar thermal system, a Trombe wall helps to utilize the Sun's energy by placement of a thermal mass (e.g., a stack of drums filled with water, used as a thermal storage capacitor) behind a wall of glass facing the Sun. (In the northern hemisphere, a Trombe wall usually faces south facing, whereas in the southern hemisphere, it usually faces north.) In this system, the air in this area is heated and then passes into the rest of the building. At night, the thermal inertia of the water-filled drums continues to heat the air. In another step-up design, a water heater contains a small pump and moves colder water into the collector when a controller senses that the panel is hotter than the tank. In another technique, a rooftop heater is used, which contains thermosiphon units, and a storage tank is placed above the panels, so that heated water rises into the tank. An Axicon conical reflector is also used in solar thermal systems. It consists of a polished mirror cone, which reflects the Sun’s energy onto a water-filled pipe and boils the water.

A solar power tower can produce up to 5 MW of power via fluxing to 280 W/cm^2. It contains a heliostat, which is separately driven to focus its beam on a receiver (Beerbaum and Weinrebe 2000).

6.4 Wind Energy

As has been reported, wind energy can be exploited on a large scale. Current reports show that it is the second-fastest-growing source of electric power generation in the world. By the end of 2015, its global capacity was about 4.33×10^5 MW of electricity. Wind power capacity of 7.5×10^4 MW was operational in the USA by 2016. Wind energy technologies have been utilized to generate electric power, charge batteries, pump water, and grind food grain in practical and commercial applications. Recently, significantly increased efforts have been made to utilize wind resources in Greece. An extensive study was conducted to gauge public opinion on application of wind energy (as shown in Fig. 6.3) on the mainland and several inlands in Greece that had high wind potential and investment interest. The study found that although there was public acceptance of existing wind parks, the public did not favor new wind park installations (Kaldellis 2005).

Generation of mechanical or electric power can be achieved by utilization of the kinetic energy of wind, and wind power is proportional to the wind speed cubed. Thus, the power harnessed by a wind generator increases by a factor of eight when the wind speed doubles. The hub of the turbine rotates, and the rotor blades are moved directly by the passing wind, generating electric power. The blowing of the wind is formed by a pocket of lower pressure air, with the capacity to pull the blade in a downwind direction and cause the rotor to turn in a lift mechanism. The force of the lift is much stronger than the wind’s force against the front side of the blade,

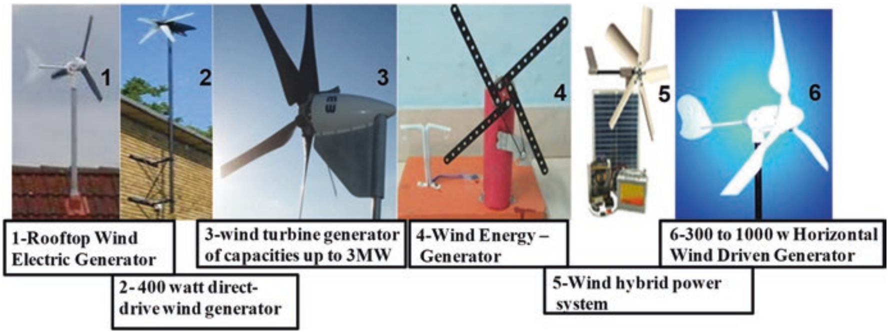


Fig. 6.3 Harnessing of wind power as a sustainable energy resource

which is called the drag force. The combination of lift and drag causes the rotor to spin.

6.4.1 Wind Energy Storage Devices

Taiwan's government is actively involved in capturing wind energy via utilization of renewable energy technology innovations to make wind energy storage devices to optimize energy resource inputs. This country has uses effective strategic energy planning. As reported in the literature, Taiwan is highly vulnerable in terms of its energy security, but it has the advantage of hilly geographical conditions, which are well suited to wind energy resource development. A study was performed to explore resistance to wind energy and key factors affecting the application of small wind energy systems in the country. The findings of the study have helped to improve the quality and quantity of Taiwan's renewable energy utilization and energy competitiveness, and have provided strategies for assessment and forecasting of wind energy–based technologies for the future (Liu and Ho 2016).

Wind energy technologies can either be used as stand-alone systems that work independently, can be connected to a utility power grid, or can be combined with a photovoltaic system. In utility-scale application of wind energy systems, turbines are built close together to form a wind farm for generation of bulk power. Wind farms supply power for customers' use and are operated by companies such as Xcel Energy, MidAmerican Energy, and Basin Electric. Xcel Energy supplies energy to power millions of homes and businesses across eight western and midwestern states of the USA. MidAmerican Energy is a private energy-generating company based in Des Moines (IA, USA). Basin Electric Power Cooperative is an electricity generation and transmission cooperative corporation in the USA, which owns and operates coal, gas, oil, nuclear, and renewable energy resources, including wind power plants.

The largest wind energy capacity in the USA is in the state of Texas, which has 8797 MW of capacity in operation plus an addition 660 MW currently under construction. Texas has seen very fast growth in the development of wind power and has achieved good understanding of techniques and processes for capturing wind energy. A study of public opinion on wind energy focused on the environmental attitudes of the population living near a wind farm development to assess how the proximity of the development influenced attitudes to wind energy. The study found that the "not-in-my-backyard" (NIMBY) phenomenon did not fully explain negative perceptions of wind energy utilization (Swofford and Slattery 2010).

Suzlon Energy is one of the largest producers of wind turbine devices in the world. It provides an all-encompassing service for wind energy farm construction with state-of-the-art blades, nacelles, towers, and foundations. Suzlon uses a multidimensional strategy in terms of its engineering with cost reduction techniques to provide a competitive advantage to its consumers. Suzlon can provide the option for choosing the sustainable energy with more sustaining conditions via utilization of renewable energy and energy deficient solution to world while also maintaining an eco-friendly approach.

6.4.2 Wind Turbine Designs

Different designs and many different sizes of wind turbine are available to accommodate different needs for energy via utilization of wind power. They can be large or small in size with a horizontal- or vertical-axis blade design. In wind turbine design construction, two or three blades can spin upwind of the tower. Small wind turbines have the capacity to generate power in the range of 250 W to 50 kW and are available for providing power off the grid, e.g., for dairy farms and remote villages, respectively. Windmills have a tail vane to keep them oriented to the wind direction. Large wind turbines have an enormous multimegawatt capability to produce large amounts of wind energy (up to 100 kW) with independent generators to provide power to a grid. Large turbines are up to 100 m tall with a blade diameter of 30–60 m. Utility-scale turbines used to generate wind power are usually found in groups or rows to take advantage of prime windy locations. Wind farms may consist of a few turbines or hundred of turbines providing enough power for whole towns or cities.

6.4.3 The S111 Wind Turbine Generator

The S111 wind turbine generator is one of the latest turbine designs from Suzlon Energy and has the capacity to provide 2.1 MW of wind power. This wind turbine generator is available in two different sizes, with different capacities to generate power. The first is 90 m tall with an all-steel tubular tower, and the second is 120 m tall with an all-steel tower that has a unique transition piece combining a lattice and tubular structure. The S97 wind turbine generator is also one of the latest products from Suzlon and also has the capability to generate 2.1 MW of power. It has been designed to make use of low-wind sites viable for generation of wind power similarly to the S111 wind turbine generator. Innovations introduced by Suzlon have resulted in increased height and stability with designs that enable greater generation of wind power. These features help to reduce costs, with simpler logistics and ease of construction, in order to make use of otherwise unusable low-wind sites viable and offer customers good returns on their investment.

6.5 Hydroelectric Energy

Worldwide, hydropower is one of the leading renewable sources of electricity. In 2015, hydropower was reported to account for up to 16.6% of the total world electric power generation and contributed to 71% of all renewable electricity generation, with an aim to reach 1.06×10^3 GW of power from the installed capacity in 2016. In this regard, China has exploited this energy resource intensively and has been reported as being the largest hydroelectric power–producing country, followed by Canada, Brazil, and the USA. More research is going on to find untapped hydropower resources with still greater abundance in Latin America, Central Africa, India, and China. However, the need for economically feasible technical processes and methods still remains (WEC 2016). Burning or combustion of renewable energy source has generated the minimal pollution concentration in atmosphere. For this type of energy power generation, water must be stored at a great height in a reservoir to run a power plant, and nature has helped in this regard by providing abundant water resources. Rainfall renews the water in the reservoir, which can almost always generate power. Innovative concepts and technical solutions have helped to improve the efficiency of hydropower greatly—for instance, in three small hydropower (SHP) developments on the Sebes river in Romania, which provide a good example of appropriate implementation of new SHP developments (Dadu et al. 2017).

There is a lot of information available on the operation and maintenance costs of hydroelectric power plants, which have relatively low costs and low energy consumption, enabling reductions in GHG emissions. However, use of hydropower facilities has forced us to think about forms of energy generation that have large impacts on the environment, affecting land use, homes, and natural habitats in the region of the dam. Most hydroelectric power plants require a dam or a reservoir, and its structure can obstruct the migrations of fish and hinder their reproduction. During the operation of hydroelectric power plants, the water temperature changes with the river flow. These changes in rivers can harm plant and animal life in and around the river. Construction of reservoirs can displace people's homes, important natural areas, agricultural lands, and archeological sites. Thus, the building of dams also involves relocating people from the dam site. From some reservoirs, methane (a powerful GHG) is emitted into the atmosphere.

In hydroelectric plants or systems, hydroelectricity is generated via the force of water falling from a height, as shown in Fig. 6.4. The production capacity of a hydropower energy system depends on the available flow and the height from which the water falls. Nowadays, there is greater application of SHP plants, which are self-contained and can play a critical role in the transition to a low-carbon future. Nigeria has the technical capacity to exploit SHP, but it is underutilized despite government policy promoting advanced power sector reform. One study traced the evolution of SHP systems to determine which component of the interactive learning mechanisms would result in the most significant outcomes. It also explored the important role of SHP in grid-based power generation to generate power for remote off-grid communities. Certain barriers (i.e., low levels of technological capability, lack of sound and

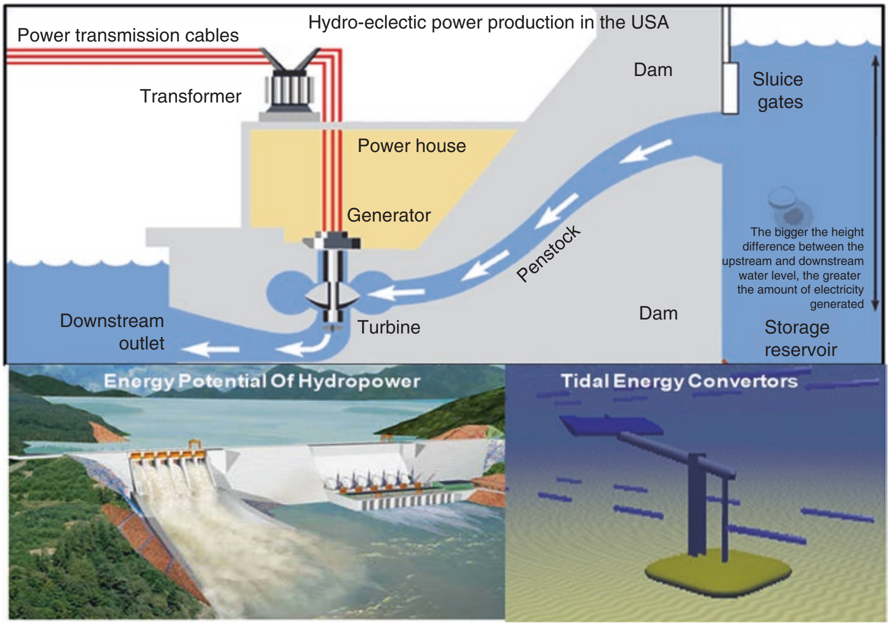


Fig. 6.4 Harnessing of hydropower to produce electricity

up-to-date financial and technical analyses, and low levels of investment) were found to be the main factors hindering deployment of SHP (Sanni 2017).

Construction of high water dams allows accumulation of greater potential energy, which can be transformed into mechanical energy via water rushing down the sluices and striking the rotary blades of turbines. The rotating turbines have the capability to spin electromagnets, generating electric current in stationary coils of wire. Finally, the current is passed through a transformer to increase its voltage for long-distance transmission through power lines. Many hydroelectric power–producing systems are operational in many countries in the world; a good example is China, which is developing large hydroelectric facilities. Greater use of hydropower has been made throughout the world in the last few decades. In the USA, the states of Idaho, Washington, and Oregon rely greatly on hydroelectricity as their main power source, as shown in Fig. 6.4 (Gielen 2012).

6.5.1 Devices for Hydropower Storage

Hydropower storage devices can be designed in a way that allows them to be utilized for storage of tidal energy. Tidal energy can be exploited by utilization of the natural ebbs and flows of coastal tidal water. Generation of tidal energy occurs through interactions of the gravitational fields of the Earth, Moon, and Sun. The best utilization of tidal energy systems takes advantage of the fact that fast ocean currents are often magnified by topographical features such as headlands, inlets, and straits, and by the shape of the seabed, with forced water flow through narrow channels. Tidal stream system devices have the capability for utilization of these currents to exploit the kinetic energy of the tidal currents. In seawater with a higher density, blade designs are kept smaller with slower turning to deliver a significant amount of power. To increase the flow and power output from the turbines, concentrators (or shrouds) are used around the blades to streamline and concentrate the flow toward the rotors (Harris 2014).Different shapes and designs are used to help capture more energy:

1. Horizontal-axis turbines have the capacity for extracting energy from moving water similarly to the way in which a wind turbine extracts energy from moving air. The tidal stream causes the rotors to rotate around the horizontal axis with generation of greater amounts of power.
2. Vertical-axis turbines are designed to optimize the extraction of energy from tides. The mounting of the turbine on a vertical axis helps the tidal stream to rotate the rotors around the axis for power generation.
3. The oscillatory nature of a hydrofoil turbine design is formed by attaching a hydrofoil to an oscillating arm, inducing lift and drag forces in the tidal current. This motion is utilized for driving fluid, as in a hydraulic system, to generate electric power.
4. Venturi effect devices are housed in a duct to concentrate the tidal flow passing through a turbine. In this design, a funnel-like collecting device is submerged in the tidal current and the water flow drives a turbine directly or under induced pressure differential conditions in the system.
5. An Archimedes screw system involves a helical corkscrew-shaped device (a helical surface surrounding a central shaft) and has the capacity to draw energy from the tidal stream by moving water up or through the spiral, turning the turbines.
6. A tidal kite design is tethered to the seabed and carries a turbine below a wing. The kite flies in the tidal stream and swoops in a figure-of-eight pattern to enhance the speed of the water flow through turbine.
7. Other different and unique turbine device designs have been created, but, so far, none of them have been shown to be technically superior to the existing designs listed above.

6.5.2 Hydropower Devices in the Ocean

Because of the sheer multiplicity of their nature, wave, tidal, and hydrokinetic power devices have shown great potential for generation of power from utilization of the world's oceans and river systems. In this regard, more than 100 different types of marine or hydrokinetic power-utilizing devices have been developed, tested, and deployed by different companies, as shown in a report from PKE Research. The

Ocean Renewable Energy Coalition contributes more than 10% of the USA's power supply and meets these energy contributions from unconventional hydropower resources.

Utilization of Point Absorber Devices for Hydropower

The floating nature of point absorber devices, together with their components or parts, makes them move relative to each other in response to wave motion, and they use a hydraulic, pneumatic, or direct-drive system. A direct-drive permanent magnet generation device has been built by Columbia Power Technology (CPT), located in Charlottesville, VA, USA. Several different types of these devices are used for generation of electric power from wave energy and in most cases, the production of energy from the seashore involves use of undersea power cables. The SeaRay exploits the average amount of energy output (measured in hundreds of kilowatts) with peak amounts of energy output (measured in megawatts) (Bozzi et al. 2013). The next step for SeaRay is to build a utility-scale model seven times larger in size, called Manta. Power buoys (PBs) are another type of point absorber, found in many areas of the ocean. The PB150 device has shown the capacity to generate 150 kW of power by bobbing quickly up and down with the motion of the waves. In this process, mechanical motion is converted into electric energy via utilization of a power takeoff device driving a generator inside the buoy. Built from steel, the buoy sizes range from the PB150 to the PB500 (which has a 500 kW maximum power capacity). The first commercial project using a power buoy device is located on the west coast of the USA and features up to 10 PB150 buoys in combination, kept in a undersea substation using submarine cables (Thorburn 2006).

Utilization of the Sea Snake Device to Produce Hydropower

The Sea Snake is a type of wave power device. It contains an attenuator, floating on top of the water, parallel to the wave direction. Several segments of the Sea Snake connect together like a long sea snake. The best example is the Pelamis wave energy converter, developed in Edinburgh, Scotland. It contains two engineered steel attenuators and is composed of five segments, which flex in two directions in response to wave motion. Its motion is converted into electric energy via use of a hydraulic power takeoff system inside each joint. This device has shown the capacity to produce power of up to 750 kW, and it is aimed to increase its capacity by a factor of 25–40%. In 2008, Pelamis opened a wave farm off the northwest coast of Portugal. It also launched an energy project with a capacity of 170 MW in UK waters. The Anaconda is another giant rubber sea snake, which floats offshore and converts wave energy to electricity. An 8 m–long (1/25th scale) model was tested in a large wave tank in Gosport, UK, and it was hoped to build a full-sized working version within 5 years (Barras 2009).

6.5.3 Generation of Electricity from Tidal Currents

Tidal energy devices have been used to harness the kinetic energy of tides to produce power. The Ocean Energy Council has estimated that the world's total potential for ocean tidal power is 64,000 MW. Water is 800 times denser than air, and tidal devices can produce far more energy at a low technological level and using a slower speed of water current than wind turbines can produce using air currents. The Earth's ocean resources provide huge amounts of renewable energy. Utilization of advanced and powerful technologies to harness the power of the ocean is at an initial stage of development. An increasing number of researchers are focusing on advanced technology or technological improvements to fill research gaps, with research efforts being directed toward capturing greater quantities of tidal current energy (Uihlein and Magagna 2016).

The Ocean Renewable Power Company (ORPC), based in Portland (OR, USA), has developed a turbine generation unit as a gearless device, consisting of corrosion-resistant composite materials. In small rivers, the RivGen system is utilized in combination with a turbine generator unit (TGU) and a support frame resting on the riverbed. In deep ocean water, the OCGen system is used to stack several TGUs on top of one another in a buoyant array pattern to take full advantage of ocean currents; a 1 MW module has been demonstrated in the Gulf Stream off the coast of Florida. A system with a 189 kW capability from a single device has been connected to the grid by Bangor Hydro Electric. A phase 3 system of up to 29 devices in a 5 MW array has been installed in the Western Passage off Kendall Head in Maine, USA (McCarthy 2015).

A free-flow kinetic hydropower system design uses a rotor and three blades in a turbine rotating at 35 revolutions per minute (RPM). It can drive a speed increaser in turn and also drives a grid-connected generator. Verdant Power's Roosevelt Island Tidal Energy (RITE) project in the East Channel of the East River in New York (NY, USA) is the first commercially licensed tidal energy plant in the USA (Lampman et al. 2011). It contains 30 turbines with a generating capability of up to 1 MW. In Canada, Verdant Power has aimed at a hydrokinetic energy system capacity of 1500 MW via utilization of the Cornwall Ontario River Energy (CORE) project and has reported energy harness efficiency of 15 MW (May 2010).

6.6 Biofuel Energy

There are many biofuels utilized as transport fuels in their pure form or blended with gasoline or other fossil fuels. Good examples are cellulosic ethanol and *n*-butanol, mostly biosynthesized from lignocellulosic materials (such as wheat or rice stalk powder, sugar bagasse, or other materials) via use of suitable microbes (Zhao et al. 2009). *Lactobacillus brevis* is known to produce 2-butanol as a biofuel energy source, and it has also been found to be produced in fermented beverage industry products, where it affects the taste, changing the quality of the final or desired products. 2-Butanol can be biosynthesized through formation of a meso-2,3-butanol compound, which is then converted into 2-butanone with the help of the enzyme diol dehydratase. 2-Butanol production in a synthetic medium (SM2) via use of *L. brevis* is a good attempt to produce advanced biofuel energy. An evaluation of the performance of the secondary alcohol dehydrogenase and the diol dehydratase required for conversion of meso-2,3-butanediol to 2-butanol has been conducted (Ghiaci et al. 2014).

For a sustainable environment, renewable fuel energy production (production of bioethanol by utilization of lignocellulosic biomass) is an important attempt to utilize different forms of lignocellulosic materials obtained from crop production, and it exhibits a remarkable potential to fulfill current energy requirements via mitigation of GHG emissions. In the last few decades, production of bioethanol has been performed using sugarcane juice, starch-based cereal grains (e.g., corn), or tuberous foods (e.g., potatoes). However, utilization of sugarcane and food grains to produce bioethanol could cause food security issues and pressure on food prices. Thus, recent research has been more focused on utilization of lignocellulosic materials or biomass as a raw material source for biocell-mediated ethanol production via use of advanced technologies (Joshi et al. 2011).

6.6.1 Utilization of Plant Biomass as a Lignocellulosic Material

Plant biomass is a good source of lignocellulosic material and is available in green or dry forms in huge quantities worldwide. This biomass consists mainly of lignin, cellulose, hemicellulose, pectin, and other components (Fig. 6.5). Lignocellulosic biomass is an abundant source of organic materials and is used as a raw material for sustainable production of bioenergy and biofuels such as biogas (about 50–75% CH_4 and 25–50% CO_2). Recently, many efficient bioconversion technologies (for hydrolysis, fermentation, and other processes) have been developed and used for biofuel and bioenergy production. One example is anaerobic digestion (AD), which is known as the most cost-effective bioconversion technology and is now being implemented worldwide for production of commercial-scale electric energy, heat, and compressed natural gas (CNG) from utilization of waste organic material (Zheng et al. 2014).

Nepal is rich in lignocellulosic material resources, but, to date, its investment in development of renewable energy has been almost nonexistent. However, this country has good potential for development of biofuels, as approximately 30% of Nepal's land is climatically favorable for cultivation of the *Jatropha* plant (locally known as *sajiyon* or *sajiba*), which has previously been utilized in ethanol biofuel production. Nepal's land is also suitable for cultivating plants with seeds containing inedible oil

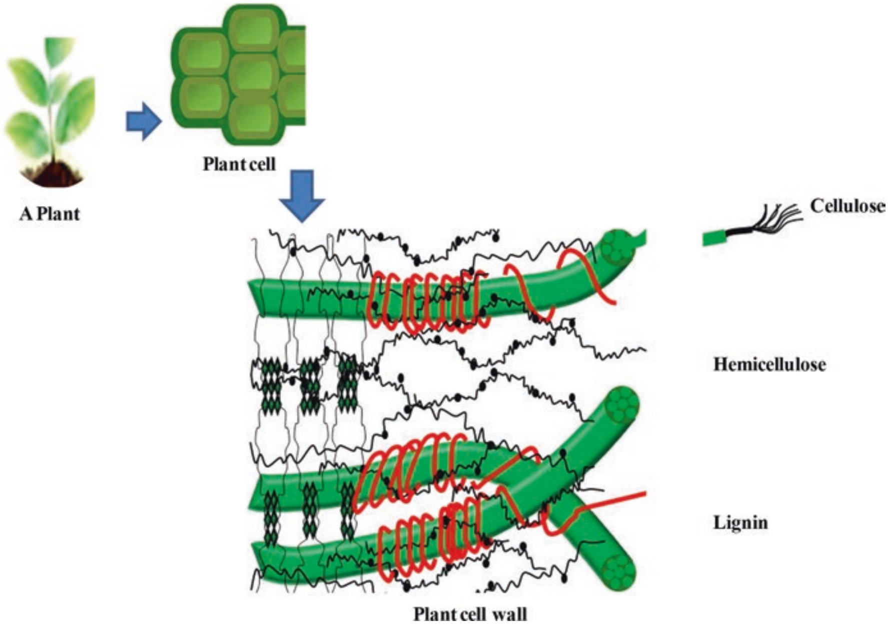


Fig. 6.5 Components of plant biomass

(e.g., soap nut (*Sapindus mukorossi*), *dhaka* (*Argemone mexicana*), and *nagkassar* (*Mesua ferrea*), which are known as excellent sources of lignocellulosic materials) (Sharma et al. 2015). Many efficient microorganisms harbored in the guts of ruminant herbivores are good at degrading different types of plant biomass. The process of converting lignocellulosic biomass into bioethanol requires isolation and identification of microorganisms that have the capability to convert pretreated biomass into a suitable fermentable sugar form, to then be fermented into bioethanol. Different microorganisms have been isolated from the guts of goats, which can hydrolyze different plant constituents (Kaphle et al. 2014).

As a component of lignocellulosic material, cellulose is a principal component in plant cell wall materials, with a concentration in the range of 40–50% of plant dry weight. Cellulose is a homopolysaccharide made up of repeating β-D-glucopyranose units, with variations in the degree of polymerization or crystallization in different plant species. These have a significant impact on hydrolytic processes via use of biological enzymes and acidic or alkaline agents (Zhang et al. 2004). Hemicellulose is another component, found in fewer complexes in nature, and its concentration in lignocellulosic biomass is in the range of 25–35%. It can be utilized as an easily hydrolyzable or convertible component in fermentable sugars. Hemicellulose is a heteropolysaccharide and consists of pentose sugars (D-xyloses and D-arabinoses), hexoses (D-mannoses, D-glucoses, and D-galactose), and sugar acids. Mannose is a major constituent in softwoods containing hemicellulose, whereas xylan is the main component in hardwoods (Balan et al. 2009; De Moraes-Rocha et al. 2010). Lignin is the third major component and is found in lignocellulosic biomass, with a concentration in the range of 20–35%. This is a complex polymer of phenyl propane (p-coumaryl, coniferyl, or sinapyl alcohol) and has been described as a cementing agent in the development of an impermeable barrier against enzymatic attack or hydrolysis processes (Kaya et al. 2000; Howard et al. 2003).

Plant biomass with a rich water content (e.g., sewage sludge, cow dung slurry, or lignocellulosic waste) is another sustainable resource and has important advantages with key properties. Large quantities of biogas can be produced from cow dung slurry. Hydrolysis or digestion (of complex organic material), acid-generating fermentation, and methane- and acid-generating bacterial species play critical roles in biogas synthesis processes. Substrates in cow dung slurry are digested with hydrolysis when kept under anaerobic conditions for 5 days, and biogases including methane (measured by use of a saccharometer) are produced through anaerobic digestion during this period (Gnanambal and Swaminathan 2015).

Grass is an efficient lignocellulosic material and an effective feedstock for production of biogases. It requires minimal water for its growth in comparison with other types of lignocellulosic biomass. It can also be grown on nonarable land, avoiding direct competition with food crops. Biogas biosynthesis is limited by unavailability of suitable feedstock with a complex lignocellulosic structure. Disruption of the structure of grasses can be achieved by mechanical, microwave, thermal pretreatment, or utilization of chemical or biological agents, followed by an anaerobic digestion process for biogas formation (Rodriguez et al. 2017).

6.6.2 Clean Energy from Hydrogen Biosynthesis

Bioproduction of hydrogen is a clean energy source. Hydrogen is a simple molecule and can be easily collected, stored (in a gas or liquid form), and transported. It is highly combustible and can be utilized as a fuel source or for electricity production. On mixing with oxygen, hydrogen forms water molecules and provides around 30,000 calories per gram, as compared with 11,000 or 8000 calories per gram of gasoline or coal, respectively. Further, hydrogen is an environmentally friendly fuel, since it is a nonpollutant. Hydrogen energy systems have the potential for greater use as renewable energy resources, and hydrogen has a role as an energy carrier and an energy production system (Rosen 2015). Because of its versatile nature, hydrogen fuel can be used in automobiles, airplanes, helicopters, buses, and scooters as transport means. Liquid hydrogen is considered to be an ideal fuel for subsonic and supersonic aircraft worldwide. Microbial biomass conversion has been shown to have many advantages, as microbes have the ability to consume a broad range of lignocellulosic materials, digest the biomass, and release hydrogen gas (Balat 2008).

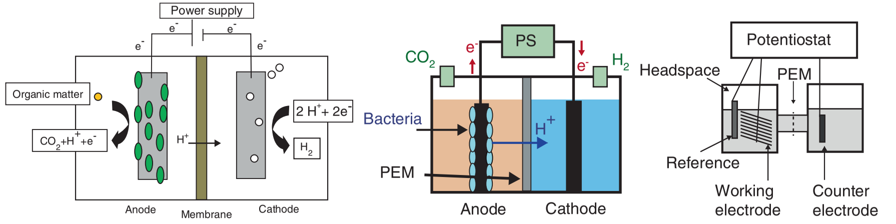
Hydrogen Production by Dark Fermentation

It has been mentioned that hydrogen biosynthesis can be achieved from nonrenewable energy resources (e.g., coal or nuclear energy sources) as well as from renewable energy resources (solar power, hydropower, wind power, agricultural biomass, ocean tides, etc.). Therefore, research is being conducted worldwide to explore utilization of hydrogen gas as an energy carrier, produced from renewable energy sources. In fermentation systems, several types of microbial cells (especially bacteria) are utilized to break down complex organic matter for hydrogen production, and corn stover (a biomass resource) or even organic matter in wastewater can be utilized by bacterial cells to release hydrogen gas in a dark fermentation process. In a direct mode of fermentation for hydrogen production, microbial cells can increase the yield of hydrogen production through breakdown of complex organic molecules via many different pathways in combination with enzyme activity (Hussy et al. 2005; Khanal et al. 2005).

Biohydrogen production has been achieved using hydrolyzed wheat straw (25% v/v) via utilization of dark fermentation and also using thermophilic mixed microbial culture strains at 70 °C in a batch or continuous mode, or in both modes, in reactors or fermenters. No hydrogen production was observed when a wheat hydrolyzed fraction of 30% v/v was used, which indicated that this hydrolysate concentration could cause inhibition of hydrogen production in the fermentation process (Kongjan et al. 2010). Biohydrogen production from D-xylose sugar as a substrate was achieved with use of a batch or continuous mode at an extreme thermophilic temperature and in the same conditions, using a mixed microbial culture, and this showed the highest hydrogen yield (1.62 mol of H_2 per mol of xylose consumption). For this hydrogen yield, an initial xylose concentration of 0.5 g/L in a synthetic medium amended with yeast extract in a 1.0 g/L concentration was used in the fermentation process. Biohydrogen has also been synthesized in a continuous stirred-tank reactor (CSTR), using a 72 h hydraulic retention time (HRT) with a xylose concentration of 1 g/L as a substrate at a temperature of 70 °C (Kongjan et al. 2009).

Hydrogen Production by Microbial Electrolysis Cells

Many designs of microbial electrolysis cell (MEC) device are utilized for harnessing energy and protons, generated by microbial cell–mediated breakdown of organic matter in combination with an additional small electricity supply for hydrogen gas generation (Fig. 6.6). MEC-based devices have the potential to produce hydrogen from different organic resources that cannot be simply utilized for fuel production. Advancements in the development of MEC systems allow them to be scaled up to commercial-level sizes with maintenance of high rates of hydrogen production and high system efficiency. Utilization of bench-scale MEC systems minimizes the costs of the reactor components (Cheng and Logan 2011).



Typical two chamber MEC construction

Bio-electrochemically assisted microbial reactor (BEAMR),

Potentiostat-controlled bio-electrochemical systems (BESs)

Fig. 6.6 Hydrogen energy production using a microbial electrolysis cell (Kadier et al. 2016)

Improvements in the hydrogen production rate and yield from fermentative processes can be achieved by application of microbial strain improvements, reactor system optimization, identification of optimal feedstock sources, and optimal processing methods with the highest yields. The MEC is cutting-edge technology, which can help to achieve a sustainable mode of clean hydrogen production from a broad range of renewable biomass resources or wastewater rich in organic material (Lai et al. 2014). The design of an MEC reactor has a direct influence on its hydrogen and current generation rates. The conventional MEC design incorporates a membrane filter, but membrane-free designs also have a high rate of hydrogen recovery. Recent advances in research have helped to produce scalable MEC designs with better configurations (Kadier et al. 2016).

Hydrogen Production by Photosynthetic Bacteria

Hydrogen has great potential as a fuel for the future, as it is a carbon-free fuel and, when oxidized, forms water as its end product. A process for bioconversion of synthetic gas (syngas) into hydrogen gas has been achieved using a continuous stirred-tank bioreactor (CSTBR) with utilization of acetate as a carbon source. An anaerobic photosynthetic bacterium species (*Rhodospirillum rubrum*) was utilized to catalyze the water–gas shift reaction (Younesi et al. 2008). A biological mode of hydrogen production has been achieved by photolysis of water molecules, using photosynthetic algae or certain bacteria, in a phenomenon known as biophotolysis. Certain microalgae and cyanobacteria (e.g., *Chlorella*, *Chlamydomonas*, *Scenedesmus*, *Microcystis*, *Oscillatoria*, and *Anabaena*) have the capability to generate molecular hydrogen. Water is the source of the raw material.

$$H_2O \overset{\text{Photolysis}}{\rightarrow} O_2 + H^+ + e^-$$

$$H^+ + H^+ \overset{\text{Hydrogenase}}{\rightarrow} H_2$$

The action of hydrogenase is inhibited by creating oxygen pressure. This condition favors greater release of free hydrogen. Isolated chloroplasts, along with the bacterial enzyme hydrogenase, have also been used in production of hydrogen. A continuous-mode photosynthetic process of hydrogen production has been studied using a batch mode of *R. rubrum*. It was found to produce hydrogen at rate of 20 ml/h per gram of dry cell weight, and the composition of the evolved gases was found to be practically constant (70–75% H_2 and 25–30% CO_2) (Zürrer and Bachofen 1979).

Hydrogen Production by Cyanobacteria

Cyanobacterial strains have proved to be highly promising microbial cells for production of hydrogen in a commercially viable process. Particularly effective cyanobacterial strains have been identified, with the potential for large-scale production of hydrogen in the future. Integration of existing knowledge can be utilized in the future for improvements in hydrogen production, so that it can be accepted as a commercial primary energy source (Dutta et al. 2005). Hydrogen fuel is a crucial energy carrier for energy security, and its availability should be ensured across the globe. The importance of the photosynthetic mode of hydrogen production is evident, and it could help to create a solar-powered hydrogen economy with development of innovative technology. In this regard, microalgal hydrogen production has been also discussed, with future prospects of being an essential technology in the development of a clean and sustainable energy economy (Bayro-Kaiser and Nelson 2017).

Solar fuels have been developed on the basis of artificial photosynthesis. Research on artificial photosynthesis has been conducted with application of the fundamental scientific principles of the natural process in the design of solar energy conversion systems. Artificial photosynthesis uses various types of materials, and more recent research efforts have turned to them for production of energy in efficient ways and in forms that are useful to humans. Fuel production can be achieved via utilization of natural or artificial modes of photosynthesis, using three main components. The first is an antenna or a reaction center complex for absorbing sunlight, the second involves excitation energy conversion into electrochemical energy (redox equivalents), and the third is a water oxidation complex for utilization of its redox potential in catalytic conversion of water into hydrogen ions, electrons (stored in reducing equivalents), and oxygen. A second catalytic system (in natural photosynthesis) uses this reducing equivalent for making fuels such as carbohydrates, lipids, or hydrogen gas (Gust et al. 2009).

