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Innovations in Environmental Biotechnology

Springer

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Dr. B. Lal Gupta, Doctor/Entrepreneur/ Visionary
An extraordinary mind where science meets service

It was on one chilly morning in December 1990 that Dr. B. Lal Gupta, then Sr. Resident at New Delhi's prestigious All India Institute of Medical Sciences, realized he had to return home to Jaipur. The humble and unassuming doctor had been for long contemplating his decision to leave a bright and successful medical career and return to his home town in a city with comparatively few and evolving pathology facilities. Throughout his graduation, he had seen many patients struggle to find the proper treatment at the right time due to the lack of reliable and modern diagnostic facilities. Bound by the solemn oath that entrusts each doctor with a duty to serve humanity to the best of his/her ability, Dr Gupta's decision was soon made.

In 1991, Dr. B. Lal Gupta, Microbiologist and Immunologist, and a former Resident of AIIMS (New Delhi), returned to Jaipur in Rajasthan to fulfil his aim and destiny of "Serve Best, Serve All" and established Dr. B. Lal Clinical Laboratory in the form of a small set-up in a rented premise in Panch Batti area, Jaipur. With the help of a few team members, Dr. Gupta laid the foundation of a Dr. B. Lal Clinical Laboratory. He and his few associates worked hard day and night to provide trustworthy diagnostic services to the people of Rajasthan. Their efforts paid and the small set-up soon showed the telltale signs of becoming a flourishing business.

With a vision to create a local niche for teaching and research in Biotechnology in Rajasthan, which could create future manpower who would use cutting-edge technology for the betterment of society, he started the Dr. B. Lal Institute of Biotechnology in the year 2008.

His Institute gradually became a niche centre for practical utilization of science to serve society. Today, Dr. B. Lal Institute of Biotechnology leads the way by imbuing its students with sound concepts and practical application of Biotechnology and follows a rigorous curriculum centred around providing industry-specific hands-on training to its students.

Dr. B. Lal Institute of Biotechnology prides itself in being a part of a dynamic Dr. B. Lal Group that sets an exquisite example of Industry-Academia collaboration wherein students benefit from close connections with the industry and leading R&D centres.

Dr. Gupta's Journey

Dr. B. Lal Gupta was born in Bharatpur district on 28th January 1959. He earned his MBBS degree (1976) and his Postgraduate degree in Microbiology in 1987 from the prestigious SMS Medical College, Jaipur. Subsequently, he joined the Department of Microbiology, AIIMS New Delhi, in 1987 as a Senior Resident. In the year 1991, he came back to Jaipur and established Dr. B. Lal Clinical Laboratory Pvt. Ltd. in the Panch Batti area of Jaipur. He also laid the foundation for the Dr. B Lal Institute of Biotechnology in 2008. For his relentless service in the field of diagnostics, Dr. Gupta was awarded the Shod Sri Award by the Governor of Rajasthan in 2008 and the Healthy Living Award by Zee Marudhara in 2015.

Foreword

Life is a mystery that does not follow any laws of physics, mathematics or chemistry. Science may be abstract, but when it becomes “technology”, it comes close to the society and works for the betterment of human evolution. Life (biology) is non-linear and we try to assign it *quasi-linear* simulations to help evolve certain solutions to natural problems, which may at times be far from reality. Nature’s engineering is extremely efficient and we can only endeavour to come as close to bio-mimicking as possible. Biotechnology derives lessons from millions of years of the evolutionary process for creating more sustainable products and processes, through which living organisms have developed circular practices to ensure their survival on the planet earth. As a replacement for relatively highly polluting chemical processes, biological interventions allow us to efficiently break down the waste materials and transform them to value added products exerting a much reduced pollution burden on the ever depleting natural resources. Biotechnology interventions have a promising future and offer perhaps the best way for human sustenance.

It is indeed fascinating to observe that microorganisms can feed on natural as well as anthropogenic substances as their substrates and convert even hazardous molecules into minerals and the essential constituents of nature. Microorganisms survive even under extreme adversities through suitable adaptive processes and by taking clues from these, we can develop appropriate protocols to bring out eco-friendly solutions. We all know that it is not easy, but these processes exemplify both the basic principles and the ultimate objectives that we must take into account in all developmental interventions. Environmental biotechnology is as enigmatic as the nature is but, if explored scientifically, can offer green solutions and lead to environmental innovations that can take human beings ahead in a sustainable manner in their quest to conquer the real-world problems.

Owing to the rigorous endeavour of committed scientists and professionals, environmental biotechnology has now acquired the prestigious position of a solution provider. Reliable documentation is increasingly accumulating on a plethora of scientific information resources and new methods are emerging as futuristic technologies to solve the field problems. This book, for all these reasons, is highly topical and incredibly well timed. The book covers biotechnological innovations as development tools and offers an in-depth overview of advances in green technology and sustainability through entrepreneurial interventions. It provides a robust framework to understand the complex nature of environmental processes and suggests suitable conversions to technological development in consonance with ecological safety. It will be very useful for researchers, practitioners and all other stakeholders who aspire to acquire a working knowledge of this fascinating and yet little-explored subject. This book covers numerous advancements in the field of environmental biotechnology and more specifically in the areas of waste resource recovery and liquid as well as solid waste treatment for achieving the objectives of sustainable development.

I strongly recommend this book. It will provide a valuable resource for professionals, researchers and students in environmental engineering in college, universities and industry settings as 109 distinguished contributors from 12 countries have provided up-to-date information on biotechnological innovations in environmental biotechnology, a timely subject. It is the hour of need that we should find sustainable innovative solutions for the emerging environmental problems.

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Preface

Environmental literacy can be described as the degree to which people have an objective and well-informed understanding of environmental issues. Today, getting an understanding of environmental issues is extremely important. This is because the human economy is engaged in a wide range of operations that inflict tremendous harm on the habitats that support the ecological heritage of both our species and the world. Pollution, global change, collapsing fisheries, deforestation, depletion of agricultural land, loss and endangerment of biodiversity and other destruction are seen all around us. Over the past two decades, the importance of environmental protection and conservation initiatives has been increasingly recognized. Recently during the crisis of the COVID-19 pandemic, restricted human interaction with nature has appeared as a blessing for nature and the environment. Reports from all over the world indicate that after the outbreak of COVID-19, environmental conditions including air quality and water quality in rivers are improving and wildlife is blooming. It is also widely accepted that plans for economic growth have to be consistent with the aim to conserve and protect the environment, and progress towards achieving SDGs. This is the right time that we can say that the pillars of the economy should be based on sustainability. This includes the integration into the process of the creation of environmental dimensions. It is necessary to make choices and decisions that, through understanding the functions of the environment, will eventually promote sound growth. In September 2015, the General Assembly adopted the 2030 Agenda for Sustainable Development that includes 17 Sustainable Development Goals (SDGs). Building on the principle of “leaving no one behind”, the new Agenda emphasizes a holistic approach to achieving sustainable development for all. The ***Sustainable Development Goals*** are the blueprint to achieve a better and more sustainable future for all. They address the global challenges we face, including poverty, inequality, climate change, environmental degradation, peace and justice. As it unveils the embodiments of nature, biotechnology has always been a fascinating choice and an important tool to progress towards sustainability. Environmental biotechnology is more interesting as it envisages how human beings use the wonder of nature. Environmental biotechnology is the application of microbiology concepts and biotechnological methods to solve environmental problems by an integrated engineering approach. It is a large and increasingly growing field that continues to revolutionize the understanding of environmental processes that sustain basic life by discovering and exploiting biomolecules and their utilities in order to provide clean technologies to resolve environmental challenges. In environmental protection, this technology plays a very crucial role. There are several applications in the environment including the use of specific bacteria to enhance nutrient supply to soil and also in pollution control, as the use of microorganisms for wastewater treatment. The technology is aimed at improving the manufacturing process with minimal waste generation, waste recycling, bio-resource growth, waste microorganism manipulation, remediation of polluted environments, chemical degradation, pesticides, heavy metal removal, and surface and groundwater remediation. Environmental destruction is induced by rapid industrialization, population development and natural resource extraction, resulting in global warming, ozone depletion, acid rain and health hazards. There is an increasing worldwide awareness that in the new millennium, biotechnology along with environmental engineering will be the key driving force. The use of biotechnology is important and increasing in the design, control and optimization of biological wastewater treatment.