6.6.3 Biofuel Production by Cyanobacteria

Cyanobacteria have been found to exhibit great potential for biofuel biosynthesis because of their rapid growth, their ability to fix CO_2 gas, and the tractability of their genetic variation. They do not require fermentable sugars or arable land for their growth. There are some challenges involved in achieving the improvements required for advanced cyanobacterial biofuel production. These mainly relate to genetic information, carbon fixation ability, metabolic flux, nutrient requirements on a large scale, and photosynthetic efficiency with use of natural light. Isobutanol (450 mg/L), 2,3-butanediol (2.4 g/L), 1-butanol (29.9 mg/L), 2-methyl-1-butanol (200 mg/L), and fatty acids (197 mg/L) have been successfully produced by *Synechocystis* species, as shown in Table 6.1 (Nozzi et al. 2013). Cyanobacteria are a diverse group of

Table 6.1 Biofuels generated using different microorganisms (Nozzi et al. 2013)

Biofuel	Microbial strain	Titer
Acetone	*Synechocystis* sp. PCC 6803	36 mg/L
2,3-Butanediol	*Synechocystis elongatus* PCC 7942	2.4 g/L
1-Butanol or *n*-butanol	*Synechocystis elongatus* PCC 7942	30 mg/L
Bioethanol	*Synechocystis* sp. PCC 6803	5.5 g/L
Fatty acids	*Synechocystis* sp. PCC 6803	197 mg/L
Isobutanol	*Synechocystis elongatus* PCC 7942	450 mg/L
Isobutyraldehyde	*Synechocystis elongatus* PCC 7942	1.1 g/L
2-Methyl-1-butanol	*Synechocystis elongatus* PCC 7942	200 mg/L

microorganisms and are also a part of marine or freshwater phytoplankton, making a significant contribution to fixation of atmospheric carbon via photosynthesis.

Many species of cyanobacteria are easier to manipulate genetically than eukaryotic algae and have shown greater efficiency in converting large amounts of carbon dioxide into biomass. Their photosynthetic ability can be used in production of carbohydrates, fatty acids, and alcohols as renewable biofuels (Sarsekeyeva et al. 2015). Existing technologies have exploited the photosynthesis process for energy production via processing of plant biomass into ethanol or synthesis of biodiesel using algal biomass. Fortification of photosynthetic organisms, with their ability to produce biofuel directly, could bypass the need to synthesize all of the complex chemicals in biomass. A promising possibility is redirection of cyanobacterial intermediate metabolism by channeling of Calvin cycle intermediates into fermentative metabolic pathways (Angermayr et al. 2009).

6.6.4 Biodiesel

High lipid content–accumulating potential has been shown in *Synechococcus* species, *Cyanobacterium aponinum*, and *Phormidium* species cultivated in BG-11 medium, with examination of the effects of four different pH values (6–9) and $NaNO_3$ substrate concentrations (0.25, 0.5, 1.0, and 1.5 g/L). The maximum yield of lipid content after 15 days was observed in the medium containing 0.25 g/L of $NaNO_3$ at a pH of 7 for growth of *Synechococcus*, a pH of 8 for growth of *C. aponinum*, and a pH of 9 for growth of *Phormidium*. The maximum lipid content and C_{16}/C_{18}-containing methyl ester yields were 43% and 49%, respectively, for *Synechococcus*; 45% and 67%, respectively, for *C. aponinum*; and 38% and 91%, respectively, for *Phormidium*. *Synechococcus*, *C. aponinum*, and *Phormidium* were reported to contain nearly 75%, 78%, and 85% saturated compounds, respectively. These crude lipids could be utilized as promising feedstock for biodiesel production (Ertuğrul and Dönmez 2011).

Utilization of photosynthetic microbial cells (particularly cyanobacterial cells), with their potential in solar energy usage, has driven the conversion of carbon dioxide into fatty acid–based biofuels. Biofuel is the ideal fuel molecule to be targeted with microbial systems, and cyanobacteria have great potential for use in biofuel biosynthesis, playing a critical role in the development of sustainable, economical, and environmentally safe sources of energy (Lu 2010). In one study, more efficient conversion of triglycerides into biodiesel by *Oscillatoria annae* BDU6 (a strain from freshwater *Cyanobacterium*) was achieved by an alkaline-mediated transesterification process (86% w/v) than by a lipase-mediated transesterification process (76.5% w/v). More efficient oil extraction from *O. annae* was achieved by an ultrasonication process in combination with use of organic solvents (51%) than by a homogenization process (40.4%). Optimization of the growth media for improved biomass growth (2.3-fold) of *O. annae* was achieved via application of response surface methodology (RSM) and a central composite rotatable design (CCRD), resulting in a biomass yield of 5.3 g of dry weight per gram of inoculum, compared with an initial biomass yield of 1.63 g of dry weight per gram of inoculum (Vimalarasan et al. 2011).

Phycobilisomes (PBS) are large antenna complexes found in cyanobacteria such as *Synechocystis* sp. PCC 6803. They are capable of harvesting light and transferring the energy to the photosynthetic reaction centers. Recently, modifications have been made in the light-harvesting machinery of cyanobacterial strains, resulting in changes in cell morphology and physiology. The effects of PBS truncation on the proteomes of three *Synechocystis* sp. PCC 6803 PBS antenna mutants were investigated via progressive truncation of phycocyanin rods in the CB and CK strains and full removal of PBS in the PAL mutant strain. The results showed an impact on core cellular mechanisms beyond the light-harvesting and photosynthetic processes. Considerable changes in membrane transport mechanisms were observed with the most severe PBS truncation in the PAL strain, with suppression of cellular utilization and regulation of bicarbonate and iron ions (Liberton et al. 2017).

6.7 Environmental Impacts of Different Sources of Energy

A typical nonrenewable energy source is made up of hydrocarbons, which are mainly compound groups composed of carbon and hydrogen atoms (e.g., fossil fuels such as petroleum oil, natural gas, and coal). They can evaporate during the fuel supply process, and there can be remnants of incompletely burned fuels in an engine. The presence of undesirable solid or gaseous particles in the environment can impair air quality and is harmful to both human health and biotic components of the environment. Hydrocarbons can be washed out of the air during rainfall and then run into surface water bodies. They can form an oily film layer on the water's surface and can react with each other to form secondary pollutants under certain conditions, creating serious problems for water-dwelling organisms. Utilization of a high oxygen concentration in fuel–air mixtures or use of valves can prevent gases from escaping into atmosphere. Further controls can be implemented by fitting of catalytic converters, with engine modifications, to reduce emission of hydrocarbons into the atmosphere.

Another impurity present in many fossil fuels is lead (a heavy metal), which is a dangerous air pollutant released by automobiles and heavy transport vehicles. High levels of lead are found in the air in cities in India such as Delhi, Mumbai, and Lucknow, where leaded petrol used in most transport vehicles is the primary source of airborne lead emissions. The same is true of other cities in India.

Ozone is another air pollutant. It is made up of three atoms of oxygen, whereas oxygen gas contains two oxygen atoms. Ozone is formed naturally from photodissociation of oxygen gas molecules in the atmosphere, and the resulting ozone layer in the stratosphere helps to protect life-forms on Earth against harmful effects of ultraviolet radiation from the Sun. In the absence of pollutants, the formation and breakdown of ozone molecules are controlled and governed by natural forces, but the presence of chlorofluorocarbons (CFCs) in the atmosphere can accelerate ozone molecule breakdown. CFC molecules are emitted from cooling equipment such as air conditioners (AC) and freezers.

6.7.1 Greenhouse Effects of Nonrenewable Fuel Utilization

Nonrenewable fuel utilization has created several adverse effects (such as the greenhouse effect) on the Earth through global warming. Global warming has been recognized as an international problem, which needs to be resolved by utilization of renewable energy sources, as discussed earlier in this chapter. Some of the effects of global warming are listed below:

- Because of global warming effects, the polar ice caps on Earth are melting, which could cause rises in sea levels and greater risks of flooding in coastal areas.
- In countries such as Bangladesh and the Maldives, catastrophic disasters could occur with rises in sea levels of up to 3 m, submerging low-lying land beneath the waves.
- Increased water temperatures could result in reduced agricultural productivity.
- The effects of global warming on solar energy distribution patterns will have effects on organism habitats, as can be seen from the occurrence of droughts on previously productive agricultural land, while other land such as deserts may experience more rainfall. These effects are likely to bring about changes in the life and distribution of natural plants, agricultural crops, insects, livestock, and microbial species.
- Huge quantities of methane gas are trapped beneath frozen soil in places such as Alaska in the USA. Melting of permafrost will result in greater releases of methane into the atmosphere, accelerating the global warming process.

Table 6.2 Air pollutant problems caused by different activities

Category	Source	Emitted pollutants
Agricultural sector	Burning of agricultural waste in open places	Particulate matter of different sizes suspended in the atmosphere, including CO and volatile organic compounds
Hydropower sector	Gas, electricity, or steam generation	Particulate matter of different sizes in the environment, such as sulfur oxides (including SO_3), nitrogen oxides, CO, volatile organic compounds, and lead
Transport sector	Use of fossil fuels to power combustion engines	Particulate matter of different sizes in the environment, such as sulfur oxides, nitrogen oxides, CO, volatile organic compounds, and lead
Community service sector	Use of municipal incinerators or burners for solid waste burning	Particulate matter of different sizes suspended in the atmosphere, such as sulfur oxides, nitrogen oxides, CO, volatile organic compounds, and lead

Use of nonrenewable resources causes flow pollution problems that affect the health of the environment. Table 6.2 lists some sources of these pollution problems. The application of the market economy to energy has resulted in maximal extraction and growth of production/synthesis. With a positive human capital externality, consumption in a market economy can approach zero in the long run, although positive growth in consumption is optimal from a social perspective. With a negative pollution externality consumption, the optimal long-run growth rate is higher. Pollution can affect utility and/or production, and it can be modeled as flow or as a stock variable. The effects of flow pollution affect the productivity of food crops, which is badly affected by problems caused by environmental events such as soil erosion or climatic changes resulting from the greenhouse effect. Different types of pollution have been found to influence the productivity of food crops in the agricultural sector, and there is greater depreciation of capital equipment, such as buildings, due to air pollution problems, etc. Balance in the rates of growth of foods and the human population can be achieved via constant growth of all variables (even at possibly zero or negative rates). Balanced growth of all things is advantageous in some theories, as it is in accordance with the traditional stylized facts of growth theory, it makes the results of the model directly comparable with other models that traditionally use this assumption, and it is easy to handle, assuming that the resource is indispensable for crop productivity (i.e., no productivity of food can take place without resource usage) (Schou 2000).

6.7.2 *Environmental Impacts of Oil Extraction and Refining*

Fossil fuel oil is found beneath the surface of the Earth and then it is extracted and refined, with separation and processing of the mixture of crude oil fractions into various natural gas, diesel, petrol, tar, and asphalt portions. The oil-refining process causes air pollution problems in most countries and generates large amounts of volatile organic hydrocarbons and toxic emissions of carcinogenic benzene. Burning of petroleum products such as gasoline and diesel in electric generators to make electricity or to power boilers for heating produces various types of toxic gas emissions with harmful effects on human and environmental health. Some of these adverse effects and their causes are listed below:

- Carbon dioxide gas is a component of GHGs, excessive emissions of which are majorly responsible for atmospheric and climate changes. Carbon dioxide gas is produced in large quantities by combustion of fossil fuels.
- Sulfur dioxide is another air pollutant, mostly produced during fossil fuel combustion. It can help to cause acid rain, which is responsible for damaging plant and animal life in water. This pollutant increases the rates of respiratory disease and heart disease (mostly in vulnerable individuals such as children and elderly people).
- Nitrogen oxide (NOx) and volatile organic compounds (VOCs) are synthesized by fossil fuel burning, contributing to increased ozone concentrations at ground level. All of these pollutants can cause irritation and lung damage.
- Particulate matter (PM) is produced by fossil fuel burning and manifests as hazy air conditions in most cities in India (particularly in Delhi) and in scenic areas. It has been found to combine with ozone, contributing to asthma and chronic bronchitis, especially in children and the elderly. Smaller particulate matter can penetrate the deepest parts of the respiratory system, causing lung cancer and emphysema. Heavy metals such as lead can have severe health impacts on children and old people via air pollution.

As mentioned earlier in this chapter, much of global warming is due to emissions of carbon dioxide from fossil fuel utilization in automobile engines. The current practice of using fossil fuels to fulfill human energy needs has greatly increased carbon dioxide emissions. Utilization of clean energy fuels and renewable energy sources can help to minimize CO_2 emissions and ameliorate the environmental problems caused by increased CO_2 emissions. Policy makers know that use of renewable energy can decrease CO_2 emissions and produce electricity, as shown by its implementation in long-term energy policies in Turkey (Bulut 2017). Generation of electricity from renewable sources can help to mitigate per capita CO_2 emissions.

6.7.3 *Effects of Renewable Energy Consumption*

Different types of renewable energy and their respective resources have been discussed earlier in this chapter. A lot of reports have described the impacts of energy utilization, some of which have been reviewed here. A panel of ten Latin American countries was used to decompose the total effects, during the period of 1991 to 2012, of renewable energy policies on emissions of carbon dioxide gas in the short and long run. Panel autoregressive distributed lag methodology was applied for renewable energy utilization with determination of its heteroskedasticity, contemporaneous correlation, first-order autocorrelation, and cross-sectional dependence with a robust dynamic Driscoll–Kraay estimator (Fuinhas et al. 2017). Other analyses such as throughput coherence and partial wavelet coherence analyses have been used with the observed frequency properties and time series properties of relevant variables to reveal the possible significant influence of biomass usage on emissions of CO_2 in the USA in short- and long-term cycles. It was reported that biomass consumption for bioenergy generation mitigated CO_2 emissions in long-term cycles after the year 2005 in the USA (Bilgili et al. 2016).

The 4As framework (availability, applicability, acceptability, and affordability) is derived from the classical hotelling nonrenewable resource economic model. Fast growth in the consumption of nonrenewable energy and its fast development have influenced the growth of indigenous energy production with uneven or inequal distribution of the exploitation of nonrenewable energy resources. The potential of the unconventional technologies of shale oil (obtained from oil shale rock fragments) and shale gas—and the roles of coal, renewable energy, and nuclear energy—have reformed local and domestic energy markets and shown greater environmental impacts in Asian economies than renewable energy harvesting technologies (Chang and Li 2014). Modern buildings in any country account for nearly 40% of the world's total annual energy consumption for lighting, heating, and cooling or AC. CO_2, NOx, and chlorofluorocarbon emissions have triggered health issues in the environment, which have forced us to develop a new interest in eco-friendly cooling and heating technologies. Use of refrigerant chemicals has been found to be very destructive to the ozone layer in the stratosphere. There is a need to reduce energy consumption in buildings by designing modern buildings that use natural or hybrid ventilation systems. Renewable energy resource exploitation in buildings contributes significantly to reducing our dependency on fossil fuels and can help to utilize agricultural waste materials (Blanc and Kjöllerström 2008).

Promotion of use of innovative and renewable resources with reinforcement of the renewable energy market can contribute to preservation of ecosystems via reductions in emissions of toxic gases at local or global levels. Building performance at a high level can be defined by indoor environmental quality (IEQ), energy efficiency (EE), and cost efficiency (CE) parameters. Indoor environmental quality is defined as the perceived level of comfort that occupants in the building experience, depending on the physical and psychological conditions of their surroundings, which are affected by air speed, temperature, relative humidity, and air quality as the main physical factors. Energy efficiency means provision of the desired environmental conditions with minimal energy consumption. Cost efficiency refers to financial expenditure on energy relative to the level of environmental comfort and productivity attained by the occupants of the building (Omer 2009). All of these parameters can be improved by greater utilization of renewable energy sources.

Impacts of Specific Air Pollutants on Public Health

Some air pollutant gases have very serious adverse effects on the health of humans, plants, and other biological organisms. The most harmful emitted gases and agents come from nonrenewable energy combustion; they include particulate matter, carbon dioxide, polycyclic organic compounds or matter, and formaldehyde (Kilbane-Dawe and Clement 2014).

- VOCs are generated/synthesized by burning of agricultural waste, which is plentiful in the Earth's environment. High levels of VOCs can cause irritation to the eyes, nose, and throat; headaches; nausea; and loss of coordination in severe cases. Over long time periods, exposure to high levels of VOCs can damage the liver or other body tissues.
- Formaldehyde exposure can cause irritation to the eyes and nose, and can increase the possibility of developing allergies and other health problems in some people.
- Prolonged exposure to lead can damage the human nervous system and cause digestive problems in some cancer patients. Lead is more hazardous to young children as they have a less-developed immune system.
- Radon is a radioactive gas. It can accumulate inside buildings and originates from rocks or soils under the building. Its level can be increased by outdoor air or other toxic gases also accumulating inside the building, and these gases can increase the risk of lung cancer.
- Exposure to ozone gas can cause itching, burning, or watering of the eyes. It can also increase the risk of respiratory disorders (e.g., asthma) and lower resistance to colds or pneumonia.
- Exposure to nitrogen oxides makes children more susceptible to respiratory disease in winter.
- Carbon monoxide is a toxic gas, which combines with blood hemoglobin to form carboxyhemoglobin and prevents the blood from carrying oxygen. It can enter the blood via the lungs. The binding of carbon monoxide to heme proteins can cause changes in the function of the affected organs (such as the brain or cardiovascular system) and in the developing fetus. Exposure to CO can impair concentration, slow reflexes, and cause confusion and sleepiness. In higher concentrations of carbon monoxide, it can be lethal.
- The toxic gas sulfur dioxide in polluted air can be formed by fossil fuel combustion and can oxidize to form sulfuric acid mist. The presence of sulfur dioxide in polluted air can lead to lung disorders such as wheezing and shortness of breath. The effects of long-term exposure are more difficult to ascertain, because SO_2 is often combined with suspended particulate matter (SPM).
- Suspended particulate matter consists of dust, fumes, mist, and smoke, and can contain lead, nickel, and arsenic from diesel exhaust. When suspended particulate matter is breathed in, it can lodge in the lungs and cause lung damage and respiratory problems. Suspended particulate matter is a major and critical air pollutant, and special measures are needed to reduce its levels in the air. It affects

more people globally than other pollutants on an ongoing basis, according to the available monitoring data, and more epidemiological evidence has been collected on exposure to this pollutant than on exposure to any other type of pollutant (Kampa and Castanas 2008).

Increased energy consumption is the basis of industrial civilization; without energy, modern life cannot continue. Energy exploration, exploitation, conversion, and use influence our environment in numerous ways. Nonrenewable energy utilization threatens both environmental and human health. Only increased utilization of alternative renewable energy options in sustainable ways can meet our current and future energy demands safely (Olugbenga 2009).

6.8 Conclusions

Energy is necessary for all activities of living beings and nonliving objects. At present, most of our energy needs for use in nonliving objects are fulfilled by energy obtained from conventional fossil fuel sources (coal, diesel, natural gas, kerosene, etc.). This chapter has focused on energy needs for use in nonliving objects. For this purpose, fossil fuels are utilized, and their combustion causes environmental problems by releasing harmful by-products that increase air pollution and contribute to global warming. Solar energy is a renewable energy source and powers the water cycle, allowing us to harness the energy from moving water. Plants use solar radiation via photosynthesis and store it as potential energy in their biomass, providing a renewable energy resource. Use of renewable energy has made a visible impact in the Indian energy scenario, and at a national level the installed electricity capacity, can be derived the renewable energy (approximately 12.5% of the total capacity) provides benefits to millions of people in Indian villages by meeting their energy needs in eco-friendly ways. Numerous geothermal power plants are now operational and produce more than 10 GW in at least 24 countries, accounting for 6.5% of the total electric power generation worldwide. This form of energy will play a bigger role in India in the future. Tidal energy generation is extremely site specific and requires tidal size differences of more than 4 m with favorable topographical conditions (such as estuaries or certain types of bay in order to reduce the costs of dam construction, etc.). India has the capability to generate 150 GW or more from all of its renewable resources (tidal, wave, geothermal, and solar power systems). Thus, India has a huge potential for renewable energy generation, only 22% of which has been developed so far. Hydrogen can be produced from either nonrenewable resources (coal or nuclear energy) or renewable resources (solar systems, hydropower, wind power, biomass, tidal power, etc.), but hydrogen production from renewable energy resources can be eco-friendly. Hydrogen is a clean energy carrier and produces only water as a by-product of its combustion. Anaerobic bioconversion of organic waste matter into hydrogen gas is an attractive option for use of stabilized waste or wastewater. Utilization of hydrogen gas to power vehicles has tremendous potential as an environmentally acceptable form of energy with no GHG emissions. The design of MEC reactors is one of the most important factors that directly influence the quantity of hydrogen produced in these systems. Plant biomass or plant waste can be converted into liquid or gas forms of fuel after addition of heat or certain chemicals, then the liquid or gas fuel can be converted (via burning) into heat or electric energy to be used to power automobiles. Ethanol can be produced via utilization of yeast strains to ferment starches of plant origin as a substrate. GHGs and other air pollutants are produced by nonrenewable energy utilization. To minimize their adverse effects on biological and environmental health, we need to greatly increase our current utilization of different forms of renewable energy sources.

References

Angermayr SA, Hellingwerf KJ, Lindblad P, de Mattos MJ (2009) Energy biotechnology with cyanobacteria. Curr Opin Biotechnol 20(3):257–263

Balan V, Sousa LDC, Chundawat SPS, Marshall D, Sharma LN, Chambliss CK, Dale BE (2009) Enzymatic digestibility and pretreatment degradation products of AFEX-treated hardwoods (Populusnigra). Biotechnol Prog 25:365–375

Balat M (2008) Potential importance of Hydrogen as a future solution to environmental and transportation problems. Int J Hydrog Energy 33:4013–4029. https://doi.org/10.1016/j.ijhydene.2008.05.047

Barras C (2009) Sea ‘snake’ generates electricity with every wave. https://www.newscientist.com/article/dn17090-sea-snake-generates-electricity-with-every-wave/

Bayro-Kaiser V, Nelson N (2017) Microalgal hydrogen production: prospects of an essential technology for a clean and sustainable energy economy. Photosynth Res 133(1–3):49–62

Beerbaum B, Weinrebe G (2000) Solar thermal power generation in India: a technoeconomic analysis. Renew Energy 21(2):153–174

Bilgili F, Öztürk İ, Koçak E, Bulut Ü, Pamuk Y, Muğaloğlu E, Bağlıtaş HH (2016) The influence of biomass energy consumption on CO_2 emissions: a wavelet coherence approach. Environ Sci Pollut Res Int 23(19):19043–19061

Blanc DL, Kjöllerström M (2008) Using non-renewable resource revenues for sustainable local development. Sustain Dev Innov Briefs 6(08-51232)., 2008–600

Bozzi S, Miquel AM, Antonini A, Passoni G, Archetti R (2013) Modeling of a point absorber for energy conversion in Italian seas. Energies 6:3033–3051

Bulut U (2017) The impacts of non-renewable and renewable energy on CO_2 emissions in Turkey. Environ Sci Pollut Res 24(18):15416–15426

Chang Y, Li Y (2014) Non-renewable resources in Asian Economies: perspectives of availability, applicability acceptability, and Affordability. ERIA discussion paper series, ERIA-DP-2014-04

Cheng S, Logan BE (2011) High hydrogen production rate of microbial electrolysis cell (MEC) with reduced electrode spacing. Bioresour Technol 102:3571–3574

Dadu V, Dadu A, Frunza D, Catarig G, Popa F, Popa B (2017) Innovative concepts applied to recent small hydropower plants. Energy Procedia 112:426–433

De Moraes-Rocha GJ, Martin C, Soares IB, Maior AMS, Baudel HM, De Abreu CAM (2010) Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production. Biomass Bioenergy 35:663–670

Dutta D, De D, Chaudhuri S, Bhattacharya SK (2005) Hydrogen production by cyanobacteria. Microb Cell Factories 4:36

Dzene I, Barisa A, Rosa M, Dobraja K (2016) A conceptual methodology for waste-to-biomethane assessment in an urban environment. Energy Procedia 95:3–10

Ertuğrul S, Dönmez KG (2011) Microbial oil production from thermophile cyanobacteria for biodiesel production. Appl Energy 88(11):3632–3635

Fuinhas JA, Marques AC, Koengkan M (2017) Are renewable energy policies upsetting carbon dioxide emissions? The case of Latin America countries. Environ Sci Pollut Res Int 24(17):15044–15054

Fukuzumi S, Yamad Y (2016) Hydrogen Peroxide used as a solar fuel in one-compartment fuel cells. Chem Electro Chem 3(12):1978–1989

Garud S, Purohit I (2007) Making solar thermal power generation in India a reality –overview of technologies, opportunities and challenges. Energy Resour Inst (TERI):1–9. http://www.aprekh.org/files/SolarThermalPowergeneration_Final.pdf

Ghiaci P, Lameiras F, Norbeck J, Larsson C (2014) Production of 2-butanol through meso-2,3-butanediol consumption in lactic acid bacteria. FEMS Microbiol Lett 360:70–75

Gielen D (2012) Renewable energy technologies: cost analysis series, International Renewable Energy Agency https://www.irena.org/documentdownloads/publications/re_technologies_cost_analysis-hydropower.pdf

Gnanambal VS, Swaminathan K (2015) Biogas production from renewable lignocellulosic biomass. Int J Environ 4(2):341–347

Guiglion P, Berardo E, Butchosa C, Wobbe MCC, Zwijnenburg MA (2016) Modelling materials for solar fuel synthesis by artificial photosynthesis; predicting the optical, electronic and redox properties of photocatalysts. J Phys Condens Matter 28:1–11, 074001

Gust D, Moore TA, Moore AL (2009) Solar fuels via artificial photosynthesis. Acc Chem Res 42(12):1890–1898

Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S (2010) Biofuels from algae: challenges and potential. Biofuels 1(5):763–784

Harris F (2014) Catching the tide: a review of tidal energy systems. SSR 95(353):123–128

He J, Lee SK (2004) Photosynthetic utilization of radiant energy by temperate lettuce grown under natural tropical condition with manipulation of root-zone temperature. Photosynthetica 42(3):457–463

Howard RL, Abotsi E, van Jansen Rensburg EL, Howard S (2003) Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr J Biotechnol 2:602–619

Hussy I, Hawkes FR, Dinsdale R, Hawkes DL (2005) Continuous fermentative hydrogen production from sucrose and sugarbeet. Int J Hydrog Energy 30:471–483

Joshi B, Bhatt MR, Sharma D, Joshi J, Malla R, Sreerama L (2011) Lignocellulosic ethanol production: current practices and recent developments. Biotechnol Mol Bio Rev 6(8):172–182

Kadier A, Simayi Y, Abdeshahian P, Azman NF, Chandrasekhar K, Kalil MS (2016) A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alex Eng J 55(1):427–443

Kaldellis JK (2005) Social attitude towards wind energy applications in Greece. Energy Policy 33(5):595–602

Kalogirou SA (2004) Solar thermal collectors and applications. Prog Energy Combust Sci 30(3):231–295

Kampa M, Castanas E (2008) Human health effects of air pollution. Environ Pollut 151(2):362–367

Kaphle P, Rana S, Bhandari J, Bhandari A, Aishwarya R, Karki TB, Shrestha BG (2014) Isolation and optimization of cellulolytic and Xylanolytic microbes from Nepal and their utilization for lignocellulosic biomass degradation. Int J Interdiscip Multidiscip Stud (IJIMS) 1(4):1–10

Kaya F, Heitmann JA, Thomas (2000) Influence of lignin and its degradation products on enzymatic hydrolysis of xylan. J Biotechnol 80:241–247

Khanal SK, Chen WH, Li L, Sung S (2005) Biohydrogen production in continuous flow reactor using mixed microbial culture. Water Environ Res 78(2):110–117

Khare V, Nema S, Baredar P (2013) Status of solar wind renewable energy in India. Renew Sust Energ Rev 27:1–10

Kilbane-Dawe I (2014) The impacts of air pollution on health a summary of the state of current knowledge, review of recent science on the impacts of air pollution on health – Parliament Hill research, London, pp 1–18

Kojima E, Zhang K (1999) Growth and hydrocarbon production of microalga *Botryococcus braunii* in bubble column photobioreactors. J Biosci Bioeng 87(6):811–815

Kongjan P, Min B, Angelidaki I (2009) Biohydrogen production from xylose at extreme thermophilic temperatures (70°C) by mixed culture fermentation. Water Res 43(5):1414–1424. https://doi.org/10.1016/j.watres.2008.12.016

Kongjan P, Thong SO, Kotay M, Min B, Angelidaki I (2010) Biohydrogen production from wheat straw hydrolysate by dark fermentation using extreme thermophilic mixed culture. Biotechnol Bioeng 105(5):899–908. https://doi.org/10.1002/bit.22616

Kydd P, Brinckerhoff P (2014) Harnessing the power of the tides. http://www.renewableenergyworld.com/articles/print/volume-17/issue-3/hydropower/harnessing-the-power-of-thetides.html

Lai Z, Zhu M, Yang X, Wang J, Li S (2014) Optimization of key factors affecting hydrogen production from sugarcane bagasse by a thermophilic anaerobic pure culture. Biotechnol Biofuels 7:119

Lampman G, Smith R, Adonizio MA (2011) Roosevelt Island Tidal Energy (RITE) environmental assessment project. (DeIorio VA, Murray FJ) NYSERDA final report 11-04-2011. www.nyserda.org

Liberton M, Chrisler WB, Nicora CD, Moore RJ, Smith RD, Koppenaal DW, Pakrasi HB, Jacobs JM (2017) Phycobilisome truncation causes widespread proteome changes in *Synechocystis* sp. PCC 6803. PLoS One 12(3):e0173251

Liu SY, Ho YF (2016) Wind energy applications for Taiwan buildings: what are the challenges and strategies for small wind energy systems exploitation? Renew Sust Energ Rev 59:39–55

Lu X (2010) A perspective: photosynthetic production of fatty acid-based biofuels in genetically engineered cyanobacteria. Biotechnol Adv 28(6):742–746

Mastny L (ed) (2016) Renewables: global status report. http://www.ren21.net/wp-content/uploads/2016/06/GSR_2016_Full_Report_REN21.pdf

May G (2010) Bold experiment tests underwater turbines in Cornwall in new twist on tidal power in the St. Lawrence River MyNewWaterfrontHome.com. September 2010. http://www.mynewwaterfronthome.com/riverturbines.aspx

Mccarthy J (2015) Portland's Ocean renewable power launches subsidiary in Quebec. http://www.mainebiz.biz/apps/pbcs.dll/article?AID=/20151016/NEWS0101/151019984/Portland

Mishra S, Tripathy P (2012) Solar thermal electricity generating system. Int J Adv Res Technol 1(3):1–16

Nozzi NE, Oliver JWK, Atsumi S (2013) Cyanobacteria as a platform for biofuel production. Front Bioeng Biotechnol 1:7

Olugbenga FP (2009) Energy exploitation, utilization, and its environmental effects – the choice to make and the decision to take. Toxicol Environ Chem 91(5):1015–1019

Omer AM (2009) Energy use and environmental impacts: a general review. J Renew Sustain Energy 1(5):053101. https://doi.org/10.1063/1.3220701

Pitz-Paal R, Hennecke K, Heller P, Buck R (2013) Solar thermal power plants. Durst A (ed) BINE-Themeninfo- II/2013. 1–20, Eggenstein-Leopoldshafen Germany

Qureshi N, Liu S, Ezeji TC (2012) Cellulosic butanol production from agricultural biomass and residues: chapter in "recent advances in technology" book. In: Lee JW (ed) Advanced biofuels bioproducts. Springer, New York, pp 247–265

Radziemska EK (2014) Environmental impacts of renewable energy technologies. 5th international conference on environmental science and technology. IPCBEE, 69, pp 104–109. IACSIT Press, Singapore

Rodriguez C, Alaswad A, Benyounis KY, Olabi G (2017) A pretreatment techniques used in biogas production from grass. Renew Sust Energ Rev 68(2):1193–1204

Rosen MA (2015) The prospects for renewable energy through hydrogen energy systems. J Power Energy Eng 3(4):373–377

Sanni M (2017) Mapping dynamics of low-carbon energy innovation for small hydropower technology in Nigeria. Afr J Sci Technol Innov Dev 10:1–10

Sarsekeyeva F, Zayadan BK, Usserbaeva A, Bedbenov VS, Sinetova MA, Los DA (2015) Cyanofuels: biofuels from cyanobacteria. Reality and perspectives. Photosynth Res 125(1–2):329–340

Schou P (2000) Polluting non-renewable resources and growth. Environ Resour Econ 16:211–217

Sharma OP, Trikha P (2013) Geothermal energy and its potential in India. RE Feature 7(1):13–18. http://mnre.gov.in/file-manager/akshay-urja/july-august-2013/EN/14-18.pdf

Sharma D, Joshi B, Bhatt MR, Joshi J, Mall R, Bhattarai T, Sreerama L (2015) Isolation of cellulolytic organisms from the gut contents of termites native to Nepal and their utility in Saccharification and fermentation of lignocellulosic biomass. J Biomass Biofuel 2:11–20. Avestia Publishing

Sitharthan R, Geethanjali M (2014) Wind energy utilization in India: a review. Middle-East J Sci Res 22(6):796–801

Sovacool BK (2013) The avian benefits of wind energy: a 2009 update. Renew Energy 49:19–24

Swofford J, Slattery M (2010) Public attitudes of wind energy in Texas: local communities in close proximity to wind farms and their effect on decision-making. Energy Policy 38(5):2508–2519

The NEED Project (2017) Energy from the Sun. 8408 Kao Circle, Manassas, VA 20110 1.800.875.5029, pp 1–24. www.NEED.org

Thorburn K (2006) Electric energy conversion systems: wave energy and hydropower. Uppsala dissertations. ACTA Universitatis Upsaliensis, Uppsala

Uihlein A, Magagna D (2016) Wave and tidal current energy – a review of the current state of research beyond technology. Renew Sust Energ Rev 58:1070–1081

Vezmar S, Spajić A, Topić D, Šljivac D, Jozsa L (2014) Positive and negative impacts of renewable energy sources. Int J Electr Comput Eng Syst 5(2):15–23

Vimalarasan A, Pratheeba N, Ashokkumar B, Sivakumar N, Varalakshmi P (2011) Production of biodiesel from cyanobacteria (*Oscillatoria annae*) by alkali and enzyme mediated transesterification. J Sci Indust Res 70:959–967, NISCAIR-CSIR, India

World Energy Council (WEC)/World Energy Resources (2016). https://www.worldenergy.org/wp-content/uploads/2017/03/WEResources_Hydropower_2016.pdf

Yadav H (2015) Recent developments of ocean energy in India – an overview. Conf Int J Recent Innov Trends Comput Commun 3(12):6717–6721

Yilanci A, Dincer I, Ozturka HK (2009) A review on solar-hydrogen/fuel cell hybrid energy systems for stationary applications. Prog Energy Combust Sci 35(3):231–244

Younesi H, Najafpour G, Ku Ismail KS, Mohamed AR, Kamaruddin AH (2008) Biohydrogen production in a continuous stirred tank bioreactor from synthesis gas by anaerobic photosynthetic bacterium: *Rhodopirillum rubrum*. Bioresour Technol 99(7):2612–2619

Zhang X, Yu H, Huang H, Liu Y (2004) Evaluation of biological pretreatment with white-rot fungi for the enzymatic hydrolysis of bamboo culms. Int. Biodiv Biodeg 60:159–164

Zhao XB, Peng F, Cheng KK, Liu DH (2009) Enhancement of the enzymatic digestibility of sugarcane bagasse by alkali–peracetic acid pretreatment. Enzyme Microbiol Technol 44:156–164

Zheng Y, Zhao J, Xu F, Li Y (2014) Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog Energy Combust Sci 42:35–53

Zürrer H, Bachofen R (1979) Hydrogen production by the photosynthetic bacterium *Rhodospirillum rubrum*. Appl Environ Microbiol 37(5):789–793

Chapter 7
Ecofriendly Synthesis of Biopolymer Nanocomposites and Its Application as a Potent Marine Antifouling Agent

G. Kavitha and D. Inbakanadan

Contents

Abstract The undesirable colonization of anthropogenic surfaces by organisms in the marine environment is stated as marine biofouling. The biofouling of marine systems is a global concern, with economic impact estimated at billions of dollars. The widely used antifouling biocides including tributyltin tin (TBT) have been previously assimilated into marine paints. Although it has remarkable antifouling performance, it is toxic to the marine environment. Therefore, it is essential to develop ecofriendly antifouling compounds. In the recent times, the physical, chemical, and mechanical properties of nanomaterials have considerably improved the potential applications ranging from environment and energy to healthcare compared with those of bulk materials. Marine antifouling coating is the most effective way for avoiding marine organism attachment till date. However, the cost associated with

---

G. Kavitha · D. Inbakanadan (✉)
Centre for Ocean Research (DST-FIST Sponsored Centre), MoES – Earth Science & Technology Cell (Marine Biotechnological Studies), Col. Dr. Jeppiaar Research Park, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India
e-mail: inbakandan@sathyabama.ac.in

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_7

the commercial antifouling agents and their maintenance is quite high. Therefore, it is indispensable to develop ecofriendly antifouling compounds. This chapter discusses ecofriendly method to produce biopolymer and nanomaterials conglomerating the antimicrobial property of nanoparticles and the unique structure of the biopolymer PHB against marine biofouling.