The use of fossil fuels results in rising CO_2 and other greenhouse gas (GHG) emissions, causing global temperature rise and climate change that will negatively impact human health, food supply, and eventually worsen hunger and misery. In our previous two books by Springer Nature on *Biofuels: Greenhouse Gas Mitigation and Global Warming: Next Generation Biofuels and Role of Biotechnology* and subsequently *Climate Change*, *Photosynthesis and Advanced Biofuels: The Role of Biotechnology in the Production of Value-added Plant Bio-products*, we have discussed these topics in depth.

In this sequence, the recent book provides a detailed idea of this rapidly growing area of environmental biotechnology in particular. The present book aims to provide a detailed view of creative approaches to environmental biotechnology for wastewater treatment, removal of heavy metals, degradation of pesticides, removal of dyes, waste management, microbial conversion of environmental pollutants, etc. Researchers are searching for new ways to boost quality performance and the environment with advances in the field of environmental biotechnology. The prospect of using renewable raw materials as a potential source of energy has been given impetus by recent technologies. The hour of need is cost-intensive and environment-friendly technologies for the manufacture of high-quality materials and successful forms of recycling waste to reduce environmental pollution. Another feasible alternative for remediating environmental contaminants, such as heavy metals, pesticides and dyes, is the use of bioremediation technologies by microbial communities, as historically used physico-chemical technologies have many possible disadvantages, including higher costs and lower sustainability. Efficient biotechnological alternatives are therefore required to overcome growing environmental pollution. There is also a need for environmentally sustainable solutions that can reduce the adverse risks to humans and the environment caused by contaminants. The world's leading research group has contributed value added articles to this book from a variety of perspectives.

The book has two parts.

Part I: Environmental Sustainability and Green Technology

This part describes the role of biotechnology in environmental sustainability, the concept of green technology, bioplastic, biofuel, green vaccination and green tools for remediation of toxic pollutants. In addition, this part provides information about new technologies for wastewater treatment. This part also highlights the application of bioenzymes and geographic information science (GIS) to monitor the COVID-19 pandemic. This part is most relevant for the successful setup of new approaches. The book provides plentiful resources for environmental researchers and is designed to provide both general and specific information for students, teachers, academic researchers and industrial teams.

Part II: Emerging Technologies in Environmental Biotechnology

Emerging technologies in environmental biotechnology have presented Vermifiltration as a sustainable solution for wastewater treatment with its applicability/opportunities, role of nanomaterials and small-scale PVA gel-based solution and bioselectors in the treatment of wastewater. Bioremediation and biodegradation can be an alternative technology to mitigate environmental pollution, sustainable tools for land remediation with the evaluation of residual toxicity.

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We also thank and acknowledge the 109 chapter contributors from 12 countries. We would like to thank our reviewer committee members Dr. Sonika Saxena, Dr. Parul Chowdhury, Dr. Aditi Nag, Dr. Aakanksha Kalra, Ms. Jayana Rajvanshi, and Ms. Sakshi Saraswat, Ms. Devanshi Sutaria, Ms. Samvida Saxena and Ms. Ekta Meena for their constant assistance and support. We are forever thankful to our families for their frequent support.

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We heartily thank all of our co-authors and colleagues who have contributed to this book. We dedicate our book to renowned Dr. B. Lal Gupta, founder of Dr. B. Lal Institute of Biotechnology and Dr. B. Lal Clinical Laboratory Pvt. Ltd. in Jaipur, on his 63rd birthday which incidentally falls on 28th January, and we want to honour his immense contributions to the cause of biotechnology teaching and research along with diagnostics. In this felicitation of Dr. B. Lal Gupta, we are supported by Dr. Aparna Datta, Principal, Mr. Saksham Gupta, Deputy Director, Dr. Sonika Saxena, Vice Principal, and the entire college staff.

Introduction

> *Our goal in life must be to rid ourselves of all bindings, grow more compassionate towards all living creatures, the entire nature and natural beauty and embrace them with open arms. – **Albert Einstein***

Technology is a great industry as it has led to the improvement of the human lifestyle and the world at large. However, due to elevated emissions and increased human activity, it has also had adverse effects on the environment. Environmental biotechnology is the multidisciplinary combination of sciences and engineering in order to employ the environmental problems with the help of biotechnological tools. It is the branch of biotechnology that addresses environmental problems, such as the removal of pollution, renewable energy generation or biomass production, by exploiting biological processes. In the future, the specific position of environmental biotechnology will be increased, taking into account the possibilities of appending novel solutions and recommendations for the remediation of pollutant-contaminated habitats, the minimization of future waste releases and the development of alternatives for pollution prevention. The materials being processed are "waste" materials (contaminated soil and water) and have no inherent value as such. On the contrary, waste treatment is a significant financial burden for businesses, and they would historically not be treated unless they were made to do so. "Waste" products (contaminated soil and water) are the materials being handled and have no intrinsic value as such. Therefore, the industries that produce them have no economic interest in treating them because the treatment does not give companies benefit. On the contrary, waste disposal is a huge financial burden for companies, and unless they were made to do so, they will traditionally not be handled. There's no telling what to expect in the future with the exciting developments in environmental biotechnology. Today, thanks to developments in environmental biotechnology, you can better regulate emissions and practice environmental remediation. Environmental biotechnology is likely to make its greatest contribution to agriculture—especially by improving crop yields. It offers opportunities to design crops for specific environments and to make crops more efficient producers of food and energy. Biotechnology can thus manipulate primary energy flows; it can also reduce fossil-fuel energy inputs into agricultural systems. It could also contribute to the mitigation of environmental problems such as deforestation and soil erosion. Green energy involving biofuels has to replace the fossil fuels to combat pollution and global warming. Biotechnology is a vehicle for better biogeochemical cycle manipulation, with bioremediation and biodegradation being utilized to restore the health of polluted soil, land, and water environments. Industrial biotechnology has the ability to alter the manufacturing process itself, ultimately reducing pollution, conserving natural resources, trimming costs and speeding new "greener" products to market. Emerging biotechnologies with low-input techniques involving microbes and plants offer novel approaches (genetic manipulation or "engineering") for striking a balance between developmental needs and environmental conservation. The chapters of this book will address issues relating to the use of biotechnological methods via innovations in solving the problems of environmental degradation for its sustainability.

Part I: Environmental Sustainability and Green Technologies

Sustainability is development that meets the needs of the present without compromising the ability of future generations to meet their own needs. Environmental sustainability respects and cares for all kinds of life forms, further to minimize the depletion of natural resources. The best method of sustaining the environment is such that it pays back all the components (wastes) in a recyclable way so that the waste becomes useful and helps the biotic and abiotic relationship to maintain an aesthetic, healthy and intricate equilibrium required for a sustainable environment. The role of green engineering and social responsibility in developing chemicals, processes, products and systems is explained in Environmental Sustainability using Green Technologies. Examining the relationship between ecology and the environment, in the creation of a sustainable society, this book discusses many facets of environmental sustainability, examines ways to make more responsible use of resources and processes, and explains the tools needed to address different challenges. It outlines the requisite biotechnological applications, techniques and processes to ensure sustainable growth and to ensure long-term success in the future (Chaps. 2, 4, 5, and 8).

Green technology has been developed and used as an environmentally friendly technology in a way that saves the planet and conserves natural resources as well. The value of green technology cannot be overlooked as part of the clean energy division of environmental technology. We have come to a point where we need to pause and focus on the increasing importance of green technology and why it will be important for humanity. It is green technology that encourages sustainable growth, which implies recognizing sources of growth that are environmentally friendly, the creation of new industries that are environmentally friendly and the development of employment and technology. To achieve green growth, it is necessary to intensify investments and innovations that represent a foundation of sustainable development and open new economic opportunities (Chaps. 6, 7, and 8).

Due to the rapid increase in population, industrialization and urbanization have seen an upsurge in a parallel manner. Nondegradable toxic pollutants generated by anthropogenic activities show a detrimental effect on humans and the biosphere. Toxic pollutants like heavy metals, oil spills, pesticides, fungicides, solvents, plentiful plastics, and industrial effluents cause several environmental health risks. For healing the environment, a sustainable solution, i.e. green technology, has emerged. Green technology generally refers to clean technology or environmental technology that reduces environmental pollution and conserves natural resources. Nowadays green technology is very much acceptable for having advantages like zero or low emission of greenhouse gases, conservation of energy and natural resources, minimization of environmental degradation and better use of renewable resources (Chap. 3).