Keywords Polyhydroxybutyrate · Bioplastic · Ecofriendly nanocomposite

7.1 Introduction

The chemistry of biopolymer encompasses a succession of principles that tips to harmless processes and products. Approximately, these ideologies reveal that a green chemistry is vital principal to fewer perilous biochemical productions, scheming inoffensive substances and nontoxic diluents. Summarizing, the chemistry of biopolymer is a biologically welcoming substitute to dent new harmless antifouling compounds. At the culmination of twenty-first century, about 8600 million tonnes of synthetic polymers for fibers and resins remained manufactured in many engineering divisions. About 6600 Metric tons of conventional pliable waste were machine-made, which were reprocessed (8.3%), burnt (12%), and congregated in land area (79%) or the natural location.

Totally, the chief global source in 2017 was polypropylene and polyethylene, which subsidizes about 47% to the worldwide manufacture and 64% in wrapping industry. Established on the prevailing production and trends in management of waste products, around 14,000 Mt. of degradable polymers leftover remained projected to be dumped in the landfill by 2050 (Rabnawaz et al. 2017). Among the other biopolymer, unique promising one is polyhydroxybutyrate, a small chain polyhydroxyalkanoate made by process of fermentation which was revealed by Lemoigne during 1921 as an energy- and carbon-storing substance congregated internally in several microbes, such as *Pseudomonas*, *Bacillus*, and *Alcaligenes* (Chen et al. 2015).

7.2 Renewability and Sustainable Development

PHB bids numerous benefits above the conservative petrochemical thermoplastics in biodegradability, utilizing renewable assets and having improved physical properties than polypropylene. Automated assets of polyhydroxybutyrate mark them as an appropriate substitute polypropylene, nevertheless, in divergence to these conventional plastics; it can be sullied entirely to produce CO_2 and H_2O which occur naturally by mineralization of microbes. Further, PHB is decomposable and compatible, which in turn commands its ecological fortune in terms of human toxicity and ecotoxicity (Isola et al. 2017).

However, ample progression has arisen in the biosynthesis of PHB by microbes, industrially, and their utilization in different field is still at earmarked apart from realism. In this veneration, appropriate selection of the microbes for production of polyhydroxybutyrate is essential. Hence, many works were done aiming to accomplish appropriate bacteriological system for major production of polyhydroxybutyrate along with the characterization to surge the appropriateness of PHB usage with suitable chemical and physical properties (Kato et al. 1992). Precisely, bioplastics including polyhydroxyalkanoates (PHAs) are the group of broadly studied decomposable constituents used for the replacement of oil-derived polymers. At current, they do not have extensive application as of their production costs, which are reasonably higher when equated to traditional plastics. Biodegradable polymers are gaining profitable attention associated with date traditional polymers in this modern world due to their decomposable characteristics.

7.3 Polyhydroxybutyrate: Structure, Properties, and Sources

Naturally, plastics are made up of synthetic nondegradable substance. Their configuration is not easily occurring; hence they are not easily decomposable. Conventional on the advances in accepting the structure of polymer, their properties and unique assets were documented for its utility of plastics with biodegradability. Recyclable plastics will turn into CO_2, CH_4, H_2O, biomass, and inert compounds under anaerobic or aerobic conditions by the exploitation of microorganisms (Andrej Krzan 2012). Necessity on conventional plastics and their limitless practice have ensued in unwanted accretion and emissions of greenhouse gas. Modern skills are engrossed in the path of the development of biomaterials that employ minor adjacent possessions on the ecosystem.

Polyhydroxyalkanoate (PHA) has enthralling benefits owing to its equivalent mechanical properties to artificial plastics. In contrast, PHB is made from renewable resources and is utilized by microbes aerobically to CO2 and H2O upon removal. The assortments of appropriate microbial strains, low-cost carbon bases, and competent fermentation besides retrieval procedures remain substantial features that ought to be reserved into attention for the PHB commercialization (Chee et al. 2010).

Polyhydroxybutyrate is the well-known and more crystal-like copolymers of PHA, by having uses mostly delimited to the manufacture of plastic. PHA copolymers encompassing 3-hydroxybutyric acid with minor quantities of other monomer units are added compatible than PHB homopolymer. Most forms of PHA pronounced in the works are made up of 3-hydroxyalkanoic acid monomers comprising 4–13 atoms of carbon with unsaturated, aliphatic, aromatic, branched, or straight chain (Sudesh et al. 2000; Valentin and Steinbüchel 1994).

The configuration and conformation of the monomer components are mostly bigoted by the assets of PHAs, and thus ample work has been done on including the monomer configuration in the direction to accelerate the physical assortment of PHAs. Like glycolate, 2-hydroxybutyrate and lactate are components of 2-hydroxyalkanoates that remain the novel mechanisms of PHAs. PHAs comprising these monomers are flexible and further radiant than conventional plastics, properties that are perfect for best practical applications (Matsumoto and Taguchi 2013).

The aliphatic homologous polymer of polyhydroxybutyric acid is extremely crystal-like (82%), with melting point of 178 °C, and PHB has three distinctive properties analogous to polypropylene, thermoplastic process ability, confrontation to water, and easily decomposable. This biodegradable polymer can be altered into sheets films and fibers and even can be molded into the form of bottle and bag; further it takes distinctive application as absorbable medical implants (Hrabak 1992). The key factor impeding commercialization and huge-scale manufacture of PHB lies in the production price. Usage of inexpensive source, enhanced farming practices, and their downstream processing approaches are crucial for lowering the rate.

7.4 Metabolism of PHB Production

PHBs are stored in microbial cells from solvable to inexplicable form as storing constituents inside the cytoplasmic bodies through instable nourishment or to eliminating entities from reducing counterparts. PHBs are transformed to solvable constituents by PHB depolymerases besides the sullied materials which enter numerous pathways. Till now, four types of enzymes responsible for PHAs polymerization are identified. The greatest restrictive factor in PHB production is their high cost associated with the petroleum plastics (Amara 2008).

A series of enzymes are involved in diverse PHAs synthesis; however PhaC synthases are accountable for the polymerization step. PHAs are transformed again to soluble constituents by additional pathways and enzymes for the degradation process. PHA depolymerases are the responsible enzymes (Fig. 7.1). De Smet et al. (1983) characterized PHAMCL in *Pseudomonas oleovorans* for the period of growth on octane. Huisman et al. confirmed that PHAMCL accumulation was the mutual feature of pseudomonads. Polyhydroxyalkonates are also produced by gram-negative bacteria and phototrophic bacteria and archaea (Huisman et al. 1989; Shabeb et al. 2005). PhaC synthase produced from an extreme halophilic archaeon has been categorized enzymologically and biochemically. The first halo bacterial PHA synthase gene sequenced is from *Haloarcula marismortui* (Hezayen et al. 2000; Baliga et al. 2002).

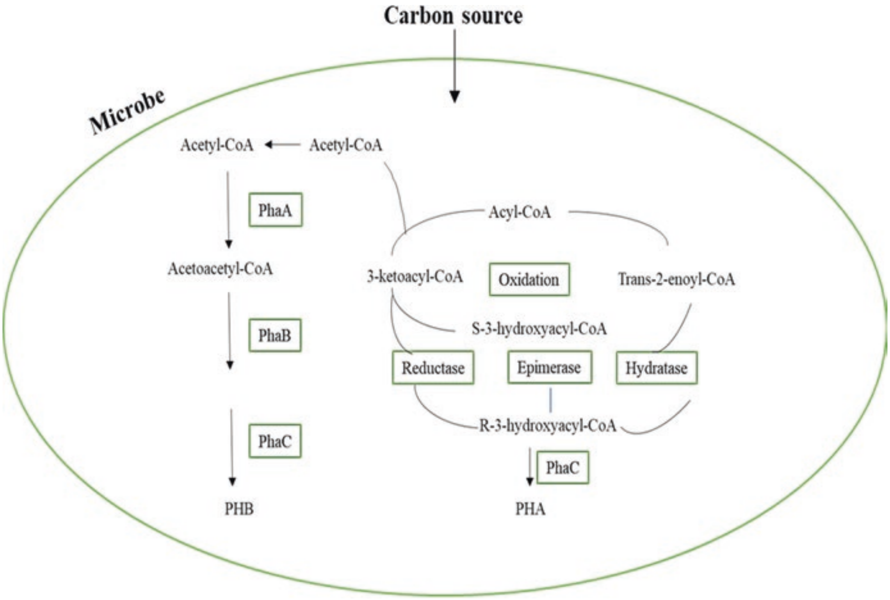


Fig. 7.1 Metabolism of PHB production in microbes

7.5 Microbial Biosynthesis of the Biopolymer: PHB

In the existing situation, the custom practice of plastics is widespread chiefly due to their thermal and automated properties (Batcha et al. 2014). The extensive utilization of plastics has accredited to ecological contamination because of their non-degradable tendency (Akaraonye et al. 2010). Biopolymers are polymers that ensue in nature which encompasses carbohydrates and lipids (Radhika and Murugesan 2012). These thermoplastic constituents such as PHB have assistances over artificial plastics owing to their degradability by many microbes underneath many environments in numerous biomes and the prospect of establishment from renewable sources (Sandhya et al. 2013).

The recyclable thermoplastics are typically made commonly from renewable assets using the microbial source comprising plants and algae which are termed as Green Plastics. Often, there is a consideration on invention of an ecologically approachable product. The biopolymers from algae aid as a unique feedstock for bioplastic fabrication that has numerous remunerations such as great harvest, mainly progressed as a by-product of microalgal biofuel fabrication. Mostly cyanobacteria remain vital producers of PHB and possibly a viable substitute to plastic bags from traditional petroleum-derived polymers. Poly-β-hydroxybutyrate (PHB) is a biopolymer made completely in prokaryotes where the Cyanobacteria are the sole prokaryotes that gather PHB as storage material in their biomass production (Panda and Mallick 2007).

The cell biomass from *Arthrospira* has unpredictability of biological compounds including biopolymers, starches, biopeptides, vital fatty acids, and minerals. The biomass of *Arthrospira* can produce polyhydroxyalkanoates that used in tissue engineering (Morais et al. 2015). A small number of microalgae and cyanobacteria can hoard PHB when full-grown with acetate; the extreme accretion has been familiar for *Synechocystis*. The PHB content can diverge from beneath 2% to above 52% which amounts on the strain, the carbon source and cultivation conditions in *Botryococcus braunii* (Drosg et al. 2015; Kavitha et al. 2016).

PHB produced from microalgae gives the possibility to decrease cost of the multifaceted substrates mandatory for the development, along with possibility to sequester detrimental CO_2. Polyhydroxybutyrate have huge bids in wrapping films, bone replacements, throwaway items, etc. Inappropriately, owing to the abundant production cost of bioplastics related to the low cost of synthetic polymers has instigated loss of responsiveness in bioplastic research. Thus, there is a vital necessity to exploit inexpensive sources for the industrialized PHB production (Van-Thuoc et al. 2012).

The most imperative element for the enriched rate of fabrication in polyhydroxybutyrate is chiefly the substrate, energy source, and carbon. Many *Cyanobacteria* including *Spirulina platensis, Synechococcus*, and *Nostoc* are also able to produce PHB (Shrivastav et al. 2010). Discovery of PHB granules in *Microcystis aeruginosa* and *Trichodesmium thiebautti* remained also distinct. *Gloeothece* and *Oscillatoria limosa* were acknowledged for PHB production (Sabirova et al. 2006).

Several bacterial strains with recombinant strains or genetic mutations or wild type have been recognized over metabolic influences for high PHB accumulation. Cyanobacteria PHB producers in industrial utilization have the advantage of altering waste CO_2 to biodegradable plastics overwhelming the energy of sunlight. Lately, non-PHB producers in cyanobacteria have remained primarily conspired through a modification in the genetic factor intricate in the PHB trail (Sabirova et al. 2006).

Recently, scientists in this subject are converging further on showing little price maintainable substitutions laterally with characterization of many factors for consuming high production of these biopolymers. Response surface methodology is unique of the vital arithmetical practices for optimization studies. Earlier works have meticulous technique of response surface methodologies or improved manufacture of polyhydroxybutyrate by diverse bacteria such as *Rhodobacter* and *Bacillus coagulans*. Only a little information was existing on the optimization studies of biopolymer from microalgae (Hosseinabadi et al. 2015).

Furthermore, progress of biopolymer-centered scaffolds utilizing PHB from *Spirulina* has the feasibility to boost cell growth while without the threat of tissue rejection. Besides, it is obvious that the accretion of PHB in *Spirulina* biomass to nanofibers fallouts in a robust upsurge in elasticity, conductivity, and infringement which were distinguished than those of commercial polyhydroxybutyrate samples (Morais et al. 2015). Cyanobacteria have countless untapped prospective for natural harvests through a massive inconsistency in organization and biological activity.

Beneath stress circumstances they are described to yield polyhydroxybutyrate, which can be formed intracellularly (Balaji et al. 2013).

Through all nourishing approaches, *R. eutropha* is capable to hoard PHB granules inside the cell. This biopolymer assists as a storage of carbon and energy. These genes are schematized in sole operon, which is expressed constitutively. The quantity of polyhydroxybutyrate can be subsidized to 90% of the bacterial cell dry mass. Besides, numerous ingredients other than 3, hydroxyl butyric acid were accrued by *Ralstonia* (Steinbüchel 1991). This comprised numerous hydroxyalkanoic acids, several of which encircled atoms of sulfur in the side chain or even mercaptoalkanoic acids (Ewering et al. 2002). In about 155 elements, the family of PHAs displays a wide-ranging functionalities and properties. Since they instigate from various resources, are recyclable, and display thermoplastic properties analogous to corporate synthetic polymer, PHB engrossed curiosity in the academic world and nevertheless also in commercial purposes (Wubbeler and Steinbüchel 2014).

The range of appropriate PHB extraction methods pivot on numerous progression constraints such as concentration of chemical elements, recovery temperature, pH, and reaction time. Mostly, the influence of process factors on the effectiveness of PHB mining procedures has been considered and demonstrated; nevertheless there is a constraint of actual figures on the effect of external aspects on PHB recovery. A novel recovery process was established to gain the aids of both assimilation approaches by means of sodium hypochlorite and chloroform in a solvent extraction. The collective process produces three separate phases, which contain hypochlorite solution at the upper phase and undisrupted cells at the middle phase and a chloroform phase encompassing PHB. The molecular weight reduction due to degradation of polymer is significantly reduced using this process (Hahn et al. 1994). Scarce mechanical approaches have been developed to complement these arrangements as autonomous structures, which are widely used to recuperate intracellular PHA. Isolated pure cultures were further used for screening the production of PHB using lipid staining method using Nile red under fluorescent microscope (Mahishi et al. 2003) at 465 nm excitation.

Meanwhile, plastics have been an essential part of our life. Nevertheless, dumping of these non-biodegradable plastics stances a danger to our environs. Thus, much attention has been increased in evolving decomposable plastics. The difficulties in PHB production is the elevation in the fabrication cost due to exclusive carbon substrates and tiresome procedures by means of pure cultures. Consequently, the applications of mixed cultures and cheap carbon sources have been discovered (Huey 2006).

7.6 Biodegradation of Polyhydroxybutyrate

Polyhydroxybutyrate is differentiated from the petroleum-based plastics based on their property of biodegradability. Under aerobic conditions, biodegradation of PHB releases CO_2 and H_2O, while in aerobic circumstances, the degradation products are CO_2 and CH_4. PHBs are biocompostable that concluded a wide variety of temperatures with extreme level of around 600 °C with moisture levels at 54%. Studies have been showing that 84.7% of PHB were despoiled in 7 weeks. PHB have been quantified to worsen in marine environments within 250 days even at temperature not beyond 600 °C (Johnstone 1990). PHAs are ruined upon acquaintance to soil or sediment. Degradation is based on the bacteria in the surroundings including pH, external surface area, temperature, and moisture (Boopathy 2000).

Inorganic compounds in the nanosize range, i.e., under 100 nm, including silver nanoparticles (AgNPs), have developed as competent tools to avert bacterial putrefaction and infectious outbreaks in food interaction plastics and surfaces. Conventionally, AgNPs are produced by chemical approaches utilizing diverse organic and inorganic reducing agents, such as ascorbate (Valodkar et al. 2011), sodium citrate (Pinto et al. 2012), and poly(ethylene glycol) copolymers (Iravani et al. 2014). Yet, utmost elements used in this kind of mixture are lethal and chief to non-ecofriendly by-products. To solve these problems, biological synthesis of nanomaterials using living organisms such as bacteria (Shivaji et al. 2011), fungi (Rajakumar et al. 2012), or plant extracts (Ahmed et al. 2016) are currently being developed.

7.7 Economical Aspects of PHB Production

It is a necessary criterion to schematize all the conditions of fermentation for the effective enactment of profitable PHB-making systems. The cost of the produce finally hinge on the cost of substrate, PHB harvest, and the product efficiency in the downstream dispensation (Lee 1996). This concludes highest units of PHB in terms of weight percentage and high productivity in terms of grams of produce per unit capacity and period. Marketable solicitations and extensive consumption of PHB is susceptible owing to its value. The price of PHB from the natural producer *A. eutropphusis* ranges from 17$/kg, that is, 20 times further profligate than synthetic polymer. With *E. coli* as a producer of PHB, price could be reduced to 4$ /kg, that is, earlier to new decomposable pliable materials such as polylactic acid and polyesters. The market value must originate to 3–5 US $ /kg (Lee 1996).

7.8 Nanotechnology

Nanotechnology is varying the approach in which resources are produced and devices are invented. Integration of nanoscale edifice blocks into efficient assemblies and further into multifunctional devices can be attained over “bottom-up approach.” Investigation on the synthesis of nano-sized substance is of great curiosity because of their distinctive properties like optoelectronic, mechanical, and magnetic which fluctuates from bulk. Nanoparticles are of great interest owing to its distinctive properties such as shape- and size-dependent photosensitive, magnetic and electrical properties which can be amalgamated into antimicrobial applications, cosmetic products electronic mechanisms, and various applications. Numerous methods have been utilized for making and improving nanoparticles synthesis (Klaus et al. 1999; Senapati et al. 2005).

The common biological approaches comprise electrochemical techniques, biological drop technique using a diversity of reducing agents, radiolysis, and physicochemical reduction methods which are widely used for the synthesis of nanoparticles. Recently, nanoparticle synthesis is among the utmost exciting systematic areas of survey, and there is increasing consideration to yield biosynthesis of nanoparticles. Material scientists researcher is steering to improve novel resources, functionality with improved properties, and subordinate rate than the prevailing ones. Diverse chemical and biosynthesis approaches have been established to enrich the recital of nanoparticles demonstrating enhanced properties with the purpose to have an improved regulation over the particle size, morphology, and distribution (Shibata et al. 2008).

7.9 Biosynthesis of Metal Nanoparticles

The first sign of biological synthesis of bacteria synthesizing silver nanoparticles was recognized by means of *Pseudomonas stutzeri* that was attained (Haefeli et al. 1984). The technologies elaborated in the conflict are modification of solubility and biosorption, reduction or oxidation, extracellular precipitation of metals, bioaccumulation, and deficiency of metal transport systems. There is also substitute feature that these microbes can foster even at negligible concentrations; their acquaintance to higher concentrations of these metals can prompt noxiousness (Husseiny et al. 2007).

7.10 Production of PHB Nanocomposite

Numerous methods have frequently been laboring in the production of nanocomposite materials. These encompass solution intercalation, deposition (He et al. 2004), ultrasonication (Lee et al. 2004), melt intercalation (Shen et al. 2002), supercritical fluid (Lin et al. 2005), magnetron sputtering (Musil and Baroch 2013), laser (Leconte et al. 2007), etc. In PHB nanocomposite fabrication, solution intercalation and melt intercalation approaches are the utmost widely discovered trials. Nevertheless, usage of in situ intercalative polymerization, supercritical fluids, and electrospinning is exposed to be promising and developing techniques. The performance and dominance of a nanocomposite hinge on exactly how well the nanofillers dissolve or blend into the environment. Therefore, these procedures create various tactics to recover the composites' thermos mechanical and physicochemical properties by supplementing efficient interfaces among the nanofiller and the polymer matrices (Taton et al. 2000).

Nowadays, the biosynthesis of silver nanoparticles has achieved farfetched fame in the world in various field owing to significant properties. The ions are believed to observe to the bacterial cell wall, varying the permeability of the cell wall and consequently prompting protein denaturation, which sways cell lysis and death. This specific quality of silver nanoparticles is subjugated in food packaging and dispensation industries. Nanocomposites of biodegradable polymers comprising silver nanoparticles exhibited antimicrobial activities, which deter the evolution of post-processing microbial contaminants, thereby encircling the shelf life of food and enlightening food safety (Rhim et al. 2013).

An innovative development for the biological synthesis of AgNPs and polyhydroxybutyrate (PHB) from the fermentation process with *Cupriavidus necator* was established and scaled up to fully automated bioreactor. Remarkably, this work verified for the first time, the intrinsic capacity of *C. necator* to diminish the silver salt and yield AgNPs without the need of adding a reducing agent. It was also observed that the method of AgNPs synthesis (with or without reducing agent) affected the dispersion of the nanoparticles and therefore their antimicrobial performance, being the biosynthesized ones the most effective in reducing the bacterial population of *S. enterica* and *L. monocytogenes*. Moreover, the incorporation of AgNPs by means of this method did not cause any detrimental effects on the thermal degradation, the optical properties, and the biodegradability of the active polymer, although a slight reduction in crystallinity was seen. Thus, the procedure here developed allows the production of AgNP-based polyhydroxyalkanotes, which are suitable for antimicrobial applications (Fig. 7.2) (Anbukarasu et al. 2015).

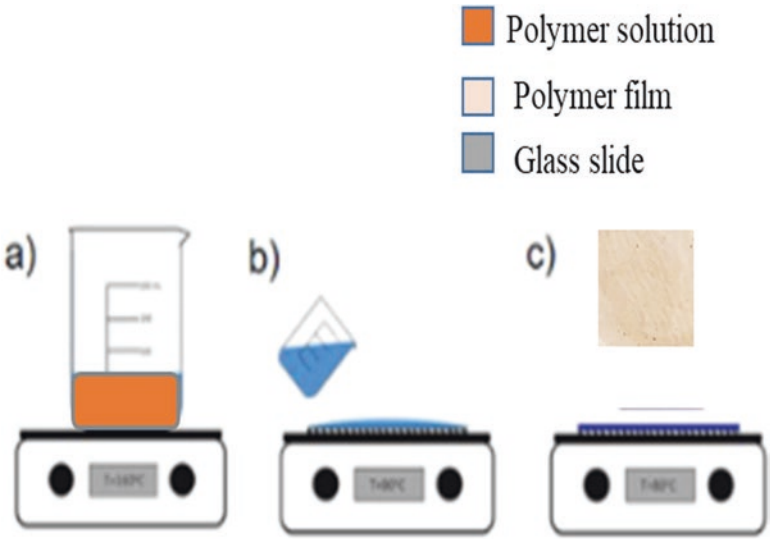


Fig. 7.2 Preparation of PHB nanocomposite film

7.11 Applications of PHB-Nanocomposites

The progression of second-generation fouling unaffected coverings is one of the utmost fruitful instances of modern biological exploration using nanochemistry to crack a stern ecological subject of global dimension. Additional ecologically approachable antifouling approach has engrossed on the classification and enlargement of goods that are created on the biological defenses of aquatic microbes that snarl their frame exteriors restricted of fouling. A diversity of novel bioactive yields has been secluded from abundant aquatic sources in the previous decades. Novel natural products have conveyed significant organizations and encouraging amalgams by the impending to be recycled as innovative antifoulants. They have remained quarantined mostly from microbes, tunicates, corals, bryozoans, and sponges (Perez-Montaño et al. 2014).

Fouling can cause many health risks and damage of assets in industrial and medical uses. In the early 1990s, foul discharge coatings were first cast off on containers, but irrespective of early exhilaration about the skill, they have had a protracted diffident market owing to price raise and mediocre performance when connected to marine biocidal coatings. In recent times, these coatings are undergoing a resurgence. Enhanced evolvement established on chemistry of nanotechnology has empowered the amalgamation of ecofriendly undercoats with enhanced foul discharge. Many studies provide a holistic indication of the research accomplishments that are driving the evolving non-biocidal tools. Biofouling hints detrimental economic and environmental impacts on marine surfaces including increase in roughness, corrosion, fuel consumption of ships, etc. Biofouling is the accretion of undesired organisms on exteriors, with biofilms generated by microorganisms and macrofouling produced by organisms (Bixler and Bhushan 2012). Nanomaterials thus conserved can be restrained nanocomposites because, naturally, they are multiphase materials in which one of the stages is the nanomaterial and/or nanostructures frequent at consistent intervals among the miscellaneous phases. Nanocomposites can reserve or even increase the activity of the discrete nanomaterials used in the complex formation. At the similar period, nanocomposites also offer aids in the disposition, conversion, and storing of active agents from the conformation process.

At the present time, a small US company called ePaint makings some 12,000 gallons per year of antifouling paints where the zinc oxide-based photoactive tints are enclosed with 7 wt % dynamic biocides zinc oxide and SeaNine 211 to deter hard-shell organisms such as zebra mussels, barnacles, and diatoms over expansion of the hull and other plants acknowledgements to the enhancing action of the biocides added in lower quantity after linked to copper oxide nanoparticles usually existing in antifouling paints in 45–79% (Morris et al. 1999). Henceforth, a systematic ecological valuation of this acquaintance will take into consideration the discharge profile of zinc oxide nanoparticles unified and its ecofriendly fortune. However, the convention of polymer nanocomposites in antifouling coatings has remained a concern so far unfamiliar. Research laboratory analyses do not offer ample evidence with veneration to the field performance of antifouling paints. The series of aspects which are significant for the fouling act is multidimensional; nevertheless field imprints are realistic (Dhams and Hellio 2009).

7.12 Conclusion

It is supposed that the aquatic environment can benefit as a pithead for determining numerous biopolymer hoarding microbes. Subsequently, PHB shows a remarkable role in fabricating a supportable atmosphere by swapping the synthetic biopolymers. Hitherto, to spot inroads into the synthetic bioplastic-dominated market, cost-effective manufacture of the ecofriendly polymers is obligatory. Decline in the rate of fabrication, ensuing in the inexpensive value of these recyclable polymers, will widen their variety of industrial application. The noteworthy assets of these polymers such as biocompatibility and biodegradability have left it practicable to envisage the widespread usage in numerous applications.

Acknowledgments We greatly acknowledge Science and Engineering Research Board and the Management of Sathyabama Institute of Science and Technology, Chennai, India, for their support. This effort was supported by Science and Engineering Research Board (SERB), Government of India for funding through PDF/2016/002806 to Dr. Kavitha Ganapathy. The authors are also grateful for the facilities provided by the DST-FIST (SR/FST/ESI-146/2016) towards Centre for Ocean Research.

Statement of Disclosure The authors account no declarations of interest.

References

Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res 7(1):17–28

Akaraonye E, Keshavarz T, Roy I (2010) Production of polyhydroxyalkanoates: the future green materials of choice. J Chem Technol Biotechnol 85:732–743

Amara AA (2008) Polyhydroyalkanoates: from basic research and molecular biology to application. IUM Eng J 9:1

Anbukarasu P, Sauvageau D, Elias A (2015) Tuning the properties of polyhydroxybutyrate films using acetic acid *via* solvent casting. Sci Rep 5:17884

Balaji S, Gopi K, Muthuvelan B (2013) A review on production of poly β hydroxybutyrates from cyanobacteria for the production of bio plastics. Algal Res 2:278–285

Baliga NS, Bonneau R, Facciotti MT, Pan M, Glusman G, Deutsch EW, Shannon A, Quaiser T, Ochsenreiter HP, Klenk A, Kletzin AHT, Meurer G, Eck J, Sensen CW, Schleper C (2002) First insight into the genome of an uncultivated crenarchaeote from soil. Environ Microbiol 4:603–611

Batcha AFM, Prasad DR, Khan MR, Abdullah H (2014) Biosynthesis of poly (3-hydroxybutyrate) (PHB) by *Cupriavidus necator* H16 from jatropha oil as carbon source. Bioprocess Biosyst Eng 37:943–951

Bixler GD, Bhushan B (2012) Biofouling: lessons from nature. Phil Trans R Soc A 370:2381–2417

Boopathy R (2000) Factors limiting bioremediation technologies. Bioresour Technol 74(1):63–67

Chee JY, Yoga SS, Lau NS, Ling SC, Abed RM, Sudesh K (2010) Bacterially produced polyhydroxyalkanoate (PHA): converting renewable resources into bioplastics. Curr Res Technol Edu Topics Appl Microbiol Microb Biotechnol 2:1395–1404

Chen GQ, Hajnal I, Wu H, Lv L, Ye J (2015) Engineering biosynthesis mechanisms for diversifying polyhydroxyalkanoates. Trends Biotechnol 33:565–574

De Smet MJ, Eggink G, Withold BB, Kingma J, Wynberg H (1983) Characterization of intracellular inclusions formed by *Pseudomonas oleovorans* during growth on octane. J Biotechnol 154:870–878

Dhams H, Hellio C (2009) Laboratory bioassays for screening marine antifouling compounds. In: Hellio C, Yebra D (eds) Advances in marine antifouling coatings and technologies. Woodsead Publishing, Cambridge, p 275e307

Drosg B, Fritz I, Gattermayr F, Silvestrini L (2015) Photo-autotrophic production of poly (hydroxyalkanoates) in cyanobacteria. Chem Biochem Eng Q 29:145–156

Ewering C, Lütke-Eversloh T, Luftmann H, Steinbüchel A (2002) Identification of novel sulfur-containing bacterial polyesters: biosynthesis of poly (3-hydroxy-S- propyl- ω-thioalkanoates) containing thioether linkages in the side chains. Microbiology 148:1397–1406

Haefeli C, Franklin C, Hardy K (1984) Plasmid determined silver resistance in *Pseudomonas stutzeri* isolated from silver mine. J Bacteriol 158:389–392

Hahn SK, Chang YK, Kim BS, Chang HN (1994) Optimization of microbial poly(3-hydroxybutyrate) recover using dispersions of sodium hypochlorite solution and chloroform. Biotechnol Bioeng 44:256–261

He LH, Lim CW, Wu BS (2004) A continuum model for size-dependent deformation of elastic films of nano-scale thickness. Int J Solids Struct 41(3–4):847–857

Hezayen FF, Rehm BH, Eberhardt R, Steinbüchel A (2000) Polymer production by two newly isolated extremely halophilic archaea: application of a novel corrosion- resistant bioreactor. Appl Microbiol Biotechnol 54:319–325

Hosseinabadi SR, Wyns K, Buekenhoudt A, Van der Bruggen B, Ormerod D (2015) Performance of Grignard functionalized ceramic nanofiltration membranes. Sep Purif Technol 147:320–328

Hrabak O (1992) Industrial production of poly-,6-hydroxybutyrate. FEMS Microbiol Rev 103:251–256

Huey C (2006) Polyhydroxybutyrate (PHB) production from cafeteria wastes under anoxic and aerobic conditions in sequencing batch reactor. Degree of Bachelor of Civil Engineering-Environmental Faculty of Civil Engineering Universiti Teknologi Malaysia

Huisman GW, De Leeuw O, Eggink G, Witholt B (1989) Synthesis of Polyhydroxyalkanoates is a common feature of fluorescent pseudomonads. Appl Environ Microbiol 55:1949–1954

Husseiny MI, El-Aziz MA, Badr Y, Mahmoud MA (2007) Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*. Spectrochim Acta A 67:1003–1006

Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B (2014) Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci 9(6):385–406

Isola C, Sieverding HL, Raghunathan R, Sibi MP, Webster DC, Sivaguru J, Stone JJ (2017) Life cycle assessment of photodegradable polymeric material derived from renewable bioresources. J Clean Prod 142:2935–2944

Johnstone B (1990) A throw away answer. Far Eastern Econ Rev 147(6):62–63

Kato N, Konishi H, Shimao M, Sakazawa C (1992) Production of 3 hydroxybutyric acid trimer by *Bacillus megaterium* B-124. J Ferment Bioeng 73:246–247

Kavitha G, Kurinjimalar C, Sivakumar K, Palani P, Rengasamy R (2016) Biosynthesis, purification and characterization of polyhydroxybutyrate from *Botryococcus braunii* kütz. Int J Biol Macromol 89:700–706

Klaus T, Joerger R, Olsson E, Granqvist CG (1999) Silver based crystalline nanoparticles, microbially fabricated. Proc Natl Acad Sci U S A 96:13611–13614

Krzan A (2012) Biodegradable polymers and plastics. Innovative Value Chain Development for Sustainable Plastics in Central Europe (PLASTiCE)

Leconte S, Veintemillas-Verdaguer M, Morales R, Costo I, Rodrıguez PB (2007) Bouchet-Fabre and N. Herlin-Boime. J Colloid Interface Sci 313:511–518

Lee SY (1996) Bacterial polyhydroxyalkanoates. Biotechnol Bioeng 49:1–14

Lee E, Mielewski D, Baird R (2004) Exfoliation and dispersion enhancement in polypropylene nanocomposites by in-situ melt phase ultrasonication. Polym Eng Sci 44:1773–1782

Lin Y, Cui X, Yen C, Wai CM (2005) Platinum/carbon nanotube nanocomposite synthesized in supercritical fluid as electrocatalysts for low-temperature fuel cells. J Phys Chem B 109:14410–14415

Mahishi LH, Tripathi G, Rawal SK (2003) Poly (3-hydroxybutyrate)(PHB) synthesis by recombinant Escherichia coli harbouring *Streptomyces aureofaciens* PHB biosynthesis genes: effect of various carbon and nitrogen sources. Microbiol Res 158:19–27

Matsumoto K, Taguchi S (2013) Biosynthetic polyesters consisting of 2-hydroxyalkanoic acids: current challenges and unresolved questions. Appl Microbiol Biotechnol 97:8011–8021

Morais MGD, Stillings C, Dersch R, Rudisile M, Pranke P, Costa JAV, Wendorff J (2015) Biofunctionalized nanofibers using Arthrospira (*Spirulina*) biomass and biopolymer. Biomed Res Int 2015

Morris RS, Walsh IV MA, Cape Cod Research Inc (1999) Zinc oxide photoactive antifoulant material. US Patent 5,916,947

Musil J, Baroch P (2013) High-rate pulsed reactive magnetron sputtering of oxide nanocomposite coatings. Vacuum 87:96–102

Panda B, Mallick N (2007) Enhanced poly-β-hydroxybutyrate accumulation in a unicellular cyanobacterium, *Synechocystis* sp. PCC 6803. Lett Appl Microbiol 44:194–198

Perez-Montaño F, Alías-Villegas C, Bellogín RA, Del Cerro P, Espuny MR, Jiménez-Guerrero I, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169:325–336

Pinto RJ, Fernandes SC, Freire CS, Sadocco P, Causio J, Neto CP, Trindade T (2012) Antibacterial activity of optically transparent nanocomposite films based on chitosan or itsderivatives and silver nanoparticles. Carbohydr Res 348:77–83

Rabnawaz M, Wyman I, Auras R, Cheng S (2017) A roadmap towards green packaging: the current status and future outlook for polyesters in the packaging industry. Green Chem 19:4737–4753

Radhika D, Murugesan AG (2012) Bioproduction, statistical optimization and characterization of microbial plastic (poly 3-hydroxy butyrate) employing various hydrolysates of water hyacinth (*Eichhornia crassipes*) as sole carbon source. Bioresour Technol 121:83–92

Rajakumar G, Rahuman AA, Roopan SM, Khanna VG, Elango G, Kamaraj C, Zahir AA, Velayutham K (2012) Fungus-mediated biosynthesis and characterization of TiO2 nanoparticles and their activity against pathogenic bacteria. Spectrochim Acta A Mol Biomol Spectrosc 91:23–29

Rhim JW, Park HM, Ha CS (2013) Bio-nanocomposites for food packaging applications. Prog Polym Sci 38:1629–1652

Sabirova JS, Ferrer M, Lünsdorf H, Wray V, Kalscheuer R, Steinbüchel A, Golyshin PN (2006) Mutation in a "tesB-like" hydroxyacyl-coenzyme A-specific thioesterase gene causes hyperproduction of extracellular polyhydroxyalkanoates by *Alcanivorax borkumensis* SK2. J Bacteriol 188(24):8452–8459

Sandhya M, Aravind J, Kanmani P (2013) Production of polyhydroxyalkanoates from *Ralstonia eutropha* using paddy straw as cheap substrate. Int J Environ Sci Technol 10:47–54

Senapati L, Schrier J, Whaley KB (2005) Reply to comment on "electronic transport, structure, and energetics of endohedral Gd@ C82 metallofullerenes". Nano Lett 5:2341–2341

Shabeb MS, Younis MA, Amara A, Shoreit A (2005) Production of PHB-co-PHV from compost bioremediation by purple non-sulfur bacteria. Egyptian J Biotechnol 19:467–478

Shen Z, Simon GP, Cheng Y-B (2002) Comparison of solution intercalation and melt intercalation of polymer-clay Nanocomposites. Polymer 43:4251–4260

Shibata Y, He LH, Kataoka Y, Miyazaki T, Swain MV (2008) Micromechanical property recovery of human carious dentin achieved with colloidal nano-β-tricalcium phosphate. J Dental Res 87:233–237

Shivaji S, Madhu S, Singh S (2011) Extracellular synthesis of antibacterial silver nanoparticles using psychrophilic bacteria. Process Biochem 46(9):1800–1807

Shrivastav A, Mishra SK, Shethia B, Pancha I, Jain D, Mishra S (2010) Isolation of promising bacterial strains from soil and marine environment for polyhydroxyalkanoates (PHAs) production utilizing Jatropha biodiesel byproduct. Int J Biol Macromol 47:283–287

Steinbüchel A (1991) Polyhydroxyalkanoic acids. In: Biomaterials. Palgrave Macmillan, London, pp 123–213

Sudesh K, Abe H, Doi Y (2000) Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. Prog Polym Sci 25:1503–1555

Taton TA, Mirkin CA, Letsinger RL (2000) Scanometric DNA array detection with nanoparticle probes. Science 289:1757–1760

Valentin HE, Steinbüchel A (1994) Application of enzymatically synthesized short-chain- length hydroxy fatty acid coenzyme A thioesters for assay of polyhydroxyalkanoic acid synthases. Appl Microbiol Biotechnol 40:699–709

Valodkar M, Modi S, Pal A, Thakore S (2011) Synthesis and anti-bacterial activity of Cu, Ag and Cu-Ag alloy nanoparticles: a green approach. Mater Res Bull 46(3):384–389

Van-Thuoc D, Huu-Phong T, Thi-Binh N, Thi-Tho N, Minh-Lam D, Quillaguaman J (2012) Polyester production by halophilic and halotolerant bacterial strains obtained from mangrove soil samples located in Northern Vietnam. MicrobiologyOpen 1(4):395–406

Wubbeler JH, Steinbüchel A (2014) New pathways for bacterial polythioesters. Cur Opin Biotechnol 29:85–92

Chapter 8
Environmental Metabolomics: With the Perspective of Marine Toxicology Assessment

**Ninian Prem Prashanth Pabbathi, Neelam M. Nathani,
Indra Ramjibhai Gadhvi, and Mootapally Chandrashekar**

Contents

Abstract Environmental metabolomics or metabonomics is the study of interactions and impact of stressors from external sources on metabolites of an organism living in it. Marine environmental stress has become a global concern in the recent past as the marine biological environment is immensely complex when compared to the other environments on this planet. The major concern can be attributed to the

---

N. P. P. Pabbathi
Department of Biotechnology, National Institute of Technology (NIT),
Warangal, Telangana, India

N. M. Nathani
Department of Life Sciences, Maharaja Krishnakumarsinhji Bhavnagar University,
Bhavnagar, Gujarat, India

I. R. Gadhvi · M. Chandrashekar (✉)
Department of Marine Science, Maharaja Krishnakumarsinhji Bhavnagar University,
Bhavnagar, Gujarat, India
e-mail: csmootapally@mkbhavuni.edu.in

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_8

comprehensive growth of anthropogenic activities in the marine environment. In the recent years, application of environmental metabolomics in the stressed marine environments has proved to increase our knowledge about toxic mechanism on the residing organisms to a greater extent.

In the current chapter, we review the present scenario of the metabolomics with the perspective of its applications in toxicology assessments, especially in the marine environments. The compilation would guide us through the plausible steps to be taken for further improvements in the toxicology study protocols, thus leading to prepare a database of signature molecules responding to specific stress/hazards as well as those specific to the uniqueness of each niche.

Keywords Metabolite profiling · Marine bioresource · Nutrition · Stress monitoring · By-products · Toxicity · Niche · Bioindicators · Biomolecules

8.1 Introduction

According to a famous oceanographer Jacques Cousteau, “Water and air, the two essential fluids on which all life depends, have become global garbage cans.” Now the time has come to clean the cans and restore the essential fluids. In early 1970, researchers started understanding and prophesying the effect of chemical combinations on the ecology. By the late 1980s, numerous scientists had started estimating the effect of toxic pollutants on organisms, populations, and communities existing in an ecosystem. It’s been more than a couple of decades since the toxic effects of pollutants were first observed, and till date the pollution rates are steadily accelerating, which is threatening a lot of communities in every ecosystem (Fig. 8.1).

Though the research started in the 1950s, significant developments took place only after 2000. There has been a gradual decrease in the number of publications for the past 4 years which indicates the decreasing research on environmental

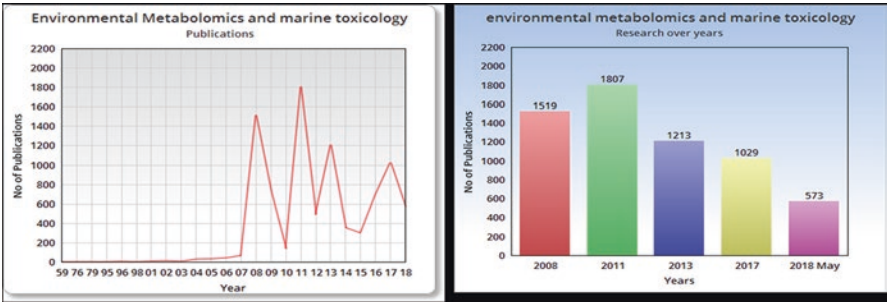


Fig. 8.1 Timeline of research work on environmental metabolomics and marine toxicology (SCHOLAR PLOTR)

metabolomics and marine toxicology. There is a need for more extensive research in this area to protect a lot of populations from extinction (Fig. 8.2).

Metals are abundantly found in nature, mostly in the form of cations. All the organisms require small amounts of metals for their survival. For instance, potassium and sodium are sufficiently required for regular nerve impulse, while magnesium, iron, zinc, etc. are required as cofactors for many enzymes. Metals can be classified into two types based on the requirement for life; they are essential and nonessential metals. As the name suggests, essential metals are essential in minor volumes for the sustenance of life, but in higher volumes they are hazardous. Whereas the nonessential metals are not required for the life sustenance and are very hazardous once their restrictive concentrations are reached, it is better explained in the figures above.

Different organisms have developed different mechanisms including proteins and transporters to uptake and transport the required metal ions based on their aquatic habitat, viz., freshwater and marine. The difference in these two habitats with respect to metals is that freshwater has fewer amounts of metals when compared to marine water. For this very reason, organisms in freshwater always tend to lose metal ions, whereas in marine water they tend to lose water. In both the cases, the organism may die either due to lack or abundance of metal ions. Aquatic metal contamination is mainly due to the mining activities and effluents from smeltery, paint industries, and liquids which leach from waste dump. Different types of metals and their source of contamination are listed below in Table 8.1.

Other aqua-toxic metals include palladium, nickel, cobalt, and uranium. Nickel and cobalt are often used as constituents in metal alloys. Uranium metal itself is a highly hazardous metal due to its radioactive nature. Apart from pure metal ions, there are some organometallic compounds that contribute to aquatic toxicology. Among all organometallic compounds, organic tin and mercury composites are the major stakes. Generally to inhibit the algal and barnaclar growth on hulls of boats and ships, antifouling paints are used. These paints contain hazardous organometallic constituents like triphenyltin (TPT), tributyltin (TBT), etc.

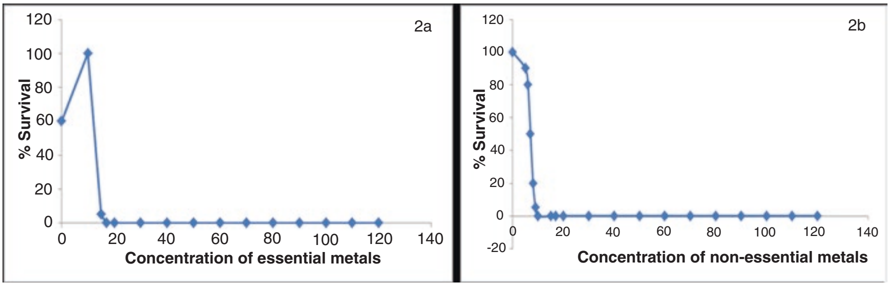


Fig. 8.2 (**a**) Effect of essential metals on the survival of organisms (Nikinmaa 2014). (**b**) Effect of nonessential metals on the survival of organisms (Nikinmaa 2014)

Table 8.1 List of major metals responsible for toxicity of seawater and their source of contamination

S. no.	Metal	Type of metal	Source of contamination
1	Copper	Essential	Water pipes, boat paints
2	Lead	Nonessential	Wastes of paint-producing industries and lead-containing paints or batteries
3	Cadmium	Nonessential	Galvanized steel, batteries, coal, and phosphate fertilizers
4	Zinc	Essential	Metal alloys of several steels
5	Iron	Essential	Mining activities, dyeing of fabrics and leather, insecticides
6	Aluminum	Nonessential	Natural processes like chemical speciation, hydrological flow paths, soil-water interactions, and the composition of the underlying geological materials
7	Silver	Nonessential	Effluent from traditional photography
8	Arsenic	Nonessential	Pesticides and components of semiconductors, copper mining by-products, and metal-smeltery effluents (e.g., from gold, lead, copper, and nickel smelteries)
9	Mercury	Nonessential	Used in batteries and light tubes, thermometers, for gold extraction

Adapted and modified from Nikinmaa (2014)

8.1.1 Organic Matter

Organic compounds contribute to be a significant part of aquatic pollution through pulp and paper mill effluents, personal hygiene products, oil, etc. Drilling of oil and its usage is the major cause for pollution. Every oil production is accompanied by minor seepage into the water nearby. With accelerated oil production, aquatic oil pollution is equally accelerating. Oil is a combination of different components like petrol, diesel, tar, etc. These are the chief compounds that contribute to plastics. The efficiency of microbes to degrade oil will increase only with increase in temperature.

Excess usage of pesticides in the fields is leading to the contamination of the nearest water body thereby contributing to aquatic pollution. Few pesticides are more toxic to the non-targeted organisms than to the targeted organisms. For instance, formulations of atrazine are used against terrestrial weeds and are highly toxic for amphibians. Most of the herbicides are photosynthesis blockers which upon entering into water bodies and inhibit aquatic autotrophs, subsequently lead to disturbance in the ecological producer-consumer relationship. Below lies an example of lethal toxicity of insecticide malathion on varying aquatic lifeforms (Fig. 8.3).

8.1.2 Eco-metabolomics

Metabolomics technically is a study of endogenous and exogenous cellular chemical impressions which are created as a part of cell's metabolism (Bennett 2005). A metabolome is a combination of all cellular metabolites in a cell or tissue or even

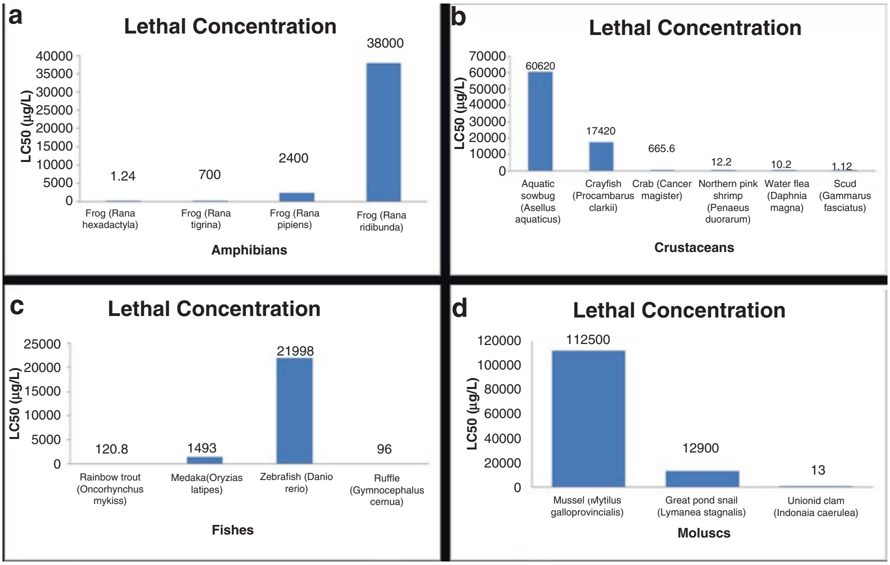


Fig. 8.3 LC50 of pesticides as observed for (**a**) amphibians (**b**) crustaceans (**c**) fishes (**d**) mollusks. (Data has been taken from http://www.pesticideinfo.org)

organism. These cellular metabolites are the end products of cellular metabolism (Jordan et al. 2009). mRNA quantification and gene expression information along with proteomic study might not reveal the real-time scenario of the cell's inside, whereas metabolomics profiling yields direct real-time physiology of the cell. The most challenging part of research is to assimilate and analyze metabolomics, transcriptomic, and proteomic data to give a whole story of the cellular metabolism.

There are even quite a few subsections under metabolomics; out of them one of the most important subsection is environmental metabolomics or eco-metabolomics. This subsection contemplates analyzing the relationships and communications of the organisms with their habitat using metabolomics. Since a decade there has been swift growth in eco-metabolomics. Out of humongous published research, a few metabolites that altered their gene expression due to environmental stress inducers are listed below; this explains the scope and impact of eco-metabolomics. These aquatic stressors are categorized into metals, pesticides, abiotic stressors, and others.

8.2 Metabolomics and Marine Environments

8.2.1 Different Strategies to Monitor Stresses on Marine Life

All these listed metabolites are capable of functioning as bioindicators of aquatic eco-stress and can be used for further studies. It is significant to consider that the level of metabolite variability differs significantly and occasionally goes unreported. The metabolite data briefed in the tables (Tables 8.1, 8.2 and 8.3) has to be used

Table 8.2 Metal stress inducers assessed on different organisms using varied metabolites

Sl. no.	Stress inducer	Organism	Affected metabolites	Reference
1	Cadmium	*Corbicula fluminea* (small Asian clam)	Malonate, Gly, Bet	Spann et al. (2011)
2	Zinc	*Corbicula fluminea*	No significant changes were reported	
3	Cadmium and zinc	*Corbicula fluminea*	Amino acids A, Q, N, I, P, V, L, E, T, succinate, malonate, ADP/ATP, benzoic acid, Glu, Gly, turanose, Bet, 2-methyl-glycoside	
4	Nickel	*Mytilus galloprovincialis*	Amino acids V, I, L, F, P, W, Y, Q, adenosine, ATP/ADP, guanine, hypotaurine, taurine, allantoin, R, Bet, Glu, homarine, malate, PAG, succinate, uracil	Jones et al. (2008)
5	Copper and cadmium (1 week, separate exposures)	*Perna viridis* (Green mussel)	R, H, E, Q, G, hypotaurine, dimethylglycine, ATP/ADP	Wu and Wang (2010)
6	Copper and cadmium (1 week, combined exposure)	*Perna viridis*	Lac, succinate, NAD, branched chain amino acids, Glu, Gly, ATP/ADP	
7	Metal contamination (site specific)	*Mytilus spp.*	Amino acids A, R, T, Y, F, Q, acetate, acetoacetate, Bet, inosine, succinate, taurine	Kwon et al. (2012)
8	Mercury exposure	*Suaeda salsa*	Amino acids V, I, L, T, A, Q, F,G, succinate, fructose, Glu, fumarate, ferulate	Wu et al. (2012a)
9	Lead	*Suaeda salsa*	Isocaproate, Glu, fructose, malate, citrate, sucrose	Wu et al. (2012b)
10	Zinc	*Suaeda salsa*	Isocaproate, Glu, fructose, phosphocholine, Bet, malate, citrate, sucrose, choline	
11	Lead and zinc	*Suaeda salsa*	No significant changes were reported	
12	Arsenic	*Daphnia magna*	Amino acids A, K	Nagato et al. (2013)
13	Cadmium	*Daphnia magna*	Glutathione, ɣ-glutamylcysteine, fructose phosphate, chitobiose, R, arginine phosphate, N-acetylglucosamine, N-acetylglucosamine phosphate, arachidonic acid, arachidonic acid products	Taylor et al. (2010)

(continued)

Table 8.2 (continued)

Sl. no.	Stress inducer	Organism	Affected metabolites	Reference
14	Cadmium	*Daphnia magna*	P, M, H, R, F, W, K, lauric acid, myristic acid, palmitic acid, stearic acid	Poynton et al. (2011)
15	Copper	*Daphnia magna*	Amino acids T, P, M, L/I, K, R, F, Y, W, N-acetylspermidine, ophthalmic acid	Taylor et al. (2009)
16	Copper	*Daphnia magna*	Amino acids V, G, T, Q, A, K, L, M, F, E, glycerophosphocholine, uracil	Nagato et al. (2013)
17	Lithium	*Daphnia magna*	Amino acids G, A, L, M, F, E, lycerophosphocholine, uracil	

Bet betaine, *Gly* glycogen, *Glu* glucose, *Lac* lactate, *A* alanine, *R* arginine, *N* asparagine, *D* aspartic acid, *C* cysteine, *Q* glutamine, *E* glutamic acid, *G* glycine, *H* histidine, *I* isoleucine, *L* leucine, *K* lysine, *M* methionine, *F* phenylalanine, *P* proline, *O* pyrrolysine, *S* serine, *U* selenocysteine, *T* threonine, *W* tryptophan, *Y* tyrosine, *V* valine, *B* aspartic acid or asparagine, *Z* glutamic acid or glutamine

Table 8.3 Pesticide stress inducers assessed on different organisms using varied metabolites

Sl. no.	Stress inducer	Organism	Affected metabolites	Nature of chemical	Reference
1	Dinoseb exposure	*Oncorhynchus tshawytscha* (Chinook salmon embryos)	Lac, succinate, creatine, phosphocreatine, ATP	Herbicide	Viant et al. (2006)
2	Ethinylestradiol exposure	*Oncorhynchus mykiss* (rainbow trout)	Plasma extract: Vitellogenin, A Lipid extract: Unsaturated fatty acids, cholesterol, cholesterol esters	–	Samuelsson et al. (2006)
3	Vincolozin	*Pimephales promelas*	Lac, acetate, creatine, taurine, formate, A, significant changes in unidentified metabolites	Fungicide	Ekman et al. (2007)
4	Atrazine	*Diporeia spp.*	Heptacosane, 9,12-octadecadienoic acid, urea, N,N-bis(tert-butyldimethylsilyl), nonadecane, D	Herbicide	Ralston-Hooper et al. (2008)
5	Chlorpyrifos	*Mytilus galloprovincialis* (Mediterranean mussel)	Amino acids F, P, Y, V, R, E, I, L, Q, adenosine, ATP/ADP, choline, guanine, homraine, hippurate, Lac, phenylacetylglycine	Pesticide	Jones et al. (2008)

(continued)

Table 8.3 (continued)

Sl. no.	Stress inducer	Organism	Affected metabolites	Nature of chemical	Reference
6	Chloropyrifos and nickel combined	*Mytilus galloprovincialis*	Amino acids A, I, V, E, choline, PAG, adenosine, β-hydroxybutyrate, guanine, nicotinate, N-methylnicotinamide, uracil	Pesticide	Jones et al. (2008)
7	Lindane	*Mytilus edulis* (blue mussel)	Amino acids A, D, Bet, homarine, taurine, succinate	Insecticide	Tuffnail et al. (2009)
8	Atrazine	*Mytilus edulis*	Amino acids I, L	Herbicide	Tuffnail et al. (2009)
9	Fenvalerate	*Daphnia magna*	Arginine phosphate, fructose phosphate, glucosamine phosphate, N-acetylglucosamine, N-acetylgluocosamine phosphate, fructose, ɣ-glutamylcysteine, linoleic acid, arachidonic acid	Insecticide	Taylor et al. (2010)
10	Dinitrophenol	*Daphnia magna*	Fructose phosphate, arginine phosphate, chitobiose, N-acetylglucosamine, N-acetylglucosamine phosphate, fructose, R, glutathione, 2-glutamylcysteine, arachidonic acid, linoleic acid, 6-keto-prostaglandin, retinoic acid	Can be used as herbicide	Taylor et al. (2010)
11	Propranolol	*Daphnia magna*	Arachidonic acid, linoleic acid, prostaglandin, retinoic acid, fructose, arginine phosphate, glutathione, ɣ-glutamylcysteine, N-acetylglucosamine, N-acetylglucosamine phosphate, fructose phosphate	Used as medicine for cardiovascular issues	Taylor et al. (2010)

(continued)

Table 8.3 (continued)

Sl. no.	Stress inducer	Organism	Affected metabolites	Nature of chemical	Reference
12	Pyrene	*Daphnia magna*	Amino acids K, A, N, aminomalonic acid, glucofuranoside, heptanedioic acid, turanose, uridine, gluconic acid, ornithine	Used in Dye making	Vandenbrouck et al. (2010)
13	Pyrene and fluoranthene mixtures	*Daphnia magna*	Amino acids F, T, P, V, N, I, glucofuranoside, N-acetylglucose, choline, talose, turanose1glyceric acid, 2,3,4-trihydroxybtylic acid, glucoronolactone, putrescine, purine-2-amine, spermine, uridine, myo-inositol, turanose, cadaverine, galactose oxime	Pollutant	Vandenbrouck et al. (2010)
14	Esfenvalerate	*Gammarus pulex*	Contaminated streams 2.7 fold more sensitive to pesticide exposure	Insecticide	Russo et al. (2018)
15	Combination of tebuconazole, trifloxystrobin, lambda-cyhalothrin, thiamethoxam and clorantraniliprole	*Cyprinus carpio*	Acethylcholinesterase activity in the brain was lower. Lipid peroxidation, catalase activity, glutathione S-transferase increased.	Insecticides and fungicide	Clasen et al. (2018)
16	Sedaxane	*Danio rerio*	Influences reactive oxygen species, malondialdehyde, peroxidase, glutathione S-transferase, superoxide dismutase, and glutathione in live larvae, decreases succinate dehydrogenase activity	Pesticide	Yao et al. (2018)
17	Mesotrione	*Cyprinus carpio*	Increases superoxide dismutase, catalase	Herbicide	Wang et al. (2018)

Bet betaine, *Gly* glycogen, *Glu* glucose, *Lac lactate*, *A* alanine, *R* arginine, *N* asparagine, *D* aspartic acid, *C* cysteine, *Q* glutamine, *E* glutamic acid, *G* glycine, *H* histidine, *I* isoleucine, *L* leucine, *K* lysine, *M* methionine, *F* phenylalanine, *P* proline, *O* pyrrolysine, *S* serine, *U* selenocysteine, *T* threonine, *W* tryptophan, *Y* tyrosine, *V* valine, *B* aspartic acid or asparagine, *Z* glutamic acid or glutamine with care as the methods employed to conceive and analyze the data differ from each other. These tables not only suggest the summary of eco-metabolomics but also accentuate the necessity of standard methods for data compilation and analysis for the easy comparison in the future (Morrison et al. 2007) (Tables 8.4, 8.5 and 8.6).

Fishes and humans share more similar biochemical processes and are apt for aquatic toxicity studies particularly when pharmaceuticals are involved (Samuelsson et al. 2006). In aquatic metabolomics, fishes have been more involved for the past decade as they are very diverse in speciation. For instance, in 2012 Martinovic-Weigelt et al. . with the help of 1H, NMR explored male fathead minnows and came up with some metabolites like taurocholic acid, trimethylamine, etc., which are responsible for more dominant nature and eye-catching to females for mating process (Martinovic-Weigelt et al. 2012). This exceptional work explains the viability of metabolomics to understand the behavior and aquatic ecology. Most of the aquatic metabolomic studies are emphasized on eco-toxicity and pollutant stress on aquatic life.

Mussels are aquatic sedentary mollusks; they are distributed in both fresh- and marine water. Mussels are being used for eco-toxicity studies because of ease in handling and relocation; they even amass toxic compounds (Spann et al. 2011;

Table 8.4 Abiotic stress inducers assessed on different organisms using varied metabolites

Sl. no.	Stress inducer	Organism	Affected metabolites	Reference
1	Sewage effluent	*Oncorhynchus mykiss*	Variable changes in HDL, LDL, VLDL, cholesterol	Samuelsson et al. (2011)
2	Crude oil exposure	*Atherinops affinis* (topsmelt)	Lac, phosphocreatine	Van Scoy et al. (2012)
3	PAH-contaminated water exposure (field study)	*Mytilus galloprovincialis* (Mediterranean mussel)	Amino acids V, L, F, acetoacetate, adenine, thymidine	Fasulo et al. (2012)
4	Salinity increases	*Suaeda salsa* (plant)	Amino acids V, I, L, T, E, Q, D, N, E, Y, F, Bet, fructose, Glu, malonate, choline, 2phosphocholine, fumarate, ferulate	Wu et al. (2012a)
5	Salinity increases and mercury exposure (combined)	*Suaeda salsa* (plant)	Amino acids V, I, L, T, E, D, N, G,Y,W, Bet, malonate, choline, phosphocholine, fructose, Glu, fumarate, ferulate	Wu et al. (2012a)
6	Temperature increases (from 12 to 18 °C)	*Salmo salar* (Atlantic salmon)	Amino acids A, Q, G, F, Y, glycoprotein acetyls, HDL, various lipids, creatine, choline, Glu	Kullgren et al. (2013)

Bet betaine, *Gly* glycogen, *Glu* glucose, *Lac* lactate, *A* alanine, *R* arginine, *N* asparagine, *D* aspartic acid, *C* cysteine, *Q* glutamine, *E* glutamic acid, *G* glycine, *H* histidine, *I* isoleucine, *L* leucine, *K* lysine, *M* methionine, *F* phenylalanine, *P* proline, *O* pyrrolysine, *S* serine, *U* selenocysteine, *T* threonine, *W* tryptophan, *Y* tyrosine, *V* valine, *B* aspartic acid or asparagine, *Z* glutamic acid or glutamine

Table 8.5 Other stress inducers assessed on different organisms using varied metabolites

Sl. no.	Stress inducer	Organism	Affected metabolites	Reference	Additional information
1	17-Ethynylestradiol	*Pimephales promelas* (male fathead minnow)	Amino acids A, E, Gly, Glu, Lac, Bet	Ekman et al. (2009)	Used widely in birth control pills
2	17-Ethynylestradiol	*Pimephales promelas* (fathead minnow)	Phosphatidylcholine, cholesterol, diglycerides, triglycerides	Ekman et al. (2008)	–
3	17-Trenbolone cyproterone acetate	*Pimephales promelas*	Taurine, monounsaturated lipid	Collette et al. (2010)	Used in birth control pills.
4	17-Ethynylestradiol	*Danio rerio* (zebrafish)	Amino acids Q, V, L, I, A, E, G, R, Lac, phosphocholine, cholesterol, succinate, creatine, Bet, choline, FA	Teng et al. (2013)	–
5	Graphene oxide	*Daphnia magna*	Increases oxidative stress	Lv et al. (2017)	–
6	Seleno-methionine	*Daphnia pulex*	Early life brood: Reduced lifespan and absence of reproduction	Nelson et al. (2018)	–
			Old life brood: Increased resistance to heat-induced stress		
7	Benzoylecgonine	*Daphnia magna*	Induces oxidative stress and inhibits acetylcholinesterase (AChE) activity affecting swimming behavior and the reproduction of Daphnia magna individuals	Parolini et al. (2018)	Cocaine is metabolized, excreted product

Bet betaine, *Gly* glycogen, *Glu* glucose, *Lac* lactate, *A* alanine, *R* arginine, *N* asparagine, *D* aspartic acid, *C* cysteine, *Q* glutamine, *E* glutamic acid, *G* glycine, *H* histidine, *I* isoleucine, *L* leucine, *K* lysine, *M* methionine, *F* phenylalanine, *P* proline, *O* pyrrolysine, *S* serine, *U* selenocysteine, *T* threonine, *W* tryptophan, *Y* tyrosine, *V* valine, *B* aspartic acid or asparagine, *Z* glutamic acid or glutamine

Fasulo et al. 2012). For instance, Fasulo et al. in 2012 exposed Mediterranean mussel to PAH (polycyclic aromatic hydrocarbon) and studied the variation in its metabolites. This study concluded that, on exposing PAH to Mediterranean mussel, its anaerobic metabolism upsurged significantly.

In the year 2012, Kwon et al. also conducted similar experiments using *Mytilus* species from South Korean bay. These studies demonstrated the potential of using wild nurtured organisms in metabolomics. These organisms were used to identify changes in inhabitants with respect to varying climates.

Table 8.6 Various marine organisms involved in eco-metabolomics studies

Sl. no.	Different organisms used	Reference
1	Flatfish (fish)	Stentiford et al. (2005)
2	Chinook salmon (fish)	Viant et al. (2006)
3	Zebrafish (fish)	Ekman et al. (2007)
4	*Mytilus edulis* (blue mussel)	Tuffnail et al. (2009)
5	Rainbow trout (fish)	Kullgren et al. (2010)
6	Fathead minnows (fish)	Van Scoy et al. (2012)
7	Topsmelt (fish)	Kokushi et al. (2012)
8	Japanese medaka (fish)	Uno et al. (2012)
9	Whale sharks (fish)	Dove et al. (2012)
10	*Mytilus galloprovincialis* (Mediterranean mussel)	Fasulo et al. (2012)
11	*Suaeda salsa* (plant)	Wu et al. (2012a)
12	Atlantic salmon (fish)	Kullgren et al. (2013)
13	*Daphnia magna* (crustacean)	Lv et al. (2017)
14	*Danio rerio* (fish)	Yao et al. (2018)
15	*Cyprinus carpio* (fish)	Wang et al. (2018)
16	*Gammarus pulex* (crustacean)	Russo et al. (2018)
17	*Daphnia pulex* (crustacean)	Nelson et al. (2018)

8.2.2 Host and Environmental Factor Interactions

Host and its microbiome relationship can be generally studied under two headings. One is symbiosis, and the other is dysbiosis.

Symbiosis

The dynamic state of host and microbiome communication, wherein immune and metabolic interactions are maintained normal.

Dysbiosis

Dysbiosis might occur due to events like stress, infection, etc. In this state of relationship, the interactions between host and microbiome vary significantly.

These concepts of symbiosis and dysbiosis regarding host-microbiome relationships have been initially conceptualized and mostly used in human beings and models that resemble them (Hamdi et al. 2011; Nicholson et al. 2012; Scharschmidt and Fischbach 2013). Many of the sea creatures can be considered as humanized models as they have a lot of resemblance to humans (Egan and Gardiner 2016) (Fig. 8.4).

Based on the complexity of host anatomy and its immune system, a number of factors and processes that influence the state of host-microbiome interactions will also increase. For instance, sponges are less complex when compared to sharks, so

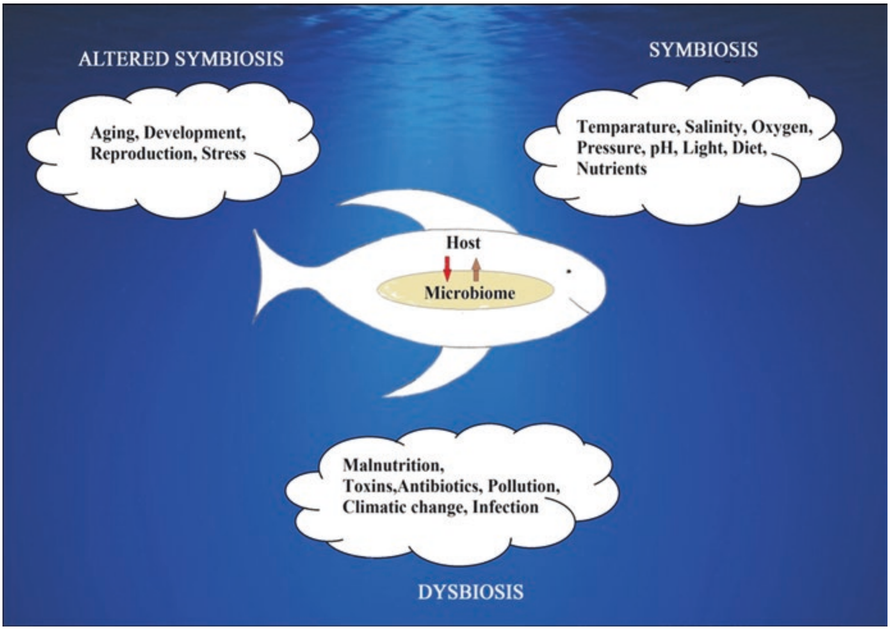


Fig. 8.4 Diagrammatic representation of host microbiome interaction and possible mechanisms

the number of factors that influence either symbiosis or dysbiosis will be less in sponges when compared to sharks. Usually, the relationship between an aquatic organism and its microbiome can be stated as symbiotic systemically, but the characteristics of the relationship may even vary for individual cell. It can be better explained with an example, where the microbes existing and surviving on the organism and in its gut have less associated intimacy when compared to the microbes that exist intracellularly.

The symbiotic state is normally influenced by a number of natural variations, which can be demarcated by the habitat characteristics. The study of upper zone of ocean can serve as best example for this. In this zone, organisms are exposed to different temperatures and different intensities of light. Particularly in ectothermal animals, host-microbiome interactions differ based on the seasonality and its respective temperature and light. According to David et al. (2014) the host-microbiome relation may even fluctuate from organism to organism individually. The diet changes of the organism, perhaps due to less availability of food, can even influence the microbiota in gut which may lead to alteration in metabolic exchange between host and its microbiome.

Another major factor influencing the relationship is stress. Stress is observed to be a part of the organism's day-to-day life, either chasing a prey or fleeing from being a prey, and even secretion of certain hormones like cortisol can show a significant effect on these relationships (Moloney et al. 2014). The regular life events of the organism like reproduction, development, and aging are also expected to influence these relationships. According to Heintz and Mair (2014), these factors have shown significant variations in nonmarine subjects. Most of the times, these changes are extreme enough to tip to transformed symbiosis from symbiosis. This state of transformed symbiosis may exist for small or extended period. According to Koren et al. (2012), in humans the gut microbiome of pregnant female generally varies from the normal female. Events like conflict which might be due to pressures of a specific environment can also cause more transformed state of symbiosis. According to Nicholson et al. (2012), the transformed symbiosis which is caused due to variation in regular events of life might be considered as healthy variations. This is suggested based on the data acquired from human beings and resembling models, but the mechanism of exchange between microbiome and host due to the variations is not clearly understood.

In an organism's lifetime, if we consider both symbiosis and transformed symbiosis as normal healthy host–microbiome alteration, then dysbiosis may be considered as the collapse of host–microbiome association, which might be interrelated to a single or multiple stressors. This can significantly change the health of the host and may even lead to an ill condition (Holmes et al. 2011). The stress inducers might be an exterior origin like agents which lead to infection, pollutants, or even an environmental change, and there are a large number of other factors which fall under this class. For instance, as the temperature of seawater increases, the corals get bleached. The reason for this is the disrupted association of unicellular algae with corals. This is one of the most evident signs of dysbiosis state.

Hashimoto et al. in 2012 linked microbial ecology to the one of the current human major stressor malnutrition, which changes even the immunity levels. Generally, in most of the studies involving host–microbiome, efforts were applied in understanding the association between dysbiosis, health of the host, and symbiosis states, but the drastic changes in the aquatic environment due to pollution have obligated this area for research so that marine life can be saved from human made pollution.

8.3 Applications of Metabolomics in Aquaculture Research

8.3.1 Larviculture Application

The prime task in effective larviculture of marine organisms is developing complete life cycle nurturing procedures. Regrettably, for commercial reasons the methodological advances have not been produced in major scientific research literature (Allan and Burnell 2013). Only since the past few years, substantial technological developments took place in the production of hatcheries. Still there are considerable opportunities in enhancing quality brood fish, their nutritional and cultural management (Hamre et al. 2013). In larviculture, enormous death rates in the process of rearing larvae are influencing the production yields. Due to this reason production yields are immensely hindered in several species. The hindering levels are sometimes as high as they almost reach 90% at times (Salze et al. 2011; Purcell et al. 2012; Sørensen et al. 2014). The reason for this high mortality rate is not the same for every species. For some new species insufficient understanding of their basic survival requirements and culturing procedures (Calado et al. 2009; Manley et al. 2014) may be the reason, while for other cases, the increased mortality can be ascribed to different factors like disease associated (Blindheim et al. 2015; Richards et al. 2015a, b; Solomieu et al. 2015), sometimes genetic, and mostly environmental and nutritional (O'Connor et al. 2012; Kousoulaki et al. 2015).

With the help of metabolomics-derived functional metabolite data, various issues which have been discussed above could be solved and could even assist in developing some curative measures for betterment of hatchery production. It has already been reported that metabolomics has been and is being employed to explore biology of aquatic larvae. Recent encroachment has been made by Papan and Chen 2009 et al., with the research made on developmental biology of *Danio rerio* (zebrafish). During embryogenesis, the zebrafish larval metabolome is found to be changing dynamically. This data can be used for the developmental stage prediction and can expose change in gene expression and thereby protein production (Soanes et al. 2011; Huang et al. 2013; Raterink et al. 2013). Though the research work is focused on zebrafish which is not a commercially cultured species, this research throws light on potential of using metabolomics for various applications to study initial life phases of many other commercially viable aquatic organisms.

In 2015, Young et al. (2015a) employed metabolomics for the analysis of developmental biology of *Perna canaliculus* (mussel) larvae. Rapidly developing and sluggish larvae of same culture were collected at different time intervals to analyze their metabolite profiles using gas chromatography–mass spectrometry (GC–MS) technique (Young et al. 2015b). Using the univariate and multivariate methods, empirical Bayesian analysis of metabolites (EBAM), supervised projection to latent squares discriminant analysis (PLS-DA), volcano plot analysis, significant analysis of metabolites (SAM), and better biomarkers were identified for separating the two groups of larvae. Using the above methods, different levels of various metabolites can be used as biomarkers for estimating the quality of mussel larvae. Some of those metabolites are myristic acid, alanine, succinic acid, glycine and pyroglutamic acid, etc.

In-depth analysis regarding the role of these metabolites provides information about the contribution of different metabolic pathways in the development of mussel larva. There are diverse pathways involved in this, and some of them are osmotic regulation, cell–cell communication, energy metabolism, and immune function. This research not only demonstrates the capability of using metabolomics for evaluating the quality of mollusk larvae but also hints the potential of metabolomics to provide analytical representations of larval development and perceptions about pathways involved. It would be a great advantage and achievement to the hatchery industry if a tool kit is developed for evaluation of larval status in the entire rearing process.

Metabolomics has even promoted other areas of developmental biology concerning disorders of reproductive systems (Courant et al. 2013). It has helped in understanding and estimating quality of oocyte and even predicts the viability of embryos (Bertoldo et al. 2013; Cortezzi et al. 2013). In 2015, Kumar et al. have identified biomarkers for estimating the fertility of sperm. Metabolomics is expected to be the most promising tool in evaluating and advancing not just hatchery production technology but various streams of biotechnology.

8.3.2 Nourishment Applications

In aquaculture, research is being emphasized on nutrition for the cultured species health improvement to upsurge the growth and thereby improving production. Supplying appropriate quantity of good quality nutrition will have comprehensive implications on other rearing factors like augmentation of feedstock and quality of sperm/ovum, discreet performance development, prevention of disease, developing reliable, and fine-quality feed substitutes.

Newly evolved aquaculture species have unfamiliar dietary necessities. So, there exists a critical necessity to identify important nutritional requirements for the sustenance of these organisms. There is a very less understanding of interactions between environment, health, and nutrition due to their complexity in both established and unknown species. As of now there is inadequate information of nutritional requirements and dietary inclinations of larvae, which is hindering upsurge production yields. Hence, there exists a significant opportunity to understand the nutritional requirements in various phases of their life cycle. Metabolomics provides an exclusive platform to evaluate the metabolome differences for different dietary regimes and helps in developing novel enhanced nutritional management.

Metabolomic methodologies have already been applied for evaluating beneficial and adverse effects of lack of food in various organisms like salmon, trout, and mussels (Kullgren et al. 2010; Thunathong et al. 2012; Cipriano et al. 2015). In abalone and sea bream, analysis of interactions between diseases, diet, and environment was done (Silva et al. 2014). Presently, metabolomic research on nutrition of aquatic fauna is in budding stage. So there exists a huge scope for identifying different food regimes of various less cultivable aquatic fauna and can even be commercialized.

8.3.3 Clinical Pathology and Immunological Applications

One of the major challenges for aqua culturists is to manage the health and diseases meticulously. When all the functions in a biological system are carried out optimally, it is said to be healthy. Any imbalance in this is considered as disease. One of the common causes of a disease is through a pathogen (bacteria, virus, or parasite). Pathogens can spread through compromise in sanitary measures and through pathogen-infected feed and water. Transmission from wild stocks and possibly through infected animals from other farms. Overstocking of animals results in poor hygienic conditions, and lack of adequate nutrition further increases the risk of disease. China, which provides for two-thirds of world's aquaculture production, loses approximately 20% of its produce due to diseases (Li et al. 2011). Incidences of disease outbreaks causing huge loss of population resulting in collapse of industry with severe socioeconomic impacts are known, for example, Herpes virus outbreak in oysters and white spot syndrome virus in shrimp (Sanchez-Martınez et al. 2007; Peeler et al. 2012).

Research suggests that a number of subtle, interrelated metabolic imbalances can be responsible for onset and development of a particular disease condition. A number of factors such as genetic, nutritional, or environmental can affect the immune system of the organism making it more susceptible to the pathogen. A pathogen may not cause disease until the host organism experiences stress causing shift in equilibrium, or it may immediately result in disease by overwhelming its immune system. The relationship between imbalances in metabolic networks and proliferation of disease can be obtained through metabolomics. Metabolomics provides insights into effect of pathogen exposure, mechanism of resistance, and efficacy of disease treatment. The field of metabolomics with applications related to host-pathogen interactions in aquaculture research is developing rapidly. Metabolomic studies revealed the common imbalances caused from pathogen exposure to be in host energy metabolism, cell signaling, osmotic regulation, oxidative stress, and respiratory processes.

Recent metabolomics-based studies on the immunological responses of bivalves to infections with various bacterial pathogens revealed that the molecular response mechanisms of the host depends on bacterial strain (Ji et al. 2013; Wu et al. 2013a; Liu et al. 2013a, 2014a, b), the host species and pedigree (Liu et al. 2013b; Wu et al. 2013b), the sex of the host (Ellis et al. 2014; Liu et al. 2014a), and between different tissues (Ji et al. 2013; Liu et al. 2014b). These studies highlight the requirement of specific disease management strategies for particular conditions. Other examples of metabolomic studies include evaluation of effect of superintensive aquaculture systems on the health of shrimp (Schock et al. 2013), identification of biomarkers involved in the host responses of shrimp white spot syndrome virus (Liu et al. 2015), study of interaction of nutrition and temperature on withering syndrome disease in abalone (Rosenblum et al. 2005, 2006), characterization of metabolic demands of hepatic tumors in flatfish (Southam et al. 2008), identification of simple strategies for potentiating the sensitivity of aquatic pathogens to antibiotics (Su et al. 2015), discovering metabolite biomarkers associated with enhanced host defense against bacterial infection in carp (Guo et al. 2014), revealing the biochemical mechanisms underlying the efficacy of bacterial vaccines in fish (Guo et al. 2015), identification of metabolic responses of crabs to vibriosis (Schock et al. 2010), enhancing the health of cobia through dietary manipulation (Schock et al. 2012), identification of simple treatments for increasing disease resistance of tilapia against bacterial pathogens (Peng et al. 2015), and determining the mechanism of action (Ma et al. 2015) and developing nutritional and non-nutritional treatments for enhancing survival of diseased tilapia (Cheng et al. 2014; Zhao et al. 2015).

A study conducted by Schock et al. (2013) to assess health parameters in cultured marine invertebrates is a good example of metabolomic application. NMR-based metabolomics was used in this study to monitor the shrimp from nursery to harvest for health factors. A superintensive aquaculture system with minimal water exchanges was used to grow the shrimp. This system provides increased production and reduced disease susceptibility. It also results in enhanced water quality due to reduction of waste products. In addition a biofloc system was used to promote growth of beneficial bacteria. Shrimp samples were collected every week throughout nursery and grow-out raceways. To evaluate experimental biases in extraction process and timing, a robust quality control procedure was performed with the use of standard reference material. Metabolomic studies require such protocols for verification of data collection procedures. NMR spectra of samples from nursery and raceway phases were compared. The results showed important differences which can be concluded to be stressful conditions in shrimp. Various biomarkers for stress were found from these studies. These include iosine and trehalose. It was observed that increase in total ammonia nitrogen, reduced feeding period and trnasition of shrimp from nursery to raceways were the three main events related to stress. Thus, specific areas which needed focus to improve production efficiency were identified through metabolomic investigations. The results are obtained faster and at reduced cost as compared to traditional approaches.

Another study conducted by Guo et al. (2014) made use of GC–MS platform to compare metabolite profiles of crucian carp (*Carassius auratus*) to assess their ability to survive infections caused by the bacterium *Edwardsiella tarda*. Various biomarkers that signal infection involved in immune response in the host were identified. For metabolomics data analysis, web-based MetaboAnalyst 2.0 pipeline, and to identify biochemical pathways that indicate infections, the pathway library of zebrafish (surrogate for carp) were used. Increased levels of palmitic acid and decreased levels of D-mannose were identified as biomarkers for this infection. These correspond to the increase in unsaturated fatty acid biosynthesis pathway and decrease in fructose and mannose metabolism pathways. It can be concluded from these results that the early signs of pathogens and infections could be effectively identified through metabolomic studies. This knowledge can be used to mitigate the infection at the onset. It can also be used to select optimal conditions for growth of cultured organism. Providing a wide scan of physiological activities and identification of areas where problems maybe encountered (production issues such as health threats) is considered to be the stronghold of metabolomics approach.

Exposure of aquatic organisms to immuno-toxic contaminants, which suppress the immune system, enhances the deleterious effects of pathogens and viruses (Girón-Pérez 2010; Morley 2010). Aquaculture industry in many regions suffers due to poor quality of water resulting from release of various organic and synthetic pollutants that are serious threat to living organisms (Li et al. 2011), into aquatic ecosystems as a direct consequence of rapid industrialization and urbanization. The biological fate and effects of many new and emerging contaminants is not known completely, and such contaminants are increasing in number. Metabolomics is progressively used to study mechanisms of toxicity and to develop environmental contamination assessment methods using fish and shell fish.

Metabolomics-based studies have recently proved to be highly appreciable for applications in:

(a) Identification of physiological responses of various marine crustaceans in response to agricultural runoff, heavy metals, and endocrine disruptors (Wu et al. 2013b; Hanana et al. 2014; Ji et al. 2014, 2015a, 2015b; Leonard et al. 2014; Zhou et al. 2014; Campillo et al. 2015; Song et al. 2016).
(b) Characterization impact of pesticide exposure in carps (Kokushi et al. 2015).
(c) Identification of novel and highly accurate biomarker species of clams for monitoring environmental contamination (Ji et al. 2015c).
(d) Assessment of petrochemical contamination at industrialized sites and their influence caged mussels (Fasulo et al. 2012; Cappello et al. 2013, 2015).
(e) Detection of marine locations that have possible multi-contaminant sediment loadings (Watanabe et al. 2015).
(f) Development of noninvasive strategies with the main objective of pollution assessment by profiling metabolites in the excreted mucus of fish skin.

In the times to come, metabolomics has a great scope in being used as a tool for health monitoring of various organisms. It has the capability of providing better methods for pollution estimation and can even augment performance ecotoxicological evaluations. This field has a lot to do in areas like biosafety and food safety, capable of throwing light on factors related to diseases which are more common for cultivated aquatic organisms.

8.3.4 Post-harvest Quality Control

In aquaculture industry, the prime task is to produce, preserve, and deliver a good-quality product from farm to the customer. Different factors are considered while classifying the product quality. Nutritional value is dependent on the presence of minerals, good percentage of polyunsaturated fatty acids like omega-3 fatty acids, and highly digestible proteins and vitamins. Based on additional compositional features like fat to lean ratio, water percentage, etc., the quality of meat may be defined. The acceptable factors of customer profundity may include appearance, odor, firmness, juiciness, softness, and savor (Nollet and Toldra 2010). However, the product quality is multifarious; still there is much to learn. To get better nutritional and compositional qualities, the culture conditions are optimized before harvesting the product from culture. The product quality and food safety are affected by post-product recovery processes like shipping, storage, and packing.

As metabolites are involved in different aspects like nutritional value, flavor, aroma, and even in the process of degradation, metabolomics provides an exciting research opportunity for food quality estimation and helps in designing novel approaches for food quality conservation. A swift upsurge has been observed in the food quality analysis, post-harvest processing, and preservation methods these days because of the metabolomics supplement (Rubert et al. 2015; Trimigno et al. 2015; Kim et al. 2016). Metabolomic study of frozen fish revealed that few nutrients, savors, and aromas are being degraded in the process for storage (Castejon et al. 2010; Leduc et al. 2012; Shumilina et al. 2015). Investigating association of culture conditions, post-harvest quality, and preservation (Savorani et al. 2010; Picone et al. 2011) and designing quick analysis methods and in the process of long-term cold storage of fish, there is a need to identify potent biomarkers to test the fish freshness and quality (Duflos et al. 2010; Leduc et al. 2012; Heude et al. 2014). To improve the shrimp meat quality and cost even after shipping, prominent research has to be done on the metabolic factors being influenced in the process of storage (Chen et al. 2015). In salmon preservation irradiation is an important procedure done to eliminate the infection-causing microbes, to reduce storage effects, and to improve product safety. During this process metabolites in the salmon meat may vary. So a study of metabolic variation can prove beneficial (Villa et al. 2013; Castejon et al. 2016).

Heude et al. devised a rapid and simple method to assess freshness and quality of fish. This method is based on indicative metabolite quantification using high-resolution magic angle spinning (1H HR-MAS) NMR spectroscopy. The well-known indicators of freshness and quality, k value (measure of spontaneous disintegration of cells and tissues after death), and trimethylamine nitrogen (TMA-N, indicative of bacterial spoilage), respectively, are quantified in this method in fish samples stored at 0 °C. Metabolite concentrations in four commercially important fish species – sea bream, sea bass, trout, and red mullet – were measured to identify these parameters. Similar results were obtained from NMR approach as compared to traditional methods. However, with a mean time of 40 min per sample and facility to use unprocessed fish without prior extraction procedures, NMR has an added advantage over traditional analytical methods that require time-consuming extraction procedures. NMR approach also requires small sample sizes of 10–15 mg which can easily be obtained from biopsy.

Such NMR approach was also used to test the effects of irradiation on cold-smoked Atlantic salmon as a means to prevent bacterial and parasite infections (Villa et al. 2013). The muscle samples from live fish after different irradiation treatments (0, 1 and 4 kGy) and non-irradiated controls were taken and NMR spectra obtained. The results were statistically analyzed using principal component analysis. To identify the differences in degradation of fish samples during storage, ANOVA was used. Compounds like creatine, trimethylamine oxide, phosphoryl choline, and glycerophosphorylcholine were identified as indicators of irradiation treatment from these studies. This approach provides for nondestructive analysis to assess food processing and physiological changes in fish due to storage.

Identification of origin of the product or traceability is an important requirement of food quality control. The producers may be asked to trace the product and specify the culture methods to ensure food safety and prevent fraud. Protection of product from low-quality produce of unknown source is a grave concern faced by farmers. Metabolomics-based approaches are used to devise superior methods for food authentication and traceability (reviewed by Castro-Puyana and Herrero 2013; Cubero-Leon et al. 2014). Examples of application of metabolomics include classification of wild-caught and cultured salmon and sea bass and exposure of fraudulent labelling of seafood products (Aursand et al. 2007, 2009; Mannina et al. 2012) and differentiation of wild and farmed sea bream which are marketed as same high-quality product but have different qualities (Melis et al. 2014), distinguishing dried, salted mullet roe (in Sardinia, Italy) processed using special methods from locally produced roe.

Use of metabolomics in traceability is demonstrated by the study done by Locci et al. 2011. In the study, salted and dried mullet roe samples and local roe samples were taken, and their 1H-NMR spectra was used to obtain the knowledge of their metabolite markers. Using the technique different metabolites from different mullet sources that are stored and processed differently were identified. This study shows that metabolomic approach can be used not only to trace the origin of fish samples, without using DNA or protein analysis, but also it allows customization of the manufacturing and processing style of this traditional Italian artisanal fishery product. Therefore, metabolomic approach can have an efficient role in providing traceability and authentication in current global food markets.

8.4 Conclusion

Environmental metabolomics are thus providing insights into the interactions of marine organisms with their niche. Major advantages in employing the approach lie in the fact that the function and health of the marine organisms can be assessed at the molecular level. Metabolomic studies in marine provide information on the response of organisms to abiotic and biotic and has wide applications in the marine sciences including toxic assessments and prevention strategy developments.

Acknowledgments The authors are thankful to the Science and Engineering Research Board, Government of India, for support in the form of Early Career Research Award – National Post Doctoral Fellowship to CSM (Grant No. PDF/2016/001239) and NMN (Grant No. PDF/2016/000190).

References

Allan G, Burnell G (eds) (2013) Advances in aquaculture hatchery technology. Woodhead Publishing Limited, Oxford

Aursand M, Standal IB, Axelson DE (2007) High-resolution 13Cnuclear magnetic resonance spectroscopy pattern recognition of fish oil capsules. J Agric Food Chem 55(1):38–47

Aursand M, Standal IB, Prael A, McEvoy L, Irvine J, Axelson DE (2009) 13C NMR pattern recognition techniques for the classification of Atlantic salmon (*Salmo salar L.*) according to their wild, farmed, and geographical origin. J Agric Food Chem 57(9):3444–3451

Bennett D (2005) Growing pains for metabolomics. Scientist 19(8):25–28. http://www.the-scientist.com/article/display/15427/

Bertoldo MJ, Nadal-Desbarats L, Gerard N, Dubois A, Holyoake PK, Grupen CG (2013) Differences in the metabolomic signatures of porcine follicular fluid collected from environments associated with good and poor oocyte quality. Reproduction 146(3):221–231

Blindheim S, Nylund A, Watanabe K, Plarre H, Erstad B, Nylund S (2015) A new aquareovirus causing high mortality infarmed Atlantic halibut fry in Norway. Arch Virol 160(1):91–102

Calado R, Vitorino A, Reis A, Lopes da Silva T, Dinis MT (2009) Effect of different diets on larval production, quality and fatty acid profile of the marine ornamental shrimp *Lysmata amboinensis* (De Man, 1888), using wild larvae as a standard. Aquac Nutr 15(5):484–491

Campillo JA, Sevilla A, Albentosa M, Bernal C, Lozano AB, Canovas M et al (2015) Metabolomic responses in caged clams, *Ruditapes decussatus*, exposed to agricultural and urban inputs in a Mediterranean coastal lagoon (*Mar Menor*, SE Spain). Sci Total Environ 524:136–147

Cappello T, Mauceri A, Corsaro C, Maisano M, Parrino V, Lo Paro G et al (2013) Impact of environmental pollution on caged mussels *Mytilus galloprovincialis* using NMR based metabolomics. Mar Pollut Bull 77(1–2):132–139

Cappello T, Maisano M, Giannetto A, Parrino V, Mauceri A, Fasulo S (2015) Neurotoxicological effects on marine mussel *Mytilus galloprovincialis* caged at petrochemical contaminated areas (eastern Sicily, Italy): 1H NMR and immunohistochemical assays. Comp Biochem Physiol Part C: Toxicol Pharmacol 169:7–15

Castejon D, Villa P, Calvo MM, Santa-Marıa G, Herraiz M, Herrera A (2010) 1H-HRMAS NMR study of smoked Atlantic salmon (*Salmo salar*). Magn Reson Chem 48(9):693–703

Castejon D, Garcıa-Segura JM, Herrera A, Cambero MI (2016) NMR-detection of methylamine compounds in Atlantic salmon (*Salmo salar*) subjected to E-beam irradiation. Food Control 60:455–460

Castro-Puyana M, Herrero M (2013) Metabolomics approaches based on mass spectrometry for food safety, quality and traceability. Trends Anal Chem 52:74–87

Chen S, Zhang C, Xiong Y, Tian X, Liu C, Jeevithan E et al (2015) A GC–MS-based metabolomics investigation on scallop (*Chlamys farreri*) during semi-anhydrous living-preservation. Innov Food Sci Emerg Technol 31:185–195

Cheng ZX, Ma YM, Li H, Peng XX (2014) N-acetylglucosamine enhances survival ability of tilapias infected by *Streptococcus iniae*. Fish Shellfish Immunol 40(2):524–530

Cipriano RC, Smith ML, Vermeersch KA, Dove AD, Styczynski MP (2015) Differential metabolite levels in response to spawning-induced in appetence in Atlantic salmon Salmo salar. Comp Biochem Physiol Part D: Genom Proteom 13:52–59

Clasen B, Loro VL, Murussi CR, Tiecher TL, Moraes B, Zanella R (2018) Bioaccumulation and oxidative stress caused by pesticides in *Cyprinus carpio* reared in a rice-fish system. Sci Total Environ 626:737–743. https://doi.org/10.1016/j.scitotenv.2018.01.154

Collette TW, Teng Q, Jensen KM, Kahl MD, Makynen EA, Durhan EJ (2010) Impacts of an anti-androgen and an androgen/anti-androgen mixture on the metabolite profile of male fathead minnow urine. Environ Sci Technol 44(17):6881–6886. https://doi.org/10.1021/es1011884

Cortezzi SS, Cabral EC, Trevisan MG, Ferreira CR, Setti AS, Braga DPDAF et al (2013) Prediction of embryo implantation potential by mass spectrometry fingerprinting of the culture medium. Reproduction 145(5):453–462

Courant F, Antignac J-P, Monteau F, Le Bizec B (2013) Metabolomics as a potential new approach for investigating human reproductive disorders. J Proteome Res 12(6):2914–2920

Cubero-Leon E, Penalver R, Maquet A (2014) Review on metabolomics for food authentication. Food Res Int 60:95–107

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE et al (2014) Diet rapidly and reproducibly alters the human gut microbiome. Nature 505:559–563. https://doi.org/10.1038/nature12820

Dove ADM, Leisen J, Zhou MS, Byrne JJ, Lim-Hing K, Webb HD et al (2012) Biomarkers of whale shark health: a metabolomic approach. PLoS One 7(11):e49379. https://doi.org/10.1371/journal.pone.0049379

Duflos G, Leduc F, N'Guessan A, Krzewinski F, Kol O, Malle P (2010) Freshness characterisation of whiting (*Merlangius merlangus*) using an SPME/GC/MS method and a statistical multivariate approach. J Sci Food Agric 90:2568–2575

Egan S, Gardiner M (2016) Microbial dysbiosis: rethinking disease in marine ecosystems. Front Microbiol 7:991. https://doi.org/10.3389/fmicb.2016.00991

Ekman DR, Teng Q, Jensen KM, Martinovic D, Villeneuve DL, Ankley GT, Collette TW (2007) NMR analysis of male fathead minnow urinary metabolites: a potential approach for studying impacts of chemical exposures. Aquat Toxicol 85(2):104–112. https://doi.org/10.1016/j.aquatox.2007.08.005

Ekman DR, Teng Q, Villeneuve DL, Kahl MD, Jensen KM, Durhan EJ et al (2008) Investigating compensation and recovery of fathead minnow (*Pimephales promelas*) exposed to 17-ethynylestradiol with metabolite profiling. Environ Sci Technol 42(11):4188–4194. https://doi.org/10.1021/es8000618

Ekman DR, Teng Q, Villeneuve DL, Kahl MD, Jensen KM, Durhan EJ et al (2009) Profiling lipid metabolites yields unique information on sex- and time-dependent responses of fathead minnows (*Pimephales promelas*) exposed to 17-ethynylestradiol. Metabolomics 5(1):22–32. https://doi.org/10.1007/s11306-008-0138-y

Ellis RP, Spicer JI, Byrne JJ, Sommer U, Viant MR, White DA et al (2014) 1H NMR metabolomics reveals contrasting response by male and female mussels exposed to reduced sea water pH, increased temperature, and a pathogen. Environ Sci Technol 48(12):7044–7052

Fasulo S, Iacono F, Cappello T, Corsaro C, Maisano M, D'Agata A et al (2012) Metabolomic investigation of *Mytilus galloprovincialis* (Lamarck 1819) caged in aquatic environments. Ecotoxicity Environ Saf 84:139–146. https://doi.org/10.1016/j.ecoenv.2012.07.001

Girón-Pérez MI (2010) Relationships between innate immunity in bivalve molluscs and environmental pollution. Invertebr Surviv J 7:149–156

Guo C, Huang X-Y, Yang M-J, Wang S, Ren S-T, Peng X-X (2014) GC/MS-based metabolomics approach to identify biomarkers differentiating survivals from death in crucian carps infected by *Edwardsiella tarda*. Fish Shellfish Immunol 39(2):215–222

Guo C, Peng B, Song M, Wu CW, Yang MJ, Zhang JY et al (2015) Live *Edwardsiella tarda* vaccine enhances innate immunity by metabolic modulation in zebrafish. Fish Shellfish Immunol 47(2):664–673

Hamdi C, Balloi A, Essanaa J, Crotti E, Gonella E et al (2011) Gut microbiome dysbiosis and honeybee health. J Appl Entomol 135:524–533. https://doi.org/10.1111/j.1439-0418.2010.01609.x

Hamre K, Yufera M, Rønnestad I, Boglione C, Conceicao LE, Izquierdo M (2013) Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. Rev Aquac 5(s1):S26–S58

Hanana H, Simon S, Kervarec N, Cerantola S (2014) Evaluation of toxicological effects induced by tributyltin in clam *Ruditapes decussatus* using high-resolution magic angle spinning nuclear magnetic resonance spectroscopy: study of metabolic responses in heart tissue and detection of a novel metabolite. Toxicol Rep 1:777–786

Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M et al (2012) ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature 487:477–481. https://doi.org/10.1038/nature11228

Heintz C, Mair W (2014) You are what you host: microbiome modulation of the aging process. Cell 156:408–411. https://doi.org/10.1016/j.cell.2014.01.025

Heude C, Lemasson E, Elbayed K, Piotto M (2014) Rapid assessment of fish freshness and quality by 1H HR-MAS NMR spectroscopy. Food Anal Methods 8(4):907–915

Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK (2011) Understanding the role of gut microbiome–host metabolic signal disruption in health and disease. Trends Microbiol 19:349–359. https://doi.org/10.1016/j.tim.2011.05.006

Huang SM, Xu F, Lam SH, Gong Z, Ong CN (2013) Metabolomics of developing zebra fish embryos using gas chromatography-and liquid chromatography-mass spectrometry. Mol BioSyst 9(6):1372–1380

Ji C, Wu H, Wei L, Zhao J, Wang Q, Lu H (2013) Responses of *Mytilusgallo provincialis* to bacterial challenges by metabolomics and proteomics. Fish Shellfish Immunol 35(2):489–498

Ji C, Wei L, Zhao J, Wu H (2014) Metabolomic analysis revealed that female mussel *Mytilusgallo provincialis* was sensitive to bisphenol a exposures. Environ Toxicol Pharmacol 37:844–849

Ji C, Wu H, Zhou M, Zhao J (2015a) Multiple biomarkers of biological effects induced by cadmium in clam *Ruditapes philippinarum*. Fish Shellfish Immunol 44(2):430–435

Ji C, Wang Q, Wu H, Tan Q, Wang W-X (2015b) A metabolomics investigation of the effects of metal pollution in oysters *Crassostrea hongkongensis*. Mar Pollut Bull 90(1–2):317–322

Ji C, Cao L, Li F (2015c) Toxicological evaluation of two pedigrees of clam *Ruditapes philippinarum* as bioindicators of heavy metal contaminants using metabolomics. Environ Toxicol Pharmacol 39(2):545–554

Jones OAH, Dondero F, Viarengo A, Griffin JL (2008) Metabolic profiling of *Mytilusgallo provincialis* and its potential applications for pollution assessment. Mar Ecol Prog Ser 369:169–179. https://doi.org/10.3354/meps07654

Jordan KW, Nordenstam J, Lauwers GY, Rothenberger DA, Alavi K, Garwood M, Cheng LL (2009) Metabolomic characterization of human rectal adenocarcinoma with intact tissue magnetic resonance spectroscopy. Dis Colon Rectum 52(3):520–525. https://doi.org/10.1007/DCR.0b013e31819c9a2c

Kim S, Kim J, Yun EJ, Kim KH (2016) Food metabolomics: from farm to human. Curr Opin Biotechnol 37:16–23

Kokushi E, Uno S, Harada T, Koyama J (2012) 1H NMR-based metabolomics approach to assess toxicity of bunker a heavy oil to freshwater carp, *Cyprinus carpio*. Environ Toxicol 27(7):404–414. https://doi.org/10.1002/tox.20653. PMID:20882592

Kokushi E, Uno S, Pal S, Koyama J (2015) Effects of chlorpyrifos on the metabolome of the freshwater carp, *Cyprinus carpio*. Environ Toxicol 30(3):253–260

Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK et al (2012) Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 150:470–480. https://doi.org/10.1016/j.cell.2012.07.008

Kousoulaki K, Bogevik AS, Skiftesvik AB, Jensen PA, Opstad I (2015) Marine raw material choice, quality and weaning performance of Ballan wrasse (*Labrus bergylta*) larvae. Aquac Nutr 21(5):644–654

Kullgren A, Samuelsson LM, Larsson DG, Björnsson BT, Bergman EJ (2010) A metabolomics approach to elucidate effects of food deprivation in juvenile rainbow trout (*Oncorhynchus mykiss*). Am J Physiol Regul Integr Comp Physiol 299(6):R1440–R1448

Kullgren A, Jutfelt F, Fontanillas R, Sundell K, Samuelsson L, Wiklander K et al (2013) The impact of temperature on the metabolome and endocrine metabolic signals in Atlantic salmon (*Salmo salar*). Comput Biochem Physiol Part A Mol Integr Physiol 164(1):44–53. https://doi.org/10.1016/j.cbpa.2012.10.005

Kumar A, Kroetsch T, Blondin P, Anzar M (2015) Fertility-associated metabolites in bull seminal plasma and blood serum:1H nuclear magnetic resonance analysis. Mol Reprod Dev 82(2):123–131

Kwon YK, Jung YS, Park JC, Seo J, Choi MS, Hwang GS (2012) Characterizing the effect of heavy metal contamination on marine mussels using metabolomics. Mar Pollut Bull 64(9):1874–1879. https://doi.org/10.1016/j.marpolbul.2012.06.012. PMID:22770532

Leduc F, Krzewinski F, Le Fur B, N'Guessan A, Malle P, Kol O et al (2012) Differentiation of fresh and frozen/thawed fish, European sea bass (*Dicentrarchus labrax*), gilthead seabream *(Sparus aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*), using volatile compounds by SPME/GC/MS. J Sci Food Agric 92:2560–2568

Leonard JA, Cope WG, Barnhart MC, Bringolf RB (2014) Metabolomic, behavioral, and reproductive effects of the aromatase inhibitor fadrozole hydrochloride on the unionid mussel *Lampsilis fasciola*. Gen Comp Endocrinol 206:213–226

Li X, Li J, Wang Y, Fu L, Fu Y, Li B et al (2011) Aquaculture industry in China: current state, challenges, and outlook. Rev Fish Sci 19(3):187–200

Liu X, Ji C, Zhao J, Wu H (2013a) Differential metabolic responses of clam *Ruditapes philippinarum* to *Vibrio anguillarum* and *Vibrio splendidus* challenges. Fish Shellfish Immunol 35(6):2001–2007

Liu X, Zhao J, Wu H, Wang Q (2013b) Metabolomic analysis revealed the differential responses in two pedigrees of clam *Ruditapes philippinarum* towards *Vibrio harveyi* challenge. Fish Shellfish Immunol 35(6):1969–1975

Liu X, Sun H, Wang Y, Ma M, Zhang Y (2014a) Gender-specific metabolic responses in hepatopancreas of mussel *Mytilus galloprovincialis* challenged by *Vibrio harveyi*. Fish Shellfish Immunol 40(2):407–413

Liu X, Ji C, Zhao J, Wang Q, Li F, Wu H (2014b) Metabolic profiling of the tissue-specific responses in mussel *Mytilus galloprovincialis* towards *Vibrio harveyi* challenge. Fish Shellfish Immunol 39(2):372–377

Liu PF, Liu QH, Wu Y, Jie H (2015) A pilot metabolic profiling study in hepatopancreas of *Litopenaeus vannamei* with white spot syndrome virus based on 1H NMR spectroscopy. J Invertebr Pathol 124:51–56

Locci E, Piras C, Mereu S, Marincola FC, Scano P (2011) 1HNMR metabolite fingerprint and pattern recognition of mullet (*Mugil cephalus*) bottarga. J Agric Food Chem 59(17):9497–9505

Lv X, Yang Y, Tao Y, Jiang Y, Chen B, Zhu X et al (2017) A mechanism study on toxicity of graphene oxide to *Daphnia magna*: Direct link between bioaccumulation and oxidative stress. Environ Pollut 234. https://doi.org/10.1016/j.envpol.2017.12.034

Ma YM, Yang MJ, Wang S, Li H, Peng XX (2015) Liver functional metabolomics discloses an action of l-leucine against *Streptococcus iniae* infection in tilapias. Fish Shellfish Immunol 45(2):414–421

Manley CB, Rakocinski CF, Lee PG, Blaylock RB (2014) Stocking density effects on aggressive and cannibalistic behaviors in larval hatchery-reared spotted sea trout, *Cynoscion nebulosus*. Aquaculture 420–421:89–94

Mannina L, Sobolev AP, Capitani D (2012) Applications of NMR metabolomics to the study of foodstuffs: truffle, kiwifruit, lettuce, and sea bass. Electrophoresis 33(15):2290–2313

Martinovic-Weigelt D, Ekman DR, Villeneuve DL, James CM, Teng Q, Collette TW, Ankley GT (2012) Fishy aroma of social status: urinary chemo-signalling of territoriality in male fathead minnows (*Pimephales promelas*). PLoS One 7(11):e46579. https://doi.org/10.1371/journal.pone.0046579

Melis R, Cappuccinelli R, Roggio T, Anedda R (2014) Addressing market place gilthead sea bream (*Sparus aurata L.*) differentiation by 1H NMR-based lipid fingerprinting. Food Res Int 63(B):258–264

Moloney RD, Desbonnet L, Clarke G, Dinan TG, Cryan JF (2014) The microbiome: stress, health and disease. Mamm Genome 25:49–74. https://doi.org/10.1007/s00335-013-9488-5

Morley NJ (2010) Interactive effects of infectious diseases and pollution in aquatic molluscs. Aquat Toxicol 96(1):27–36

Morrison N, Bearden D, Bundy JG, Collette T, Currie F, Davey MP et al (2007) Standard reporting requirements for biological samples in metabolomics experiments: environmental context. Metabolomics 3(3):203–210. https://doi.org/10.1007/s11306-007-0067-1

Nagato EG, D'eon JC, Lankadurai BP, Poirer DG, Reiner EJ, Simpson AJ, Simpson MJ (2013) 1H NMR-based metabolomics investigation of *Daphnia magna* responses to sub-lethal exposure to arsenic, copper and lithium. Chemosphere. https://doi.org/10.1016/j.chemosphere.2013.04.085

Nelson JR, Schwartz TS, Gohlke JM (2018) Influence of maternal age on the effects of seleno-l-methionine in the model organism Daphnia pulex under standard and heat stress conditions. Reprod Toxicol 75:1–9. https://doi.org/10.1016/j.reprotox.2017.11.001

Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W et al (2012) Host-gut microbiota metabolic interactions. Science 336:1262–1267. https://doi.org/10.1126/science.1223813

Nikinmaa M (2014) Chapter 2: What causes aquatic contamination? In: Nikinmaa M (ed) An introduction to aquatic toxicology. Academic, pp 19–39. https://doi.org/10.1016/B978-0-12-411574-3.00002-5. ISBN: 9780124115743. http://www.sciencedirect.com/science/article/pii/B9780124115743000025

Nollet LM, Toldra F (eds) (2010) Sensory analysis of foods of animal origin. CRC Press, Boca Raton

O'Connor S, Moltschaniwskyj N, Bolch CJ, O'Connor W (2012) Dietary influence on growth and development of flat oyster, *Ostrea angasi* (Sowerby, 1871), larvae. Aquac Res 43(9):1317–1327

Papan C, Chen L (2009) Metabolic fingerprinting reveals developmental regulation of metabolites during early zebrafish embryogenesis. OMICS J Integr Biol 13(5):397–405

Parolini M, Felice B, Ferrario C, Salgueiro-González N, Castiglioni S, Finizio A et al (2018) Benzoylecgonine exposure induced oxidative stress and altered swimming behavior and reproduction in *Daphnia magna*. Environ Pollut 232:236–244. https://doi.org/10.1016/j.envpol.2017.09.038

Peeler JE, Reese RA, Cheslett DL, Geoghegan F, Power A, Trush MA (2012) Investigation of mortality in Pacific oysters associated with Ostreid herpesvirus-1 Var in the Republic of Ireland in 2009. Prev Vet Med 105(1–2):136–143

Peng B, Ma YM, Zhang JY, Li H (2015) Metabolome strategy against *Edwardsiella tarda* infection through glucose enhanced metabolic modulation in tilapias. Fish Shellfish Immunol 45(2):869–876

Picone G, Engelsen SB, Savorani F, Testi S, Badiani A, Capozzi F (2011) Metabolomics as a powerful tool for molecular quality assessment of the fish *Sparus aurata*. Nutrients 3(2):212–227

Poynton HC, Taylor NS, Hicks J, Colson K, Chan SR, Clark C et al (2011) Metabolomics of microliter hemolymph samples enables an improved understanding of the combined metabolic and transcriptional responses of Daphnia magna to cadmium. Environ Sci Technol 45(8):3710–3717. https://doi.org/10.1021/es1037222

Purcell SW, Hair CA, Mills DJ (2012) Sea cucumber culture, farming and sea ranching in the tropics: progress, problems and opportunities. Aquaculture 368–369:68–81

Ralston-Hooper K, Hopf A, Oh C, Zhang X, Adamec J, Sepúlveda MS (2008) Development of GC×GC/TOF-MS metabolomics for use in ecotoxicological studies with invertebrates. Aquat Toxicol 88(1):48–52. https://doi.org/10.1016/j.aquatox.2008.03.002

Raterink RJ, van der Kloet FM, Li J, Wattel NA, Schaaf MJM, Spaink HP et al (2013) Rapid metabolic screening of early zebrafish embryogenesis based on direct infusion-nano ESIFTMS. Metabolomics 9(4):864–873

Richards GP, Watson MA, Needleman DS, Church KM, Häse CC (2015a) Mortalities of Eastern and Pacific oyster larvae caused by the pathogens *Vibrio coralliilyticus* and *Vibrio tubiashii*. Appl Environ Microbiol 81(1):292–297

Richards RG, Davidson AT, Meynecke J-O, Beattie K, Hernaman V, Lynam T et al (2015b) Effects and mitigations of ocean acidification on wild and aquaculture scallop and prawn fisheries in Queensland, Australia. Fish Res 161:42–56

Rosenblum ES, Viant MR, Braid BM, Moore JD, Friedman CS, Tjeerdema RS (2005) Effects of temperature on host-pathogen-drug interactions in red abalone, *Haliotis rufescens,* determined by 1H NMR metabolomics. Environ Sci Technol 40(22):7077–7084

Rosenblum ES, Tjeerdema RS, Viant MR (2006) Characterizing the metabolic actions of natural stresses in the California red abalone, *Haliotis rufescens* using 1H NMR metabolomics. Metabolomics 1(2):199–209

Rubert J, Zachariasova M, Hajslova J (2015) Advances in high resolution mass spectrometry based on metabolomics studies for food – a review. Food Addit Contam Part A 32(10):1685–1708

Russo R, Becker JM, Liess M (2018) Sequential exposure to low levels of pesticides and temperature stress increase toxicological sensitivity of crustaceans. Sci Total Environ 610-611:563–569. https://doi.org/10.1016/j.scitotenv.2017.08.073

Salze G, Craig SR, Smith BH, Smith EP, McLean E (2011) Morphological development of larval cobia *Rachycentron canadum and* the influence of dietary taurine supplementation. J Fish Biol 78(5):1470–1491

Samuelsson LM, Förlin L, Karlsson G, Adolfsson-Erici M, Larsson DGJ (2006) Using NMR metabolomics to identify responses of an environmental estrogen in blood plasma of fish. Aquat Toxicol 78(4):341–349. https://doi.org/10.1016/j.aquatox.2006.04.008

Samuelsson LM, Björlenius B, Förlin L, Larsson DGJ (2011) Reproducible 1H NMR-based metabolomic responses in fish exposed to different sewage effluents in two separate studies. Environ Sci Technol 45(4):1703–1710. https://doi.org/10.1021/es104111x. PMID:21261249

Sanchez-Martınez JG, Aguirre-Guzm an G, Mejıa-Ruız H (2007) White spot syndrome virus in cultured shrimp: a review. Aquac Res 38(13):1339–1354

Savorani F, Picone G, Badiani A, Fagioli P, Capozzi F, Engelsen SB (2010) Metabolic profiling and aquaculture differentiation of gilthead sea bream by 1H NMR metabonomics. Food Chem 120(3):907–914

Scharschmidt TC, Fischbach MA (2013) What lives on our skin: ecology, genomics and therapeutic opportunities of the skin microbiome. Drug Discov 10:e83–e89. https://doi.org/10.1016/j.ddmec.2012.12.003

Schock TB, Stancyk DA, Thibodeaux L, Burnett KG, Burnett LE, Boroujerdi AF et al (2010) Metabolomic analysis of Atlantic blue crab, *Callinectes sapidus*, hemolymph following oxidative stress. Metabolomics 6(2):250–262

Schock TB, Newton S, Brenkert K, Leffler J, Bearden DW (2012) An NMR-based metabolomic assessment of cultured cobia health in response to dietary manipulation. Food Chem 133(1):90–101

Schock TB, Duke J, Goodson A, Weldon D, Brunson J, Leffler JW et al (2013) Evaluation of Pacific white shrimp (*Litopenaeus vannamei*) health during a super intensive aquaculture Growout using NMR-based metabolomics. PLoS One 8(3):e59521

Shumilina E, Ciampa A, Capozzi F, Rustad T, Dikiy A (2015) NMR approach for monitoring postmortem changes in Atlantic salmon fillets stored at 0 and 4°C. Food Chem 184:12–22

Silva TS, da Costa AMR, LEC C~a, Dias JP, Rodrigues PML, Richard N (2014) Metabolic fingerprinting of gilt head sea bream (*Sparus aurata*) liver to track interactions between dietary factors and seasonal temperature variations. Peer J 2:e527

Soanes KH, Achenbach JC, Burton IW, Hui JPM, Penny SL, Karakach TK (2011) Molecular characterization of zebrafish embryogenesis via DNA microarrays and multiplatform time course metabolomics studies. J Proteome Res 10(11):5102–5117

Solomieu VB, Renault T, Travers MA (2015) Mass mortality in bivalves and the intricate case of the Pacific oyster, *Crassostrea gigas*. J Invertebr Pathol 131:2–10

Song Q, Chen H, Li Y, Zhou H, Han Q, Diao X (2016) Toxicological effects of benzo(a)pyrene, DDT and their mixture on the green mussel *Perna viridis* revealed by proteomic and metabolomic approaches. Chemosphere 144:214–224

Sørensen SR, Skov PV, Lauesen P, Tomkiewicz J, Bossier P, deSchryver P (2014) Microbial interference and potential control in culture of European eel (*Anguilla anguilla*) embryos and larvae. Aquaculture 426–427:1–8

Southam AD, Easton JM, Stentiford GD, Ludwig C, Arvanitis TN, Viant MR (2008) Metabolic changes in flatfish hepatic tumours revealed by NMR-based metabolomics and metabolic correlation networks. J Proteome Res 7(12):5277–5285

Spann N, Aldridge DC, Griffin JL, Jones OAH (2011) Size-dependent effects of low level cadmium and zinc exposure on the metabolome of the Asian clam, *Corbicula fluminea*. Aquat Toxicol 105(3–4):589–599. https://doi.org/10.1016/j.aquatox.2011.08.010

Stentiford GD, Viant MR, Ward DG, Johnson PJ, Martin A, Wenbin W et al (2005) Liver tumors in wild flatfish: a histopathological, proteomic, and metabolomic study. OMICS 9(3):281–299. https://doi.org/10.1089/omi.2005.9.281

Su YB, Peng B, Han Y, Li H, Peng XX (2015) Fructose restores susceptibility of multidrug-resistant *Edwardsiella tarda* to kanamycin. J Proteome Res 14(3):1612–1620

Taylor NS, Weber RJM, Southam AD, Payne TG, Hrydziuszko O, Arvanitis TN, Viant MR (2009) A new approach to toxicity testing in *Daphnia magna*: application of high throughput FT–ICR mass spectrometry metabolomics. Metabolomics 5(1):44–58. https://doi.org/10.1007/s11306-008-0133-3

Taylor NS, Weber RJM, White TA, Viant MR (2010) Discriminating between different acute chemical toxicities via changes in the *daphnid* metabolome. Toxicol Sci 118(1):307–317. https://doi.org/10.1093/toxsci/kfq247

Teng Q, Ekman DR, Huang W, Collette TW (2013) Impacts of 17 α-ethinyl estradiol exposure on metabolite profiles of zebrafish (*Danio rerio*) liver cells. Aquat Toxicol 130–131:184–191. https://doi.org/10.1016/j.aquatox.2013.01.011

Thunathong T, Francis DS, Senadheera SPSD, Jones PL, Turchini GM (2012) Short-term food deprivation before a fish oil finishing strategy improves the deposition of n-3 LC-PUFA, but not the washing-out of C18 PUFA in rainbow trout. Aquacult Nutr 18(4):441–456

Trimigno A, Marincola FC, Dellarosa N, Picone G, Laghi L (2015) Definition of food quality by NMR-based foodomics. Curr Opin Food Sci 4:99–104

Tuffnail W, Mills GA, Cary P, Greenwood R (2009) An environmental 1HNMR metabolomic study of the exposure of the marine mussel *Mytilus edulis to* atrazine, lindane, hypoxia and starvation. Metabolomics 5(1):33–43. https://doi.org/10.1007/s11306-008-0143-1

Uno S, Shintoyo A, Kokushi E, Yamamoto M, Nakayama K, Koyama J (2012) Gas chromatography–mass spectrometry for metabolite profiling of Japanese medaka (*Oryzias latipes*) juveniles exposed to malathion. Environ Sci Pollut Res 19(7):2595–2605. https://doi.org/10.1007/s11356-012-0834-z

Van Scoy AR, Anderson BS, Philips BM, Voorhees J, McCann M, De Haro H et al (2012) NMR-based characterization of the acute metabolic effects of weathered crude and dispersed oil in spawning top smelt and their embryos. Ecotoxicol Environ Saf 78:99–109. https://doi.org/10.1016/j.ecoenv.2011.11.009

Vandenbrouck T, Jones OAH, Dom N, Griffin JL, De Coen W (2010) Mixtures of similarly acting compounds in *Daphnia magna*: from gene to metabolite and beyond. Environ Int 36(3):254–268. https://doi.org/10.1016/j.envint.2009.12.006

Viant MR, Pincetich CA, Tjeerdema RST (2006) Metabolic effects of dinoseb, diazinon and esfenvalerate in eyed eggs and alevins of Chinook salmon (*Oncorhynchus tshawytscha*) determined by 1H NMR metabolomics. Aquat Toxicol 77(4):359–371. https://doi.org/10.1016/j.aquatox.2006.01.009. PMID: 16488491

Villa P, Castejon D, Herraiz M, Herrera A (2013) 1H-HRMASNMR study of cold smoked Atlantic salmon (*Salmo salar*)treated with E-beam. Magn Reson Chem 51(6):350–357

Wang C, Harwood JD, Zhang Q (2018) Oxidative stress and DNA damage in common carp (*Cyprinus carpio*) exposed to the herbicide mesotrione. Chemosphere 193:1080–1086. https://doi.org/10.1016/j.chemosphere.2017.11.148

Watanabe M, Meyer KA, Jackson TM, Schock TM, Johnson WE, Bearden DW (2015) Application of NMR-based metabolomics for environmental assessment in the Great Lakes using zebra mussel (*Dreissena polymorpha*). Metabolomics 11(5):1302–1315

Wu H, Wang W-X (2010) NMR-based metabolomic studies on the toxicological effects of cadmium and copper on green mussels *Perna viridis*. Aquat Toxicol 100(4):339–345. https://doi.org/10.1016/j.aquatox.2010.08.005

Wu H, Liu X, Zhao J, Yu J (2012a) Toxicological responses in halophyte *Suaeda salsa* to mercury under environmentally relevant salinity. Ecotoxicol Environ Saf 85:64–71. https://doi.org/10.1016/j.ecoenv.2012.03.016

Wu H, Liu X, Zhao J, Yu J, Pang Q, Feng J (2012b) Toxicological effects of environmentally relevant lead and zinc in halophyte *Suaeda salsa* by NMR based metabolomics. Ecotoxicology 21(8):2363–2371. https://doi.org/10.1007/s10646-012-0992-2

Wu H, Ji C, Wei L, Zhao J, Lu H (2013a) Proteomic and metabolomics responses in hepatopancreas of *Mytilus galloprovincialis* challenged by *Micrococcus luteus* and *Vibrio anguillarum*. J Proteome 94:54–67

Wu H, Ji C, Wang Q, Liu X, Zhao J, Feng J (2013b) Manila clam *Venerupis philippinarum* as a biomonitor to metal pollution. Chin J Oceanol Limnol 31(1):65–74

Yao H, Yu J, Zhou Y, Xiang Q, Xu C (2018) The embryonic developmental effect of sedaxane on zebrafish (*Danio rerio*). Chemosphere 197:299–305. https://doi.org/10.1016/j.chemosphere.2018.01.046

Young T, Alfaro AC, Villas-Boas S (2015a) Metabolic profiling of mussel larvae: effect of handling and culture conditions. Aquac Int. https://doi.org/10.1007/s10499-015-9945-0

Young T, Alfaro AC, Villas-Boas S (2015b) Identification of candidate biomarkers for quality assessment of hatchery-reared mussel larvae via GC/MS-based metabolomics. N Z J Mar Freshw Res 49(1):87–95

Zhao XL, Han Y, Ren ST, Ma YM, Li H, Peng XX (2015) L-proline increases survival of tilapias infected by *Streptococcus agalactiae* in higher water temperature. Fish Shellfish Immunol 44(1):33–42

Zhou J, Chen B, Cai Z (2014) Metabolomics-based approach for assessing the toxicity mechanisms of dibutyl phthalate to abalone (*Haliotis diversicolor supertexta*). Environ Sci Pollut Res 22(7):5092–5099

Chapter 9
Epiphytism in Seaweed Farming: Causes, Status, and Implications

Sunil Kumar Sahu, Kapilkumar N. Ingle, and Vaibhav A. Mantri

Contents

Abstract An epiphyte is a nonparasitic plant that dwells on another plant and has been well studied in terrestrial plants. However, in the marine ecosystem, these epiphytes thrive on algal thallus for their support and growth, and their infestation has a prime economic impediment in commercial cultivation. They usually belong to various groups, namely, bacteria, fungi, algae, ascidians, bryozoans, sponges, protozoa, molluscs, crustaceans, and other marine sessile organisms. The seaweed farming industry is currently growing at ca. 9% per annum, with global production of 31.2 million wet tons worth US$ 11.7 billion. The first report of an epiphytic outbreak in commercial farms of *Kappaphycus* in the 1970s caught the attention of

---

S. K. Sahu (✉)
BGI-Shenzhen, Shenzhen, China

State Key Laboratory of Agricultural Genomics, China National GeneBank, BGI-Shenzhen, Shenzhen, China
e-mail: sunilkumarsahu@genomics.cn

K. N. Ingle
Department of Ecology, University of Szeged, Szeged, Hungary

V. A. Mantri
Applied Phycology and Biotechnology Division, CSIR-Central Salt and Marine Chemicals Research Institute, Bhavnagar, India

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_9

several researchers on this devastating epiphyte which causes retarded growth and significant loss of stocking biomass, ultimately leading to the production of inferior quality of raw material. High-density planting in commercial farms is often responsible for recurring epiphytic infestations. Nevertheless, it is almost certain that the entire crop collapses due to epiphyte outbreak in a short span of time. Therefore, the lack of reliable global statistics exerts trade deficit in commercial seaweed farming. This chapter highlights the causes of epiphytic infestations, the current status of outbreaks, methods to control epiphytes, and its economic implications.

Keywords Seaweed · Epiphytes · Control strategies · Seaweed farming · Economic implications

9.1 Introduction

Seaweeds are marine macrophyte algae. They are exceptionally diverse in their forms and functions, are renewable in nature, and provide inimitable prospects for its utilization as a source of nutrition, food, cosmetics, fertilizer, medicinal, nutraceuticals, pharmaceutical, biofuels, personal care, and allied industries. Commercial harvesting of seaweeds has reached a new milestone with 31.2 million tonnes year^{-1} production (95% accounts to farming) with a market value of over US$ 11.7 billion (FAO 2018). About 221 species of seaweed are being utilized commercially. Of these, about 145 species are used for food, while nearly 110 species are exploited for phycocolloid extraction. Almost all of the seaweed production (94%) is produced through aquaculture practice, while harvesting from the wild stocks is minuscule.

Asian countries alone account for over 99% of global seaweed production. The highest proportion of tonnage is constituted by food alga, namely, *Porphyra* (Nori), *Laminaria* (Kombu), and *Undaria* (Wakame), followed by seaweeds for phycocolloid extraction. As seaweed farming has been gaining impetus globally, including outside Asia, the problems faced by this industry need to be addressed; one of the several issues is an epiphytic infestation. The first report of an epiphytic outbreak in commercial farms of *Kappaphycus* in the 1970s caught the attention of several researchers on this devastating epiphytes which causes retarded growth and significant loss of stocking biomass ultimately leading to the production of inferior quality of raw material. It is also a major, worldwide problem in *Gracilaria* cultivation as well (Fig. 9.1). The epiphytic infestation has severely reduced the productivity and cost efficiency in tank cultivation systems (Fletcher 1995). Seaweed in their natural ecosystem acts as a primary producer and provides food to consumers. With this primary role in the marine ecosystem, seaweed provides shelters and habitat to many organisms including the plants and animals. The organisms which colonize the seaweeds can be called as epibiotic communities or epibionts or even referred as epiphytes (Peteiro and Freire 2013) although this word is not clearly defined (Steel

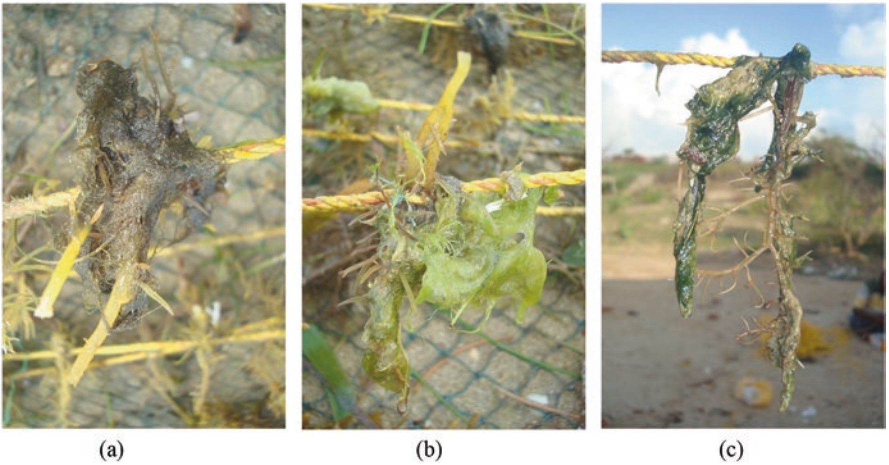

(a) (b) (c)

Fig. 9.1 Various forms of epiphytic infestation recorded during *Gracilaria edulis* farming. (**a**) Red algal type (**b**) Green algal type (**c**) Blue-green algal type

and Wilson 2003) and the phenomenon is referred as epiphytism. Epiphytism is common in marine habitat (Ingle et al. 2018), and there are various types of epiphytes from microalgae, other seaweeds to invertebrates and animals from the class of gastropod, and many small crustaceans such as amphipods and isopods associated with seaweeds. With this, there are various kinds of microorganisms from bacterial, fungal, and viral species (Gachon et al. 2010) associated with seaweeds. Almost all of these microorganisms (Goecke et al. 2013, 2010) and epibiotic communities (Wahl 1989) get suitable place, habitat, food source, as well as protection from their predators and other environmental stresses.

All these epibiotic communities of seaweed can show positive as well as negative mechanism. The positive interaction however is not so well studied, but few species particularly grazers can control the epiphytic algal species. In the cultivation of seaweeds, the decrease or increase of these communities can impact on the production. The abundance and species biodiversity of epibiotic communities are dependent on crop species morphology, season, etc. There are some other important factors in defining the epifaunal assemblages apart from the algal host such as epiphytic load and height on the shore (Cacabelos et al. 2010). In many places, the seaweeds are non-native species which can show variation in acceptance of them by native epibiotic communities (Cacabelos et al. 2010), due to their metabolites as deterrents against consumers (Paul and Fenical 1986). The physiological tolerance of these animals and the variations in the habitat environment determine the distributional pattern and abundance of this epifauna related to specific marine macrophyte (Lancellotti et al. 1993). Basically, the majority of epiphytic algae species are facultative in nature and are usually found associated with more than one species (Wahl and Mark 1999), while others are known obligate epiphytes which grow on specific single host species.

Almost all epibiotic organisms are generally deposited on the thallus part of cultivated seaweeds. In case of the epiphytic seaweeds and seaweed, crops are generally competitors of each other for the resources such as sunlight and nutrient (Kersen et al. 2007) which can make host seaweed weak, resulting in bacterial infection. The abundance of epiphytic species is determined by few abiotic factors, for instance, the direction of water movement and the availability of nutrients. Their spatial distribution is dependent on their ability to dryness or removal capacity of moisture during low tides (Molina-montenegro et al. 2005). The higher level of sunlight, high temperature, and strong desiccation at the time of low tides make the intertidal zone a stressful habitat (Bertness and Leonard 1997). The species which have the capability to keep themselves alive in such adverse conditions can impact on other species (Bertness et al. 1999; Molina-montenegro et al. 2005). Few seaweed species show tolerance to such environment and can provide shelter to various organisms such as other seaweeds and small crustaceans (Bertness et al. 1999).

The upsurge in the amount of nutrient load shows a gradual increase in the number of epiphytic seaweeds and invertebrates which are dependent on the seaweeds. However, intraspecific competition is also possible in the invertebrates for light, space, and food (Lobban and Harrison 2000; Kersen et al. 2007). The complex structure of seaweed can provide larger surface results in the diverse assemblages of invertebrates associated with that seaweed (Chemello and Milazzo 2002). The spatial variability of epifauna within the same environments might vary from a few days to several months. The small spatial scale of observation shows that the seaweed is a highly appropriate habitation for an extensive variety of faunal organisms (Chemello and Milazzo 2002), but this depends on many factors such as life cycle of epibiotic organisms, the architecture, and chemical defense of host seaweeds (Duffy and Hay 1994).

9.2 Classification of Epiphytes

Linskens (Linskens 1963) classified the epiphytes in two types on the basis of their attachment to host seaweed. The holo-epiphytes attach to the outermost layer of their seaweed host, while amphi-epiphytes acutely anchor inside the host seaweed tissue. This classification is complimentary compared to the classification given by Leonardi et al. (2006), which is based on the level of host penetration and classified in five types as per their interaction with macroalgae. Leonardi classified the epiphytes in five categories as shown in Table 9.1.

These epiphytes can be other algae, bacteria, fungi, etc. which cover the parts of seaweed densely as per their requirement and possibility of spreading. Both these classifications are related with the interaction of seaweed with epiphytes particularly epiphytic algae, and no mention about microorganisms are given separately and even no discussion on the animal's association to the seaweed. Ingle et al. (2018) defined the term pests in macroalgae cultivation, and on the basis of negative

Table 9.1 Classification of marine epiphytes in five different types

Type	Attachment characteristics	Damage possibility
1	Weak attachment to the host surface	No damage to host tissue
2	Strong attachment to the host surface	No damage to host tissue
3	Bleach the deck lamella, and outer layer of the host wall is penetrated	No damage to cortical cell
4	The deck lamella and outer layer of the host cell wall	Disturb the cortical tissue
5	The cortex is penetrated to reach the deep medullary tissue	Damage to host due to penetration up to medulla of host seaweed

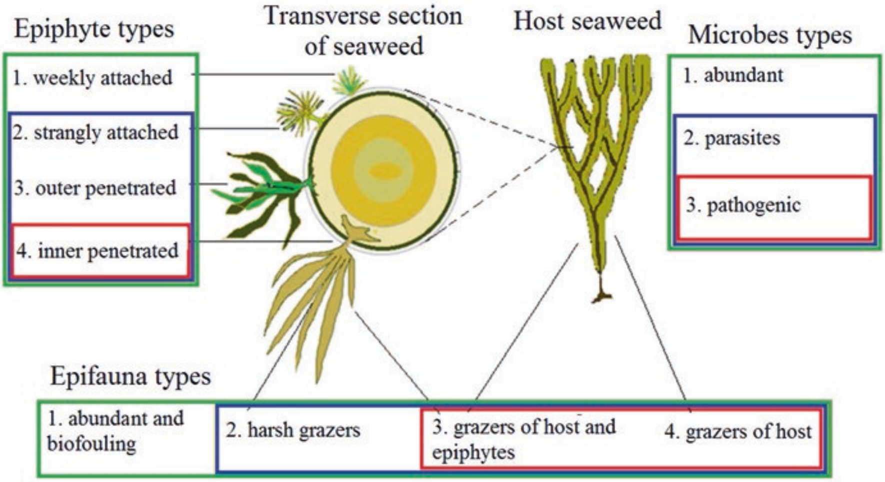


Fig. 9.2 Classification of marine pests into three main groups, plants, animals, and microorganisms, which are further categorized into subtypes which negatively interact with seaweed and might be responsible for direct or indirect harm or injuries. (Modified from Ingle et al. 2018)

interaction of epibiotic communities with crop seaweed, pests are classified in various categories as shown in Fig. 9.2.

9.3 Microorganisms and Seaweed

In the marine environment, seaweed deals with all types of microorganisms including the viruses and fungal species but mostly with bacteria. Although there is a limited study on the seaweed interaction with viruses and fungi, it is found that up to half of natural seaweed is infected by viruses (Cock et al. 2010) which denotes that viruses can strongly influence the seaweed lifestyle as well (Egan et al. 2013) (Table 9.2). In case of fungal species, only a few fungal species are yet known, but

Table 9.2 Key research works on the epiphytes in different geographical regions

Sl no.	Authors	Work done	Epiphytic composition	Area
1	Sand-Jensen and Borum (1984)	Epiphyte and photosynthesis	*Lobelia dortmanna* L., epiphytic diatoms	Denmark
2	Kitayama and Garrigue (1998)	Microscopic multicellular algae	*Plzaeophila deidroides* (Chlorophyceae, Phaeophilales), *Feldinannia irregularis*, *Feldrnaniiizia indica* (Phaeophyceae, Ectocarpales), *Stylonema alsidii* (Rhodophyceae, Porphyridiales)	Japan
3	Muñoz and Fotedar (2010)	Epiphytism of *Gracilaria cliftonii*	*Hypnea episcopalism*, *Ceramium puberulum*, *C. minuta*, *C. pusillum*, *C. isogonum*, *Polysiphoniaforfex*, *P. spinosissima*, *Laurencia clavata*, *Ulva lactuca*, *Champia parvula*, etc.	Western Australia
4	Vairappan (2006)	Epiphytic algae on *Kappaphycus alvarezii*	*Neosiphonia savatieri* followed by *Neosiphonia apiculata*, *Centroceros* sp., *Ceramium* sp., *Acanthophora* sp.	Malaysia
5	Rindi and Guiry (2004)	Spatiotemporal variability and composition of the epiphytic macroalgal assemblage of *Fucus vesiculosus*	*Polysiphonia lanosa*, *Elachista fucicola*, *Porphyra umbilicalis*, *Ulva compressa*, and *Spongonema tomentosum*	Western Ireland
6	Totti et al. (2009)	Function of the host thallus morphology in structuring the epiphytic diatom communities	*Epiphytic diatoms*	Iceland
7	Tujula et al. (2010)	Bacterial epiphytes on *Ulva*	Members of the *Alphaproteobacteria*, *Gammaproteobacteria*, and the *Bacteroidetes*	Australia
8	Ganesan et al. (2011)	Culture of *Gracilaria edulis* in open sea by rafting method	*Chaetomorpha linum*, *Enteromorpha compressa*, *Cladophora fascicularis*, *Ulva lactuca*, *Enteromorpha intestinalis*	Southeastern coast of India
9	Burke et al. (2011)	Epiphytes on *Ulva*	*Various epiphytic bacteria species*	Bare Islands, Australia

(continued)

Table 9.2 (continued)

Sl no.	Authors	Work done	Epiphytic composition	Area
10	Ganesan et al. (2014)	Epiphytism differences in *Gelidiella acerosa* in floating rafts and concrete blocks	*Caulerpa racemosa*, *C. linum*, *C. peltata*, *Acetabularia* sp., *L. Majuscula*, *P. tetrastromatica*, *Dictyota* sp., *Cladophora* sp., *Gracilaria cylindrica*, *H. pannosa*, *H. valentiae*, *J. adhaerens*	Thonithurai coast and Ervadi coast of Gulf of Mannar, India
11	Veeragurunathan et al. (2015)	Seasonality (epiphyte and epifaunal assemblages) on *Gracilaria dura*	*Chaetomorpha crassa*, *Enteromorpha intestinalis*, *Ulva reticulata*, *Hydroclathrus clathratus*, *Padina gymnospora*, *Sargassum palgiophyllum*, *Acanthophora spicifera*, *Hypnea pannosa*	Palk Bay, southeast coast of India
12	Lim et al. (2016)	Defense-related transcripts of an agarophyte, *Gracilaria chang*	*Epiphytic bacteria*	Malaysia
13	Werner et al. (2016)	Food web structure in climate change	*Interaction with grazing and epiphytes in changing seasons*	Baltic Sea
14	Anderson and Martone (2014)	Biomechanical consequences in epiphytism	*Soranthera ulvoidea*	Canada

majority of fungi are from *Ascomycota* (Loque et al. 2010; Zuccaro et al. 2008). But compared to these, the bacterial-seaweed interaction is well studied because it is one of the dominant groups, and seaweed can come under pressure due to a higher number of bacterial communities.

Many times this interaction is positive and beneficial for both. Seaweed provides favorable habitat to bacterial colonies to grow and reproduce on its surface (Englebert et al. 2008). With this, bacterial colonies get nutrients and food source from biochemical activities in the seaweed. For example, seaweed is marine photosynthetic organisms which produce oxygen is a good source of oxygen for bacteria associated to seaweed. But this interaction is not one way as for the growth and development of seaweeds, and few bacterial species are important. These symbiotic bacteria can enhance seaweed growth (Singh et al. 2011), protect seaweed from harmful bacterial and fungal species by producing certain chemicals (Lemos et al. 1985), and also help in reproduction (Joint et al. 2007). But with such positive interaction, there is negative interaction too, in which few bacterial species are responsible for many seaweed diseases (Goecke et al. 2010).

The physical association of bacteria with seaweed is generally in different ways such as tightly attached and not directly attached, and a few show syntrophic associations (Goecke et al. 2013). Bacteria can harm seaweeds by producing a chemical which is toxic (Berland et al. 1972), injuring them by degrading their cell walls

(Goecke et al. 2010). Few bacterial species can produce biofilm in the seaweed crop surrounding or on the water surface which is an intricate three-dimensional cluster in nature, responsible for both direct and indirect harms by reducing the essential light penetration (Wahl et al. 2010) and gaseous exchange. Biofilm can impact negatively on the reproduction of seaweed and also in the natural environment by enhancing the attachment of spores which later results in damage.

9.4 Epiphytic Algae

The epiphytic algae on seaweed crop can be microalgae or most specifically macroalgae or other seaweed species which are not part of cultivation. It can impacts on the host seaweed as a reduction of the growth rate of the crop, a decrease of reproduction output, or even whole or partial mortality of host seaweed (Davis et al. 1989) which can further results in quality and quantity of production or yield of the crop (Table 9.3). Generally, the ephemeral epiphytes can be seen at the host tips, while large-sized epiphytes can be found attached to the basal disk (Arrontes 1990). The overall coverage of epiphytes on these seaweed crops increases as per availability and nutrients and with the age of host seaweed. The structure of epiphytic communities depends on the position of the thallus of seaweed (Longtin et al. 2009).

9.5 Epifaunal Communities

The epifaunal species assemblage undergoes frequent temporal fluctuation because of several environmental (abiotic) factors like availability of light, temperature, abundance of epiphytes, and pressure of predation (Jones and Thornber 2010). Fluctuations in these features can influence the pattern of abundance at different spatial and temporal scales for the epifaunal community. Many of these epifaunal species such as amphipods, isopods, and even smaller gastropods live in the epiphytic algae (Orav-kotta and Kotta 2004) and can use that as a food source (Leonardi et al. 2006). In this way more dense epiphytic algae host more epifaunal communities by creating a suitable environment.

In the seaweed assemblages, amphipod crustaceans are very much common which are particularly the mesograzers and impact adversely on the seaweed (Poore et al. 2012). In all ecosystems, herbivory is a key process which transfers the primary production to further consumers in ecosystem level with impact on structure and productivity of vegetated habitat (Poore et al. 2012). Similarly as herbivores control the growth of producers in the ecosystem, they are controlled by the predators, but in this natural phenomenon, the seaweed can get harm indirectly (Ingle et al. 2018). The amphipod crustaceans are important for predatory fishes and other invertebrates as a food source (Table 9.3).

Table 9.3 Key research works on the epifaunal composition in different geographical regions

SL	Authors	Work done	Epifaunal composition	Area
1	Norton and Benson (1983)	Faunal association of the brown seaweed *Sargassum muticum* (Yendo)	Ampithoe mea, Aoroides columbiae, *Caprella laeviuscula*, *Ischyoceras anguipes*, and *Lacuna variegata*	Washington, USA
2	Sarma and Ganapati (1972)	Association of faunal species with algae at the intertidal region of Visakhapatnam	A diverse group of Mollusca, Foraminifera, Polychaeta, Nematoda, Amphipoda, and Isopoda are associated with seaweeds	Visakhapatnam, India
3	Joseph (1978)	Ecological perspective on the faunal species associated with economically important seaweeds of South India	Porifera, Bryozoa, Polychaeta, Amphipods, Isopods, Ostracoda, Harpacticoida, Tanaidacea, Gastropoda, Bivalvia, Echinodermata, and the algivorous gastropods are *Turbo intercostalis* Menker, *Pyrene versicolor* Sowerby, *Aplysia lineolata* Adams and Reeve, *Pyrene zebra* Gray, *Trochus radiatus* Gmelin	Gulf of Mannar and Palk Bay and the adjacent groups of island
4	James et al. (1986)	Fauna associated with the cultured seaweed *Gracilaria edulis*	Fish and crab fauna	Coastal waters of Gulf of Mannar and the Palk Bay
5	Taylor and Cole (1994)	Mobile epifauna on subtidal brown seaweeds *Carpophyllum plumosum* var. *Capillifolium* and *Cystophora retroflexa*	Isopods and amphipods were the most dominant animals (2000 individuals per algal wet weight)	Northeastern New Zealand
6	Viejo (1999)	Mobile epifauna inhabiting the invasive *Sargassum muticum* and two local seaweeds *Fucus vesiculosus* L. and *Cystoseira nodicaulis*	Gastropods, gammarid amphipods, and isopods accounted for 90–95% of the total number of invertebrates. *Fucus* had the lowest number of taxa in comparison with *Sargassum* “tidepool”	Northern Spain
7	Norderhaug et al. (2002)	Mobility patterns of holdfast fauna in kelp	A total of 59,664 individuals from 116 species/taxa of kelp-associated fauna were recorded	Norway

(continued)

Table 9.3 (continued)

SL	Authors	Work done	Epifaunal composition	Area
8	Anandavelu et al. (2013)	Epifaunal assemblage on morphologically distinct intertidal seaweeds of Kodiyaghat, India	The epifaunal communities mainly include *Amphipoda*, *Polychaeta*, *Mysida* *Mollusca*, *Brachyura*, *Isopoda*, *Pycnogonida* followed by other fauna *Echinodermata*, *Nemertea*, and *Sipuncula*	South Andaman, India
9	Veeragurunathan et al. (2015)	Epifaunal pattern study	15 epifauna species were observed including maxillopod, decapods, gastropod, polychaetes, anthozoa, ophiuroidea, isopods, ascidiacea, ectoprocta, and several fish species	Thonithurai Southeast coast of India

9.6 Problems/Diseases Caused by Epiphytes and Their Potential Control Strategies

Due to the rapid developments in the seaweed-based farming activities, there is also an increase in the epiphytic diseases/menace worldwide. The epiphytic invasion causes significant damages for the local farmers as it drastically affects the growth of field-grown algal species and ultimately hampers the farming activity in several cases. As with any agronomical practice, several approaches are being utilized and developed to control the epiphytes growth on seaweed, although several strategies have been suggested to control the unsolicited growth of epiphytes and have been used in controlling them in *Gracilaria* farming (Fletcher 1995). However, some of these strategies can be used only in the cultivation tanks, and its application in an open ocean farming is highly challenging. Here we discuss about some major control strategies against seaweed epiphytes.

9.6.1 Chemical Method

The epiphytes can be removed by employing chemical procedures such as by chlorine or copper rinsing or by altering the pH. Some other preventive chemicals like sodium hypochlorite have been largely used to pre-treat the seawater, tanks, and equipment (Ugarte and Santelices 1992). However, if the contamination already starts spreading, the host material is often immersed in an appropriate toxicant and has been proven to be highly useful. Ugarte and Santelices (1992) have successfully demonstrated that immersing *Ectocarpus* and *Enteromorpha* species in 4–6% commercial hypochlorite solution for 6–10 h killed 80% of epiphytes. However, in the case of *Gracilaria*, it caused minor injuries until the thiosulfate was used, and the repetitive treatment led to enhanced yield of *Gracilaria*. Similarly, the usage of 100 g/l of copper solution significantly reduces the growth of *Enteromorpha* within a week without affecting the growth of *Gracilaria* (Haglund and Pedersén 1992). Several researchers have reported that by controlling the pH level, the epiphytes can be repressed (Friedlander 1991), particularly at the high pH level (Haglund and Pedersén 1992).

Considerable interest has been shown by the researchers to optimize the nutrient regimes in order to control the growth of epiphytes. It has been proven that incessant or higher supply of nutrients is not only extravagant but could also favor the unsolicited growth of epiphytic species (Pickering et al. 1993). By following a vigilant supervision of the nitrogen inputs, mainly by decreasing the nitrogen supply, the uncontrolled loads of epiphyte can be prohibited (Pickering et al. 1993). Contrarily, high levels of ammonia (>0.5 mmol/l) have been also proven to be lethal for several epiphytes (Friedlander et al. 1991).

9.6.2 Physical Method

Epiphytes can be physically removed by using the manual methods like mechanical brushing and rapid water movement. In certain cases, sand shifting has been implemented as an effective approach to maintain epiphyte-free *Gracilaria* culture (Doty 1980). Moreover, the strategy to control epiphytic growth in seaweed cultivation system includes the direct physical elimination of the host epiphytes. For the removal of diatoms, water jets are very useful and are usually used against the host material post-harvest. However, for the majority of the macroalgal species especially those affected by a special type of filament known as rhizoidal filaments can deeply penetrate and thus requires manual hand removal, which is a labor-intensive process and is unfeasible for huge culture units and may also cause damage to the host (Ugarte and Santelices 1992). Due to this problem, many farmers favor to employ some other precautionary procedures and recommend good husbandry to tackle the occurrence of epiphytism and fouling.

In tank cultivation systems, it must be confirmed that the source of seawater is devoid of impurities (Ugarte and Santelices 1992) or the seawater is routinely exchanged. For instance, filtration of seawater using diatomaceous earth or filter cartridges and sand is often utilized for blocking the entry of epiphytic organisms. Additionally, some other precautionary procedures have been also implemented to reduce the contamination, for instance, by adjusting the abiotic factors in favor of the host species. The usage of UV light has also been endorsed for tank culture system. The reduction in irradiance levels has also been successfully demonstrated to reduce the level of epiphytism in seaweed cultivation (Friedlander 1992).

9.6.3 Biological Method

The rapid prevalence of epiphytism in seaweed mariculture has enticed special attention to develop resistant strains and to utilize existing knowledge to control epiphytism in seaweed farming (e.g., Santelices and Ugarte 1990; Fletcher 1995). Acadian marine plant extract powder (AMPEP) is a commercial extract obtained from the brown alga, *Ascophyllum nodosum*. It harbors some major macronutrients such as total nitrogen N (0.8–1.5%), phosphoric acid P_2O_5 (1–2%), and soluble potash K_2O (17–22%), which has been proven to augment the overall growth and development of eucheumatoids (Hurtado et al. 2013). Furthermore, promising results have been obtained in in vitro and field trials using AMPEP extract against epiphytes and pathogen. In addition, it has been linked to increase the rate of growth and carrageenan production (Loureiro et al. 2012). Interestingly, studies have also shown that soaking the algal seedlings in AMPEP, before planting, could efficiently improve the daily growth rate and productivity of both varieties of *Kappaphycus*. It has been also utilized to check or diminish the influence of *Neosiphonia* infection on commercial farming regions (Borlongan et al. 2011). However, more field trials with extracts from other algal species might lead to discovery of some new and potential anti-epiphytic compounds.

Maintaining an optimal density of host plants has been suggested by the phycologist to prevent epiphytes from colonizing. Grazers have been effectively utilized to control epiphyte growth. *Gammarus lawrencianus* and *Idotea baltica* are the two crustacean species which have been successfully demonstrated to selectively graze on *Ectocarpus* spp. and *Enteromorpha* spp. which epiphytically grow on the surface of *Chondrus crispus* (Shacklock and Doyle 1983). Similarly, the epiphyte growth on *Fucus* is controlled by *Idotea* which is often seen during the high nutrient load conditions (Worm et al. 2000; Orav-Kotta and Kotta 2004). Sporadic feeding by herbivores could also be beneficial for seaweeds and communities sometimes (Hay et al. 2004). The mesograzers which are the filamentous epiphytes are usually removed manually from a host. This manual removal of epiphytes allows the absorption of higher amount of light and enhances nutrient absorption by the host plant (Duffy and Hay 1990). The epiphytes associated with pond-grown seaweed species have been effectively controlled by fish such as milkfish (*Chanos chanos*) and *Tilapia mossambica* (Shang 1976).

9.7 Conclusion and Perspectives

Seaweed farming, or seagriculture, is anticipated to offer sustainable seaweed biomass, thereby enabling the rapid expansion of marine bioeconomy. But, with the increase in activities related to seaweed farming, there has been reportedly higher occurrence of epiphytic filamentous algae (EFA) disease in several parts of the world. Similar to land-based crop, the cultivation of macroalgae is also prone to diseases and infestations. This epiphytic infiltration significantly affects the algal growth and thus the local farmers and has even totally collapsed the farming activity in several parts of the globe. Moreover, because of the fragility of the marine environment, it is impractical to utilize chemical methods to control the epiphytes. In this regard, marine integrated pest management (MIPM) approach appears to be the best available option for the sustainable seagriculture. On the other hand, AMPEP (Acadian marine plant extract powder) has also been proven to be highly potential in minimizing or controlling the growth of epiphytes and the respective diseases caused by them.

References

Anandavelu I et al (2013) Epifaunal assemblage on morphologically distinct intertidal seaweeds of Kodiyaghat (South Andaman), India. Proc Int Acad Ecol Environ Sci 3(3):229–237

Anderson LM, Martone PT (2014) Biomechanical consequences of epiphytism in intertidal macroalgae. J Exp Biol:1167–1174. https://doi.org/10.1242/jeb.088955

Arrontes J (1990) Composition, distribution on host and seasonality of epiphytes on three intertidal algae. Bot Mar 33(2):205–212. https://doi.org/10.1515/botm

Berland BR, Bonin DJ, Maestrini SY (1972) Are some bacteria toxic for marine algae? Mar Biol. Springer 12(3):189–193

Bertness M, Leonard G (1997) The role of positive interactions in communities : lessons from intertidal habitats. Ecology 78(7):1976–1989

Bertness M et al (1999) Testing the relative contribution of positive and negative interactions in rocky intertidal communities. Ecology 80(8):2711–2726

Borlongan IAG, Tibubos KR, Yunque DAT, Hurtado AQ, Critchley AT (2011) Impact of AMPEP on the growth and occurrence of epiphytic Neosiphonia infestation on two varieties of commercially cultivated *Kappaphycus alvarezii* grown at different depths in the Philippines. J Appl Phycol 23:615–621. https://doi.org/10.1007/s10811-010-9649-9

Burke C et al (2011) Bacterial community assembly based on functional genes rather than species. Proc Nat Acad Sci U S A 108(34):14288–14293. https://doi.org/10.1073/pnas.1101591108

Cacabelos E et al (2010) Do grazers prefer invasive seaweeds? J Exp Mar Biol Ecol 393(1–2):182–187. https://doi.org/10.1016/j.jembe.2010.07.024.

Chemello R, Milazzo M (2002) Effect of algal architecture on associated fauna: some evidence from phytal molluscs. Mar Biol 140:981–990. https://doi.org/10.1007/s00227-002-0777-x

Cock JM et al (2010) The Ectocarpus genome and the independent evolution of multicellularity in brown algae. Nature, Nature Publishing Group 465(7298):617–621

Davis A et al (1989) Epibiosis of marine algae and benthic invertebrates: natural product chemistry and other mechanisms inhibiting settlement and overgrowth. In: Bioorganic marine chemistry. Springer, Berlin, pp 85–114

Doty MS (1980) Outplanting Eucheuma species and Gracilaria species in the tropics. In: Abbott IA, Foster MS, Eklund LF (eds) Pacific seaweed aquaculture, Proc. Symp. Useful algae. California Sea Grant College Program, Inst. Mar. Resources, Univ Calif, La Jolla, pp 19–22

Duffy JE, Hay ME (1990) Seaweed adaptations to herbivory. Bioscience 40(5):368–375

Duffy JE, Hay ME (1994) Herbivore resistance to seaweed chemical defense: the roles of mobility and predation risk. Ecology 75(5):1304–1319

Egan S et al (2013) The seaweed holobiont: understanding seaweed–bacteria interactions. FEMS Microbiol Rev. The Oxford University Press 37(3):462–476

Englebert ET, McDermott C, Kleinheinz GT (2008) Effects of the nuisance algae, Cladophora, on Escherichia coli at recreational beaches in Wisconsin. Sci Total Environ 404:10–17. https://doi.org/10.1016/j.scitotenv.2008.05.025

FAO (2018) The state of world fisheries and aquaculture 2018 – meeting the sustainable development goals. Rome

Fletcher RL (1995) Epiphytism and fouling in Gracilaria cultivation: an overview. J Appl Phycol 7(3):325–333

Friedlander M (1991) Growth rate, epiphyte biomass and agar yield of Gracilaria conferta in an annual outdoor experiment. I. Irradiance and nitrogen. Bioresour Technol 38:203–208

Friedlander M (1992) Gracilaria conferta and its epiphytes. The effect of culture conditions on growth. Bot Mar 35:423–428

Friedlander M, Krom MD, Ben-Amotz A (1991) The effect of light and ammonium on growth, epiphytes and chemical constituents of *Gracilaria conferta* in outdoor cultures. Bot Mar 34:161–166. https://doi.org/10.1515/botm.1991.34.3.161

Gachon CMM et al (2010) Algal diseases: spotlight on a black box. Trends Plant Sci 15(11):633–640. https://doi.org/10.1016/j.tplants.2010.08.005

Ganesan M, Sahu N, Eswaran K (2011) Raft culture of Gracilaria edulis in open sea along the south-eastern coast of India. Aquaculture. Elsevier B.V 321(1–2):145–151. https://doi.org/10.1016/j.aquaculture.2011.08.040

Ganesan M et al (2014) Epiphytism differences in Gelidiella acerosa cultivated with floating rafts and concrete blocks. J Appl Phycol 27:399–412. https://doi.org/10.1007/s10811-014-0279-5

Goecke F et al (2010) Chemical interactions between marine macroalgae and bacteria. Mar Ecol Prog Ser 409:267–299. https://doi.org/10.3354/meps08607

Goecke F et al (2013) Algae as an important environment for bacteria-phylogenetic relationships among new bacterial species isolated from algae. Phycologia 52(1):14–24

Haglund K, Pedersén M (1992) Growth of the red alga Gracilaria tenuistipitata at high pH: influence of some environmental factors and correlation to an increased carbonic-anhydrase activity. Bot Mar 35:579–587

Hay ME, Parker JD, Burkepile DE, Caudill CC, Wilson AE, Hallinan ZP, Chequer AD (2004) Mutualisms and aquatic community structure: the enemy of my enemy is my friend. Annu Rev Ecol Evol Syst 35:175–197

Hurtado AQ, Montaño MNE, Martinez-Goss MR (2013) Commercial production of carrageenophytes in the Philippines: ensuring long-term sustainability for the industry. J Appl Phycol 25(3):733–742

Ingle KN et al (2018) Marine integrated pest management (MIPM) approach for sustainable seagriculture. Algal Res 29(November 2017):223–232. https://doi.org/10.1016/j.algal.2017.11.010

James PSBR, Krishnamurthy Chennubhotla VS, Rodrigo JX (1986) Studies on the fauna associated with the cultured seaweed Gracilaria edulis. The symposium of coastal aquaculture:1193–1198

Joint I, Tait K, Wheeler G (2007) Cross-kingdom signalling: exploitation of bacterial quorum sensing molecules by the green seaweed Ulva. Philos Trans R Soc London B Biol Sci 362(1483):1223–1233

Jones E, Thornber CS (2010) Effects of habitat-modifying invasive macroalgae on epiphytic algal communities. Mar Ecol Progress Ser 400(Rodriguez 2006):87–100. https://doi.org/10.3354/meps08391

Joseph MM (1978) Ecological studies on the fauna associated with economic seaweeds of South India-I. Species composition, feeding habits and interrelationships. Seaweed Res Util 3:2–24

Kersen P et al (2007) Epiphytes and associated fauna on the brown alga Fucus vesiculosus in the Baltic and the north seas in relation to different abiotic and biotic variables. Mar Ecol 32:87–95. https://doi.org/10.1111/j.1439-0485.2010.00418.x.

Kitayama T, Garrigue C (1998) Marine algal endophyte and epiphytes new to New Caledonia. Bull Nat Sci Mus Tokyo Ser B 24(3):93–101

Lancellotti DA et al (1993) Distribution patterns and coexistence of six species of the amphipod genus Hyale. Mar Ecol Prog Ser 93(Lancellotti 1990):131–141

Lemos ML, Toranzo AE, Barja JL (1985) Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microb Ecol 11(2):149–163. https://doi.org/10.1007/BF02010487

Leonardi PI et al (2006) Diversity, phenomenology and epidemiology of epiphytism in farmed Gracilaria chilensis (Rhodophyta) in northern Chile. Eur J Phycol 41(2):247–257. https://doi.org/10.1080/09670260600645659

Lim E et al (2016) Global transcriptome analysis of Gracilaria changii (Rhodophyta) in response to agarolytic enzyme and bacterium. Mar Biotechnol Biotechnol 18:189–200. https://doi.org/10.1007/s10126-015-9680-6

Linskens HF (1963) Beitrag zur frage der beziehungen zwischen epiphyt und basiphyt bei marinen algen. Pubbl Stn Zool Napoli 33:274–293

Lobban CS, Harrison PJ (2000) Seaweed ecology and physiology. Cambridge University Press, Cambridge, 366 pp

Longtin CM et al (2009) Distribution of algal epiphytes across environmental gradients at different scales: intertidal elevation, host canopies, and host fronds. J Phycol 45:820–827. https://doi.org/10.1111/j.1529-8817.2009.00710.x.

Loque CP et al (2010) Fungal community associated with marine macroalgae from Antarctica. Polar Biol 33(5):641–648. https://doi.org/10.1007/s00300-009-0740-0

Loureiro RR, Reis RP, Berrogain FD, Critchley AT (2012) Extract powder from the brown alga Ascophyllum nodosum (Linnaeus) Le Jolis (AMPEP): a “vaccinelike” effect on Kappaphycus alvarezii (Doty) Doty ex PC Silva. J Appl Phycol 24(3):427–432

Molina-montenegro MA et al (2005) Positive associations between macroalgal species in a rocky intertidal zone and their effects on the physiological performance of Ulva lactuca. Mar Ecol Prog Ser 292:173–180

Muñoz J, Fotedar R (2010) Epiphytism of Gracilaria cliftonii (Withell, Millar & Kraft) from Western Australia. J Appl Phycol 22:371–379. https://doi.org/10.1007/s10811-009-9469-y

Norderhaug KM, Christie H, Rinde E (2002) Colonisation of kelp imitations by epiphyte and holdfast fauna; a study of mobility patterns. Mar Biol 141:965–973. https://doi.org/10.1007/s00227-002-0893-7

Norton TA, Benson MR (1983) Ecological interactions between the brown seaweed Sargassum muticum and its associated fauna. Mar Biol 75:169–177

Orav-kotta H, Kotta J (2004) Food and habitat choice of the isopod Idotea baltica in the northeastern Baltic Sea. Hydrobiologia 514:79–85

Paul VJ, Fenical W (1986) Chemical defense in tropical green algae, order Caulerpales. Mar Ecol Prog Ser 34:157–169

Peteiro C, Freire O (2013) Epiphytism on blades of the edible kelps Undaria pinnatifida and Saccharina latissima farmed under different abiotic conditions. J Would Aquacult Soc 44(5):706–715

Pickering TD, Gordon ME, Tong LJ (1993) Effect of nutrient pulse concentration and frequency on growth of *Gracilaria chilensis* plants and levels of epiphytic algae. J Appl Phycol 5:525–533. https://doi.org/10.1007/BF02182511

Poore AGB et al (2012) Global patterns in the impact of marine herbivores on benthic primary producers. Ecol Lett 15:912–922. https://doi.org/10.1111/j.1461-0248.2012.01804.x

Rindi F, Guiry MD (2004) A long-term comparison of the benthic algal flora of Clare Island, County Mayo, western Ireland. Biodivers Conserv 13:471–492

Sand-Jensen K, Borum J (1984) Epiphyte shading and its effect on photosynthesis and diel metabolism of Lobelia dortmanna l. during the spring bloom in a Danish lake. Aquat Biol 20:109–119

Santelices B, Ugarte R (1990) Ecological differences among Chilean populations of commercial Gracilaria. J Appl Phycol 2:17–26

Sarma LN, Ganapati PN (1972) Faunal association of algae in the intertidal region of Visakhapatnam. Proc Indian Natl Sci Acad Part B Biol Sci 38:380–396

Singh RPR et al (2011) Isolation of seaweed-associated bacteria and their morphogenesis-inducing capability in axenic cultures of the green alga Ulva fasciata. Aquat Biol 12(1):13–21. https://doi.org/10.3354/ab00312

Shacklock PF, Doyle RW (1983) Control of epiphytes in seaweed cultures using grazers. Aquaculture 31:141–151

Shang VC (1976) Economic aspects of Gracilaria culture in Taiwan. Aquaculture 8:1–7

Steel JB, Wilson JB (2003) Which is the phytes in epiphytes. Folia Geobot 38:97–99

Taylor RB, Cole RG (1994) Mobile epifauna on subtidal brown seaweeds in northeastern New Zealand. Mar Ecol Prog Ser 115:271–282

Totti C, Poulin ÆM, Romagnoli ÆT (2009) Epiphytic diatom communities on intertidal seaweeds from Iceland. Polar Biol 32:1681–1691. https://doi.org/10.1007/s00300-009-0668-4

Tujula NA et al (2010) Variability and abundance of the epiphytic bacterial community associated with a green marine Ulvacean alga. ISME J 4:301–311. https://doi.org/10.1038/ismej.2009.107

Ugarte R, Santelices B (1992) Experimental tank cultivation of Gracilaria in Central Chile. Aquaculture 101:7–16

Vairappan CS (2006) Seasonal occurrences of epiphytic algae on the commercially cultivated red alga Kappaphycus alvarezii (Solieriaceae, Gigartinales, Rhodophyta). J Appl Phycol 18:611–617. https://doi.org/10.1007/s10811-006-9062-6

Veeragurunathan V et al (2015) Feasibility of Gracilaria dura cultivation in the open sea on the Southeastern coast of India. Aquaculture. Elsevier B.V 438:68–74. https://doi.org/10.1016/j.aquaculture.2015.01.009

Viejo RM (1999) Mobile epifauna inhabiting the invasive Sargassum muticum and two local seaweeds in northern Spain. Aquat Bot 64:131–149

Wahl M (1989) Marine epibiosis. I. Fouling and antifouling: some basic aspects. Mar Ecol Progr Ser Int Res 58:175–189

Wahl M, Mark O (1999) The predominantly facultative nature of epibiosis : experimental and observational evidence. Mar Ecol Prog Ser 187:59–66

Wahl M et al (2010) Ecology of antifouling resistance in the bladder wrack Fucus vesiculosus: patterns of microfouling and antimicrobial protection. Mar Ecol Prog Ser 411:33–48

Werner FJ, Graiff A, Matthiessen B (2016) Even moderate nutrient enrichment negatively adds up to global climate change effects on a habitat-forming seaweed system. Limnol Oceanogr 61:1891–1899. https://doi.org/10.1002/lno.10342

Worm B, Lotze HK, Sommer U (2000) Coastal food web structure, carbon storage, and nitrogen retention regulated by consumer pressure and nutrient loading. Limnol Oceanogr 45(2):339–349

Zuccaro A et al (2008) Detection and identification of fungi intimately associated with the brown seaweed Fucus serratus. Appl Environ Microbiol 74(4):931–941

Chapter 10
Microbial Fuel Cell-Based Process for Wastewater Treatment and Power Generation

Geetanjali, Roma Agrahari, Sanjay Kumar, and Radha Rani

Contents

Abstract Biotechnology is constantly explored to develop methods for wastewater treatment to have a clean environment. Discharge of untreated wastewaters in the soil and water environment is the major cause of environmental pollution in soil and surface and groundwater bodies. Current wastewater treatment technologies are not sufficient to meet the ever-growing demands due to rapid industrialization and population growth, and they are also energy-consuming and cost-intensive. Therefore, it is necessary to develop an energy-efficient treatment method. The rise in global energy demand is projected to increase by 37% by 2040. As the conventional energy sources are also depleting at a fast rate, it is necessary to adopt renewable energy to meet the demands of future energy scenario. Microbial fuel cell (MFC) is a

---

Geetanjali · R. Agrahari · R. Rani (✉)
Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, Uttar Pradesh, India

S. Kumar
School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi, India

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9_10

technology in which electricity is generated from wastewater using microorganism as a biocatalyst, simultaneously achieving wastewater treatment. In this system, microorganism mediates the direct conversion of chemical energy stored in biodegradable organic matter of wastewater into electrical energy. Many researchers have developed a variety of MFC-centered hybrid wastewater treatment plants at a pilot scale, for the treatment of wastewater from different industries such as dye, brewery, and dairy industries, domestic wastewater, and landfill leachate. Irrespective of the potential of MFC as a renewable energy source and its wide applicability for wastewater treatment, the technique is not yet established successfully for field applications. Low-energy performance and excessive internal resistance are the limiting factors in its practical application. Researchers have performed multiple strategic attempts to minimize these factors by developing efficient reactor designs for minimizing internal resistance, stacking multiple reactors into one enlarge system (modularization). Modularization is one of the common strategies followed by researchers for pilot-scale MFC setup. This prevents the unnecessary distance between anode and cathode and enhances COD removal rate. Also, using various forms of electrodes such as carbon brushes, nickel foam, etc. to provide large surface area and modification of electrodes using nanomaterials have shown power density enhancement. This chapter discusses the potential applications and practical limitations of MFC for its effectiveness in wastewater treatment and contaminant removal and energy generation at bench and pilot scale.

Keywords Microbial fuel cell · Waste water treatment · Bioelectricity · Internal resistance · Power generation

10.1 Introduction

Clean water and electricity are one of the basic human requirements, but unfortunately these are denied to large swathes of world population. The rise in energy demand of the world is projected to increase by about 37% by 2040 (International Energy Agency). The current sufficiency in the supply of different energy resources available for the short term may paint an image of the future with an easy and clear path to sustainable energy development and also hide different challenges involved in doing so. The rise in the usage of different energy resources varies based on the risk of disruption involved in supply and the harm their usage can cause to the environment. For example, the growth in liquefied natural gas has been highest of those of all the fossil fuels at about more than half while the usage of coal is seen to decline. Along with the energy resource crisis, the problem of waste generation makes the situation even worse, especially wastewater. There is an increasing demand for freshwater across the globe. The usage of freshwater has tripled over the past 50 years. It is increasing by 64 trillion liters every year as per data by UN, UNESCO, and FAO. As per a report published by Central Pollution Control Board

(CPCB) of India, in March 2015, the sewage generation from urban areas in the country is estimated at 61,948 million liters per day (mld), against which a sewage treatment capacity of 23,277 mld is available with us. The remaining untreated sewage either flows into rivers and other water bodies or percolates into the ground. Wastewater treatment is an energy-intensive process and requires lots of energy each year for treatment. According to an estimate, 0.349 kWh of electrical energy per cubic meter of wastewater is required for activated sludge processes in the USA (Oh et al. 2010). There is a strong nexus between water and energy. For example, water and wastewater utilities consume 3% of the electricity in the USA, which accounts for 35% of the total municipal energy budgets. Activated sludge (aerobic digestion) is one of the well-established processes and has been used in most wastewater treatment systems. Although it is an efficient process, it is chemical- and energy-intensive with high capital and significant operational cost (Lu et al. 2017).

To overcome these problems, microbial fuel cells (MFCs) offer a potential solution as they can concurrently be used for wastewater treatment and electricity generation. MFCs have long been considered as an attractive technology for converting various organic wastes directly into electricity using electrogenic bacterial cells in the anode compartment. Most MFCs have been operated using anaerobic or facultative aerobic bacteria which oxidize various organic components including glucose, sewage sludge, brewery waste, dairy waste, and petroleum hydrocarbon. The performances of the MFCs vary with its configuration, electrode configuration, bacterial community, and specific substrate concentration (Lu et al. 2017). Apart from producing electricity, MFC can also be used as efficient wastewater treatment technology and water quality monitoring. In wastewater treatment technology, MFCs have advantage over other technologies in that it can also treat high soluble BOD content wastewater (Waller and Trabold 2013) and produce less sludge. So, various researches are focused to commercialize MFC technology due to its advantage over other technologies of treating wastewater.

Preliminary reports concerning the use of MFC for wastewater treatment were mostly focused on MFC with low volumetric capacity (less than 1 L) and using synthetic wastewater. However, for commercialization purposes, it requires scaling-up, and studies are needed on pilot scale using real wastewater or operated in treatment plants. Recently, various studies have focused on scaling-up MFCs. For example, a 90L MFC was built by stacking anode and cathode modules into a reactor vessel which was reported to produce 0.056 kWh/m^3 energy and 171±8.4 mW/m^2 power densities from each module and reached 87.6% COD removal efficiency while treating brewery wastewater (Dong et al. 2015). Zhang et al. (2013a, b) designed two 4 liter tubular MFCs to treat primary effluent wastewater and reached 65–70% COD removal efficiency in the continuous mode of operation at a HRT of 11 h, and the maximum energy production was 0.0255 kWh/m^3.

It is a common approach to stack multiple small reactors to form a bigger system, as this greatly reduces the internal resistance, and the total volume capacity of the reactor can be increased by just increasing the number of each single reactor. This also offers the advantage as each reactor can function and be maintained as an independent unit. Zhuang et al. (2012a, b) reported the energy output and COD

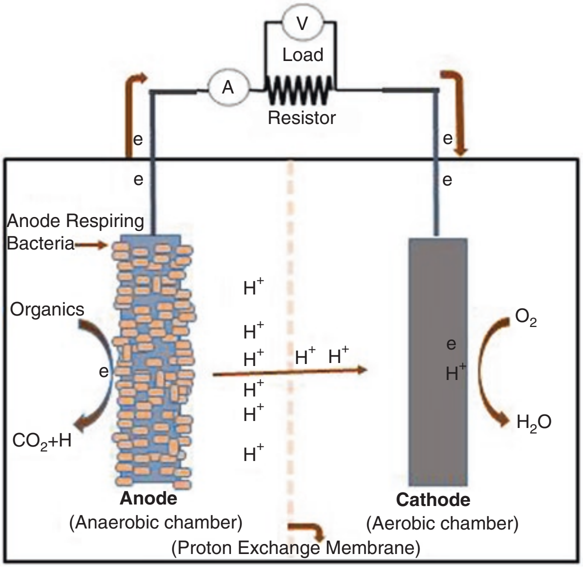


Fig. 10.1 Basic design of a microbial fuel cell

removal of 6 W/$m^3$and 87/1%, respectively, by stacking 40 tubular units in MFCs with 10 L volumetric capacity while treating brewery wastewater. Ge et al. (2013) stacked 96 tubular MFC modules to build an MFC system with 200 L liquid volume to treat primary effluent wastewater and obtained maximum power density of 6.4 W/m^3. These studies show that the stacking of small segmented reactors might be measured as a suitable option for scale-up. This approach would be better for maximizing the power production of systems; however, minimizing internal resistances and energy losses due to fluid flow and reducing dead space are difficult to achieve.

Electrode configuration and positioning is another great challenge in making MFC a scalable technology. Frequently used electrodes can be categorized according to their configurations, the plane electrode and the three-dimensional electrode. Carbon cloth and carbon paper come under plane electrode, whereas carbon brushes/felt comes under three- dimensional electrodes. A good configuration and properties for anodes include a large surface area for bacterial adhesion and efficient current collection.

This chapter focuses on design and operation of MFCs, with emphasis on its application in wastewater treatment (Fig. 10.1).

10.2 MFC: Design and Operation

Many MFC designs have been proposed and operated in laboratory with different reactor configurations. Various designs have different positioning of basic components of MFC such as anode, cathode, and separator. Below is the short introduction about the components of MFC.

10.2.1 Components of MFC

The main components of MFC include the electrodes, i.e., cathode and anode, and the separator which is a semipermeable membrane which allows the exchange of protons.

10.2.2 Electrodes

Electrode is the significant component in determining the performance and cost of MFC. Electrode design is one of the challenging problems in making MFCs a cost-effective and scalable technology. A variety of carbonaceous material are used as electrodes.

10.2.3 Anodes

Anode materials are responsible for the electron transfer efficiency which is an important factor for MFC performance. A large variety of carbon materials are used as anode in MFC due to their conductivity, chemical stability, cost-effectiveness, and biocompatibility.

(a) Granular carbon/graphite – It is generally used as an anode in MFC for its high surface area and porosity. Graphite granules of diameter between 1.5 and 5 mm provide an estimated surface area between 817 and 2720 m^2 m^{-3} of the reactor volume, which is much higher than flat electrodes (Rabaey et al. 2005). This type of anode configuration is achieved by filling the anodic chamber with carbon/graphite granules. Tubular reactor is the best suited configuration for using granule anode. Although granular anodes facilitate good energy output, filling the reactor with granules decreases the volume of the anodic chamber. Moreover, clogging of pores in the electrode may be due to suspended solids in wastewater which may hinder the long-term continuous use of reactor.

(b) Carbon cloth – It is the most common material for plain electrodes and can be used in most of the single-chamber MFC configuration due to its flexible nature. In tubular MFC configuration, carbon cloth wrapped around the tube acts as cathode, which is only possible due to its flexible nature. Kim et al. (2010) used carbon cloth as anode in tubular MFC by wrapping around a perspex cylinder and fitted centrally in the tube reactor.

(c) Carbon brushes – High surface area and efficient electron collection properties make carbon brush an ideal electrode material for MFC (Wei et al. 2011). It was first testified by Logan et al. (2007). The brushes were made of carbon fibers looped into a twisted core of two conductive titanium wires. The diameter and mass of fibers affect the performance of MFC. Liu et al. (2013) investigated that the smaller diameter brush facilitates more energy output, but the effect of fiber loading on energy output was not clear. However, studies do indicate that dense fiber loading leads to clumping and causes hindrance in bacterial access to fiber surface as well as substrate access into the fiber interior. Zhang et al. (2013a, b) used carbon brushes (100 cm long) as anode in tubular MFC deployed in the primary effluent of municipal wastewater treatment plant.

10.2.4 Cathode

The electrons produced in the anodic chamber are transferred to the cathode via external circuit where reductions of electrons occur. In dual-chamber MFC, ferricyanide is used as electron acceptor and converted to ferrocyanide. In single-chamber MFCs, water is formed by reduction reaction in the presence of atmospheric oxygen. Oxygen is most commonly used as electron acceptor due to its high affinity to acquire electron and easy availability, and its end product is only water. Graphite, carbon, and steel are commonly used as a cathode electrode (Fan et al. 2007). Reduction potential of graphite and carbon is very low, and it usually leads to high over-potential. So, platinum is used as catalyst for oxygen reduction reaction at the cathode, but due to its high cost, it is limited to lab scale only. Many researchers focused on using alternative to platinum catalyst to low-cost metal such as Fe (III), cobalt complexes (Cheng et al. 2006), and manganese oxide (Rhoads et al. 2005).

10.2.5 Separator

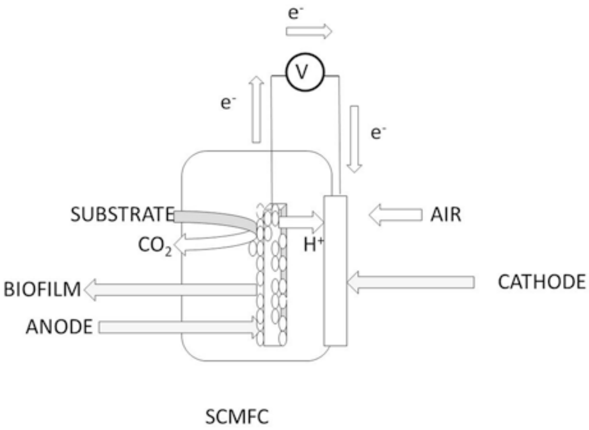
Separator is employed in MFC to physically separate the anodic and cathodic chambers or cathode for a single-chamber MFC and migration of proton from anodic to cathodic chambers. Various types of separator have been extensively studied for MFC including cation-exchange membrane (CEM) (Kim et al. 2010; Zhang et al. 2013a, b), anion-exchange membrane (AEM), microfiltration membrane (MFM), ultrafiltration membrane (UFM), and salt bridge. Separator has large contribution in the cost of MFC. In the study conducted by Ge et al. (2016), separator (CEM) contributed to 60% of the total cost (Table 10.1).

Two of the basic designs of MFC are single-chamber MFC and dual-chamber MFC.

Table 10.1 The basic components of a typical microbial fuel cell

Components	Materials
Anodic chamber	Glass, plexiglass, acrylic sheet, polycarbonate
Cathodic chamber	Glass, plexiglass, acrylic sheet, polycarbonate
Anode	Graphite, graphite felt, carbon cloth, carbon brush, carbon felt, carbon paper, Pt, steel mesh, Pt black, aluminum mesh
Cathode	Graphite, graphite felt, carbon cloth, carbon brush, carbon felt, carbon paper, Pt, steel mesh, Pt black, aluminum mesh
Proton-exchange system	Nafion membrane, Ultrex, polyethylene, polystyrene-co-divinylbenzene, salt bridge, cation-exchange membrane porcelain septum
Mediator	Neutral red, humic acid, thionin, methyl blue, methyl viologen

Fig. 10.2 Schematic diagram of single-chamber microbial fuel cell



10.2.6 *Single-Chamber Microbial Fuel Cells (SC-MFCs)*

SC-MFC offers simpler and cost-saving designs which only have an anodic chamber and cathode directly facing the air. This is the simpler type of MFC in which cathode is manifested directly to air and is now widely used. Nowadays researchers have shown that oxygen can act as terminal electron acceptor that eliminates the necessities of catholyte solution. Liu and Logan (2004) designed the first SC-MFC of cylindrical shape having anode placed inside it and cathode wrapped outside. A single-compartment MFC was designed by Park et al. (2018) consisting of a rectangular chamber having anode inside it and cathode which is directly exposed to the air. Cheng and Logan (2011) designed a SC-MFC consisting of a cylindrical structure with anode placed into it, and a cathode made of carbon cloth material coated with platinum was enclosed around the reactor. In single-chamber MFC, stacking MFCs is more easily achieved and offers higher voltage/current (Fig. 10.2).

10.2.7 Dual-Chamber Microbial Fuel Cells (DC-MFCs)

The traditional two-compartment MFC has "H" shape, in which a proton-exchange membrane (PEM) is used to separate the anodic chamber and the cathodic chamber. Sometimes salt bridge is also used that allows the protons to cross the membrane to cathode. This kind of MFC is used for the study of the basic parameter of microbial fuel cells such as electrodes, biofilms, proton-exchange membrane, and substrates. Dual-chamber MFCs are generally larger in size due to the presence of extra chamber; the distance between two electrodes (anode and cathode) is more, thus causing high internal resistance. Also the chemicals used in cathodic side should be environment-friendly. The use of membranes in DC-MFCs prevents the diffusion of oxygen from cathode to anode. Zhang et al. (2010) constructed a plate-shaped DC-MFC in which cation-exchange membrane (CEM) is used to separate the anode and cathode, and potassium ferricyanide was used as the electron acceptor to complete the circuit (Table 10.2).

Based on the above criteria and components, many MFC reactor designs have been proposed and operated in the laboratory; despite the great efforts and research, they are still challenging for MFC to be used in practical application. Many MFC systems have been developed at milliliter scale, but in order for practical applications to be comprehended, it is required that it should be capable of treating large quantities of wastewater. System scaling-up is one of the major obstacles for MFC

Table 10.2 MFC components used in liter-scale reactors

S.No	Volume (L)	Anode	Cathode	Membrane	Reference
1.	90	Carbon brushes	Activated carbon and PTFE	Two layers of textile separator (glass)	Dong et al. (2015)
2.	72	Granular-activated carbon	Biocathode	Cation-exchange membrane (CEM)	Wu et al. (2016)
3.	0.22	Carbon cloth	Carbon cloth	CEM (CMI-7000)	Kim et al. (2010)
4.	2	Carbon brushes	Carbon cloth	CEM	Zhang et al. (2013a, b)
5.	1.8	Carbon brushes	Carbon cloth	CEM(CMI-7000)	Ge et al. (2013)
6.	72	Granular-activated carbon	Granular-activated carbon	CEM	Wu et al. (2016)
7.	120	Carbon felt	Stainless steel wool	Rhinohide membrane	Heidrich et al. (2014)
8.	250	Carbon brushes	Carbon mesh	Polypropylene porous plastic plate	Feng et al. (2014)
9.	45	GFB (graphite fiber brush)	Carbon cloth	Membrane not used	Hiegemann et al. (2016)
10.	4	Carbon brush	Carbon cloth	CEM	Zhang et al. (2013a, b)

to be used for practical application. It has been observed that there is a logarithmic relationship between power output and reactor volume. The declining power output in large scale is due to the fact that a proper mixing and large distance between anode and cathode hamper the electricity generation. For scaling-up MFC, research approaches the modularization strategies in which multiple reactors are stacked into one system.

10.2.8 Multimodule MFC Reactors

This is a promising configuration for scale-up, as optimal cross-sectional dimensions could be maintained while increasing the volume by extending the tube in the orthogonal dimension. This configuration geometric feature also helps in mass transfer with minimum pressure losses. Carbon brush anode is mostly used in tubular MFC either at the lab scale or pilot scale (Zhang et al. 2013a, b). Carbon cloth anode is used in tubular MFC by wrapping it on a thin pipe and fitted centrally on the tubular MFC. To increase the volume in tubular MFC, researchers generally vary the length of the tube, and the diameter of the tube is generally maintained at 3–6 cm. The size of the tube length has large impact on COD removal rate. In municipal wastewater treatment plant, 53% COD removal was observed by 100-cm-long tubular MFC (Zhang et al. 2013a, b), while in the laboratory, 49% COD removal is observed by 23-cm-long tubular MFC (Kim and Logan 2013). When raw sludge from primary sedimentation tank was used as substrate, about 69% COD removal was observed with 70 cm tube length.

10.3 Strategies for Improving Power Output

High MFC power output is due to the efficient harvesting of electrons from the anode and transferring them to the cathode, where it is finally accepted by oxygen within a certain limit of time. Wei et al. (2011) reported that to improve the performance of MFCs in terms of power generation, it is necessary to modify electrodes to increase their electron capturing capacity. Zhuang et al. (2012a, b) reported that to enhance the power generation and pollutant removal from wastewater, MFC units could be connected either in parallel or in series. Alatraktchi et al. (2014) reported a power density of 461.6 mW/m^2 using electrodes modified by coating them with gold nanoparticles, which was 1.88 times higher than the unmodified electrode (carbon paper). Wu et al. (2012) achieved a maximum power density of 320 mW/m^3 using white-rot fungus, which was significantly higher as compared to the abiotic control, i.e., 50 mW/m^3. Ren et al. (2013) reported that the charge and discharge frequency significantly influences the current output, COD removal efficiency, and charge recovery efficiency.

Below are some of the given factors which should be considered while scaling-up MFC to get improved power output.

(a) *Internal Resistance*

It is one of the main factors that affects the power density in liter-scale MFC, and as already discussed, increasing the volume of reactor leads to increased internal resistance. The same will also occur in the multiple cell systems stacked up by combining small MFC modules in series or parallel connection (Zhuang et al. 2012a, b). Therefore, it is necessary to quantify the internal resistance of each component of MFC reactor to identify the limiting factors of the system (Fan et al. 2012). Many studies have been conducted to minimize the internal resistance by improving the reactor configuration (Dong et al. 2015; Feng et al. 2014), increasing conductivity of substrate (Zuo et al. 2014), adding ion-exchange resins to chamber (Zuo et al. 2014), and improving the electrode configuration. Feng et al. (2014) compared the internal resistance distribution between a lab-scale MFC module and a pilot MFC module with similar structure to identify the limiting factors during enlargement for further enhancing the performance in the application-oriented pilot MFC system and derived the following equation to identify the increase internal resistance during enlargement:

$$P_{p.\max} = E_{p.emf}^{2} \div 4R_{p..real} = K \times E_{p.emf}^{2} \div 4\left(R_{cubic} + fK\right)$$

where $P_{p.\max}$ is the maximum power output from a pilot-scale MFC could be, E is the potential difference, K is the enlarge ratio of reactor cross-sectional area, and $R_{p,real}$ and R_{cubic} refer to the internal resistance in pilot and cubic reactors.

Zuo et al. (2014) mixed salt solution with influent to increase the conductivity of anolyte which decreased the internal resistance. The internal structure of MFC also greatly influences the internal resistance, thus indicating that abiotic factors such as catalysts, electrodes, electrolytes, and reactor types, along with biotic factors, should also be considered, if one wishes to optimize power generation.

(b) *Biofouling*

Bacterial consortia endeavor to grow on every surface in its surrounding area. Membrane is also exposed to the wastewater in the anode chamber, so biofilm formation on its surface is quite obvious, which leads to biodegradation of the membrane, i.e., biofouling. Biofouling of both membrane and cathode electrode over time decreases the current. Biofouling leads to a decrease in the surface area required for migration of ions to cathode and thus a decrease of the efficiency of MFC (Sonawane et al. 2013). It is one of the major problems in of MFC in the long run, especially when operated in wastewater treatment plant. Biofouling of cathode affects the power generation as it reduces the surface for oxygen uptake. Zhang et al. (2013a, b) observed that by simply washing the cathode with water, it quickly restored the current generation, but biofilm formation occurs quickly again once the

MFC is installed back. Biofouling is one of the serious problems that needs to be improved by developing new membranes with lower internal resistance or less subject to biofouling to achieve long-term performance of MFC.

(c) *Modularization*

Another tactic for improving power output in MFC is to stack multiple small reactors together to form a bigger system rather than having a singular large unit. This strategy is followed by most researchers to improve the power output at a large scale. Apart from electricity generation, COD removal efficiency also depends on the number of modules and reactor configuration. Dong et al. (2015) reported COD removal of 87.6%, at an HRT of 72 h, while Zhuang et al. (2012a, b) achieved 87.1% COD removal with an HRT of 48 h only, both using brewery wastewater. The volumetric capacity of the above two studies was 90 L and 10 L, respectively, but the reactor configuration was different. In the study by Dong et al. (2015), anode and cathode modules were placed in a single vessel, and in the report by Zhuang et al. (2012a), 40 units of tubular MFC were stacked to form a serpentine-type MFC. It should be noted that when 96 tubular MFC modules of total volumetric capacity of 200 L were used to treat primary effluent, 76.8% COD removal efficiency was achieved at an HRT of 18 h only (Ge et al. 2016). These efforts have proved that it is more appropriate to stack multiple MFC modules, since it allows maintaining minimum distance between anode and cathode, and each single reactor can function and be maintained independently. Although Haeger et al. (2014) constructed a single reactor instead of having modules in which anode and cathode are rolled up around a central manifold in a spiral fashion, the electrolytes flow through it producing power density of 170 W/m^3. These studies indicate that efficiency of MFC largely depends on its configuration, and more studies are required to achieve a module configuration in which maximum COD removal efficiency is achieved with a minimum HRT and maximum power production.

(d) *Use of Modified Electrodes*

Approaches to modify the surface of electrodes to enhance surface area and improve conductivity have recently gained popularity. Electrode configuration relates to flat electrode, three-dimensional electrode (carbon felt), and brush electrode. The surface properties of electrode material are one of the deciding factors that affect the bacterial attachment to anode and electron reduction at cathode. One of the approaches to enhance power production in MFCs is using modified electrode to facilitate electron transfer to the anode and improve bacterial adhesion capability (Wei et al. 2011). Strategies used to modified anode include (i) use of metal oxide/graphene as electroactive coatings for electrode, (ii) surface treatment with chemical or physical methods (iii), use of conductive polymer to enhance conductivity of electrode, (iv) and increase surface area by enhancing configuration such as carbon brush, carbon mesh, stainless steel mesh, etc. For cathode, generally flat electrode is used with platinum on carbon coating to enhance its activity.

(e) *Hydraulic Retention Time (HRT)*

HRT is one of the important parameters in the wastewater treatment process; it also greatly affects the operational cost and power generation in MFC (Akman et al. 2013). Excessively long HRTs in MFC reduce the organic loading rate, thereby causing fast depletion of substrate and reducing the cell performance, as the microbes attached to the anodic chamber could not receive sufficient electron sources to produce sufficient amounts of electrons, whereas shorter HRTs favor the development of non-exoelectrogenic microorganism, which causes reduction in the electrochemical performance, thus leading to lower columbic efficiency and less efficient COD removal. Also, when an excessively low HRT is applied to MFCs during the continuous wastewater inflow, it prevents the attachment of microbes to the anode surfaces, thus preventing the formation of confluent biofilm on anodic surface. In this way, HRT readily affects the power generation and effluent quality. In a continuous wastewater flow, MFC having HRT between 0.5 and 2 days, when HRT was decreased, enabled the organic load of the anode chamber to facilitate the microbes to generate more electrons by degrading organic matter, thus releasing more electrons and enhancing the power generation efficiency. Akman et al. (2013) and Hiegemann et al. (2016) reported the power density of 80, 82, and 73 mW/m^2 with HRT of 12, 22, and 44 h, respectively.

The effect of temperature on MFC performance is also reported. MFCs operated higher temperatures were reported to give better performance in terms of power generation than those which operated under ambient temperature conditions (Ahn and Logan 2010).

10.4 Application in Wastewater Treatment

The MFC technology utilizes the potential of exoelectrogenic bacteria by releasing the energy containing wastewater while simultaneously treating it. Many researchers have demonstrated power generation using wastewater as substrate and reported COD and pollutant removal simultaneously (Hiegemann et al. 2016; Akman et al. 2013). The microorganisms growing on the anode oxidize the organic matter content of wastewater and transfer the electrons generated to the anode. As the electrons flow from the anode to the cathode through an external circuit, a current is produced which is sometimes stored in capacitors. Therefore, MFCs can be one of the most promising technologies for getting renewable energy and treating wastewater with great eco-friendly benefits. Previously researchers mainly focused on optimizing the power generation and neglecting wastewater treatment; however, lately, attention is being given to both aspects.

Application of MFC in wastewater treatment was first demonstrated by Habermann and Pommerin (1991). Logan (2008) reported a maximum power generation of 330 kW/day using an MFC installed at the wastewater treatment plant of a food processing plant.

Heilmann and Logan (2006) demonstrated electricity generation from protein-rich municipal wastewaters. They used three different protein-rich waste streams, i.e., bovine serum albumin, peptone, and slaughterhouse wastewater with three different BODs of 1100 mg/L, 500 mg/L, and 1420 mg/L, respectively, and reported power densities of 354, 269, and 80 mW/m^2, respectively, with BOD removal efficiencies of 90%, 86%, and 93%, respectively. They concluded that less complex protein generates more power than the complex protein. It may thus be concluded that the higher the complexity of wastewater, the lower will be the columbic efficiency. Zhuang et al. (2012a, b) constructed an MFC system of 10 L volume that consists of 40 individual tubular MFCs to treat synthetic wastewater and reported a maximum power density of 4.1 W/m^3.

Jiang et al. (2011) constructed a 16 L MFC module to treat municipal wastewater and achieved 80% of COD removal but reported low-energy generation due to excessive internal resistance. Zhang et al. (2013a, b) designed two tubular microbial fuel cells (MFCs) of volume 4 L to treat municipal wastewater and achieved similar COD removal efficiency of 65–70% with both types of MFCs. They used activated carbon powder as catalyst in one MFC while platinum along with carbon powder on other MFC. Both the MFCs removed 65–70% chemical oxygen demand at a hydraulic retention time of 11 h and reduced 50% suspended solids (Table 10.3).

10.5 Conclusion

MFC reactor design and configuration are the greatest challenges in making the MFC effective for power generation and COD removal efficiency. In wastewater treatment plant, COD removal is one of the important parameters, and it will be beneficial that the energy required for its treatment is obtained from the wastewater itself. Scaling-up MFC is still facing lots of challenges such as increasing internal resistance, dead space, biofouling, and long-term usability. Further studies on more optimization of reactor configurations are expected to address these challenges and enhance the potential of large-scale MFCs with conventional wastewater treatment systems.

Table 10.3 Performance of MFCs treating different wastewaters along with different operational modes

Volume (L)	Design	Power density	Operational mode	Recirculation rate (L/min)	Coulombic efficiency (%)	Substrate	Reference
250	Stackable horizontal MFC	116 mW each module	Continuous	N.A.	79	Domestic wastewater	Feng et al. (2014)
90	Stackable baffle	171 ± 8.4 mW/m^2 each module	Continuous	N.A.	84.7	Brewery wastewater treatment	Dong et al. (2015)
72	Stacked MFC	50.9 W/m^3	Fed-batch	0.85	78–97	Synthetic wastewater	Wu et al. (2016)
1	Tubular	1.37 mW	Continuous	1.05	N.A.	Sucrose solution	Kim et al. (2010)
2	Tubular	0.37 W/m^3	Continuous	0.2	N.A.	Primary effluent	Zhang et al. (2013a, b)
1.8	Tubular	6.4 W/m^3	Continuous	0.15	69.8	Raw sludge	Ge et al. (2013)
1.6	Single chamber	6.8 W/m^3	Batch	N.A.	N.A.	Domestic wastewater	Cheng and Logan (2011)
7.5	Two-chamber MFC stack	2-10 W/m^3	Continuous	N.A.	69–97	Synthetic	Clauwaert et al. (2009)
10	Tubular MFC stack	6 W/m^3	Continuous	N.A.	86.4	Brewery wastewater	Zhuang et al. (2012a, b)
4	Tubular MFC	NA	Continuous	0.17	65–70	Primary effluents	Zhang et al. (2013a, b)
3	Tubular stack	158 mW/m^2	Fed-batch mode	N.A.	22	anaerobic sludge diluted with synthetic media	Yazdi et al. (2015)
45	Rectangular	10.92 mW	Continuous	N.A.	0.41	Mixed anaerobic sludge	Ghadge et al. (2016)
120	Stacked	N.A	Continuous	N.A.	41.2	Domestic wastewater	Heidrich et al. (2014)

References

Ahn Y, Logan BE (2010) Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures. Bioresour Technol 101:469–475. https://doi.org/10.1016/j.biortech.2009.07.039

Akman D, Cirik K, Ozdemir S, Ozkaya B, Cinar O (2013) Bioelectricity generation in continuously-fed microbial fuel cell: effects of anode electrode material and hydraulic retention time. Bioresour Technol 149:459–464. https://doi.org/10.1016/J.BIORTECH.2013.09.102

Alatraktchi FA, Zhang Y, Angelidaki I (2014) Nanomodification of the electrodes in microbial fuel cell: impact of nanoparticle density on electricity production and microbial community. Appl Energy 116:216–222. https://doi.org/10.1016/j.apenergy.2013.11.058

Cheng S, Logan BE (2011) Increasing power generation for scaling up single-chamber air cathode microbial fuel cells. Bioresour Technol 102(6):4468–4473. https://doi.org/10.1016/j.biortech.2010.12.104

Cheng S, Liu H, Logan BE (2006) Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. Environ Sci Technol 40:364–369. https://doi.org/10.1021/es0512071

Clauwaert P, Mulenga S, Aelterman P, Verstraete W (2009) Litre-scale microbial fuel cells operated in a complete loop. Appl Microbiol Biotechnol 83:241–247. https://doi.org/10.1007/s00253-009-1876-0

Dong Y, Qu Y, He W et al (2015) A 90-liter stackable baffled microbial fuel cell for brewery wastewater treatment based on energy self-sufficient mode. Bioresour Technol 195:66–72. https://doi.org/10.1016/j.biortech.2015.06.026

Fan YZ, Hu HQ, Liu H (2007) Enhanced Coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration. J Power Sources 171:348–354. https://doi.org/10.1016/j.jpowsour.2007.06.220

Fan Y, Han S-K, Liu H (2012) Improved performance of CEA microbial fuel cells with increased reactor size. Energy Environ Sci 5:8273–8280. https://doi.org/10.1039/c2ee21964f

Feng Y, He W, Liu J, Wang X, Qu YRN (2014) A horizontal plug flow and stackable pilot microbial fuel cell for municipal wastewater treatment. Bioresour Technol 156:132–138. https://doi.org/10.1016/j.biortech.2013.12.104

Ge Z, Zhang F, Grimaud J, Hurst J, He Z (2013) Long-term investigation of microbial fuel cells treating primary sludge or digested sludge. Bioresour Technol 136:509–514. https://doi.org/10.1016/j.biortech.2013.03.016

Ge Z, He Z, Ren ZJ, Zeeman G, Saakes M, Sleutels TH, Buisman CJ (2016) Long-term performance of a 200 liter modularized microbial fuel cell system treating municipal wastewater: treatment, energy, and cost. Environ Sci Water Res Technol 2(2):274–281. https://doi.org/10.1039/C6EW00020G

Ghadge AN, Jadhav DA, Ghangrekar MM (2016) Wastewater treatment in pilot-scale microbial fuel cell using multielectrode assembly with ceramic separator suitable for field application. Environ Prog Sustain Energy 35:1809–1817. https://doi.org/10.1002/ep.12403

Habermann W, Pommer EH (1991) Biological fuel cells with sulphide storage capacity. Appl Microbiol Biotechnol 35:128–133. https://doi.org/10.1007/BF00180650

Haeger A, Forrestal C, Xu P, Ren ZJ (2014) High performance spiral wound microbial fuel cell with hydraulic characterization. Bioresour Technol 174:287–293. https://doi.org/10.1016/j.biortech.2014.09.153

Heidrich ES, Edwards SR, Dolfing J, Cotterill SE, Curtis TP (2014) Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12 month period. Bioresour Technol 173:87–95. https://doi.org/10.1016/j.biortech.2014.09.083

Hiegemann H, Herzer D, Nettmann E, Lubken M, Schulte P, Schmelz G, Hoffmann SG, Wichern M (2016) An integrated 45 L pilot microbial fuel cell system at a full scale wastewater treatment plant. Bioresour Technol 218:115–122. https://doi.org/10.1016/j.biortech.2016.06.052

Heilmann J, Logan BE (2006) Production of electricity from proteins using a microbial fuel cell. Water Environ Res 78:531–537. https://doi.org/10.2175/106143005X73046

Jiang D, Curtis M, Troop E, Scheible K, McGrath J, Hu B, Suib S, Raymond D, Li B (2011) A pilot-scale study on utilizing multi-anode/cathode microbial fuel cells (MAC MFCs) to enhance the power production in wastewater treatment. Int J Hydrogen Energy 36:876–884. https://doi.org/10.1016/J.IJHYDENE.2010.08.074

Kim Y, Logan BE (2013) Microbial desalination cells for energy production and desalination. Desalination 308:122–130. https://doi.org/10.1016/j.desal.2012.07.022

Kim JR, Premier GC, Hawkes FR et al (2010) Modular tubular microbial fuel cells for energy recovery during sucrose wastewater treatment at low organic loading rate. Bioresour Technol 101:1190–1198. https://doi.org/10.1016/j.biortech.2009.09.023

Liu H, Logan BE (2004) Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ Sci Technol 38(14):4040–4046. https://doi.org/10.1021/es0499344

Liu Y, Liu H, Wang C, Hou SX, Yang N (2013) Sustainable energy recovery in wastewater treatment by microbial fuel cells: stable power generation with nitrogen doped graphene cathode. Environ Sci Technol 47(23):13889–13895. https://doi.org/10.1021/es4032216

Logan BE (2008) Microbial fuel cells. John Wiley and Sons, Hoboken

Logan BE, Cheng S, Watson V, Estadt G (2007) Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environ Sci Technol 41(9):3341–3346. https://doi.org/10.1021/es062644y

Lu M, Chen S, Babanova S et al (2017) Long-term performance of a 20-L continuous flow microbial fuel cell for treatment of brewery wastewater. J Power Sources 356:274–287. https://doi.org/10.1016/j.jpowsour.2017.03.132

Oh ST, Kim JR, Premier GC, Lee TH, Kim C, Sloan WT (2010) Sustainable waste water treatment: how might MFC contribute? Biotech Adv 28:871–881. https://doi.org/10.1016/j.biotechadv.2010.07.008

Park Y, Nguyen VK, Park S, Yu J, Lee T (2018) Effects of anode spacing and flow rate on energy recovery of flat-panel air-cathode microbial fuel cells using domestic wastewater. Bioresour Technol 258:57–63. https://doi.org/10.1016/j.biortech.2018.02.097

Rabaey K, Clauwaert P, Aelterman P, Verstraete W (2005) Tubular microbial fuel cells for efficient electricity generation. Environ Sci Technol 39:8077–8082. https://doi.org/10.1021/es050986i

Ren S, Xia X, Yuan L, Liang P, Huang X (2013) Enhancing charge harvest from microbial fuel cells by controlling the charging and discharging frequency of capacitors. Bioresour Technol 146:812–815. https://doi.org/10.1016/j.biortech.2013.08.055

Rhoads A, Beyenal H, Lewandowski Z (2005) Microbial fuel cell using anaerobic respiration as an anodic reaction and biomineralized manganese as a cathodic reactant. Environ Sci Technol 39:4666–4671. https://doi.org/10.1021/es048386r

Sonawane JM, Gupta A, Ghosh PC (2013) Multi-electrode microbial fuel cell (MEMFC): a close analysis towards large scale system architecture. Int J Hydrog Energy 38:5106–5114. https://doi.org/10.1016/j.ijhydene.2013.02.030

Waller MG, Trabold TA (2013) Review of microbial fuel cells for wastewater treatment: large-scale applications, future needs and current research gaps. ASME. International Conference on Fuel Cell Science, Engineering and Technology, ASME 2013 11th International Conference on Fuel Cell Science, Eng. and Technol: V001T01A011. doi:10.1115/FuelCell2013-18185.

Wei J, Liang P, Huang X (2011) Recent progress in electrodes for microbial fuel cells. Bioresour Technol 102(20):9335–9344. https://doi.org/10.1016/j.biortech.2011.07.019

Wu C, Liu XW, Li WW, Sheng GP, Zang GL, Cheng YY (2012) A white-rot fungus is used as a biocathode to improve electricity production of a microbial fuel cell. Appl Energy 98:594–596. https://doi.org/10.1016/j.apenergy.2012.02.058

Wu S, Li H, Zhou X et al (2016) A novel pilot-scale stacked microbial fuel cell for efficient electricity generation and wastewater treatment. Water Res 98:396–403. https://doi.org/10.1016/j.watres.2016.04.043

Yazdi H, Gaviria LA, Ren ZJ (2015) Pluggable microbial fuel cell stacks for septic wastewater treatment and electricity production. Bioresour Technol 180:258–263. https://doi.org/10.1016/j.biortech.2014.12.100

Zhang F, Jacobson KS, Torres P et al (2010) Effects of anolyte recirculation rates and catholytes on electricity generation in a litre-scale upflow microbial fuel cell. Energy Environ Sci 3:1347. https://doi.org/10.1039/c001201g

Zhang F, Ge Z, Grimaud J, Hurst J, He Z (2013a) Long-term performance of liter-scale microbial fuel cells treating primary effluent installed in a municipal wastewater treatment facility. Environ Sci Technol 47(9):4941–4948. https://doi.org/10.1021/es400631r

Zhang F, Ge Z, Grimaud J et al (2013b) In situ investigation of tubular microbial fuel cells deployed in an aeration tank at a municipal wastewater treatment plant. Bioresour Technol 136:316–321. https://doi.org/10.1016/j.biortech.2013.02.107

Zhuang L, Yuan Y, Wang Y, Zhou S (2012a) Long-term evaluation of a 10-liter serpentine-type microbial fuel cell stack treating brewery wastewater. Bioresour Technol 123:406–412. https://doi.org/10.1016/j.biortech.2012.07.038

Zhuang L, Zheng Y, Zhou S et al (2012b) Scalable microbial fuel cell (MFC) stack for continuous real wastewater treatment. Bioresour Technol 106:82–88. https://doi.org/10.1016/j.biortech.2011.11.019

Zuo K, Cai J, Liang S et al (2014) A ten liter stacked microbial desalination cell packed with mixed ion-exchange resins for secondary effluent desalination. Environ Sci Technol 48:9917–9924. https://doi.org/10.1021/es502075r

Index

K. M. Gothandam et al. (eds.), *Environmental Biotechnology Vol. 1*,
Environmental Chemistry for a Sustainable World 44,
https://doi.org/10.1007/978-3-030-38192-9