This part discusses green technologies and sustainability in the innovation and improvisation of the environment by means of biotechnology tools. There are some trending green technologies like bioremediation, biomineralization, phytoremediation, bioaugmentation, bioflocculation, biosurfactant, biocatalyst, membrane bioreactor, wetlands and nano green technology that are used to rescue and secure the environment from toxic pollutants. Various biological organisms, microbes (Acinetobacter, Pseudomonas, etc.), algae (Chlorella, Desmodesmus, etc.), plants (Amaranthus, Camellia sinensis, etc.) or their by-products (exopolysaccharide, enzymes, phenolic compounds, nanomaterials, etc.) are involved in these green technologies. In this chapter, various green technologies and their widespread applicability have been complied for environmentally sustainable development.

Part II: Emerging Technologies in Environmental Biotechnology

For the sake of environmental protection, new developments in environmental biotechnology incorporate emerging technologies for the treatment and management of agricultural wastes and other environmental contaminants. Emerging approaches in bioremediation are explored, such as nanobioremediation technology, electro-bioremediation technology, microbial fuel cell technology, modified activated sludge process and phyto-technologies for industrial waste/pollutant remediation. The removal of toxicity created by natural or anthropogenic actions can be accomplished by various biotechnological approaches. These include a wide spectrum of issues like removal of pollutants by bioremediation, denaturation of toxic compounds by biodegradation and controlling pollution using a bioreactor containing living material to capture and degrade contaminants by biofiltration with subsequent wastewater treatment (Chaps. 21, 23, 24, 26, and 29).

In addition, the book contains new information as well as future directions for biotechnology science. Biotechnology harnesses the most modern cellular and biomolecular approaches to develop technologies and products that help in the sustained development of the environment. Today biotechnological approaches combined with artificial intelligence and machine learning are helping to develop cutting-edge technology for cleaning up of contamination and restoration of a pollution-free ecosystem (Chaps. 32, 38, and 40).

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Editors and Contributors

About the Editors

Sudipti Arora is an Environmental Research Scientist at Dr. B. Lal Institute of Biotechnology, Jaipur, with a specialization in wastewater treatment by Vermifiltration technology and other nature-based sanitation solutions. She is also the founder of “Prakrit: a centre of excellence in Environmental Biotechnology”. Prakrit aims to provide transformative education and innovative solutions to drive the planet towards sustainability.

Prakrit is dedicated to solving the problems pertaining to the environment, particularly water, wastewater, sanitation, health and hygiene, solid waste management, energy and climate change, soil and land use, good health and well-being fulfilling sustainable development goals.

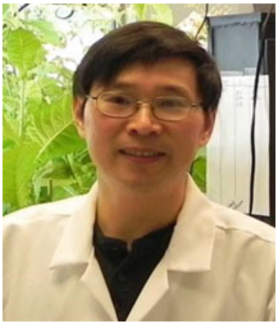
She has obtained her Ph.D. from the Indian Institute of Technology (IIT), Roorkee, and Master’s in Environmental Engineering from Malaviya National Institute of Technology, Jaipur. She is the Assistant Director at Dr. B. Lal Institute of Biotechnology, Jaipur, with teaching and research experience of more than 10 years with various research publications of international repute. She has also been the guest faculty at Malaviya National Institute of Technology, Jaipur. She has been working on indigenous wastewater and faecal sludge treatment through Vermifiltration technology for 8 years and has expertise in integrated solid waste management through circular economy and wastewater-based epidemiology, particularly pathogens in wastewater and AMR. She is a member of the International Water Association and has been involved in WASH projects since 2010. She has been involved in SARS-CoV-2 wastewater-based epidemiology research from India with other Indian and UK International partners through global challenge research funds (GCRF 2020). She is also involved in an Indo-European project titled “Identifying best available technologies for decentralized wastewater treatment and resource recovery for India (Saraswati 2.0)” funded by DST, GOI, and “Optimization of the Disinfection Process for Wastewater Treatment by Hybrid Disinfection” funded by DST, in collaboration with MNIT, Jaipur. She is also involved in an integrated technology intervention project, funded by DST, GOI, on “transforming Aandhi village in Rajasthan towards a zero waste model through green technology interventions”. She is a guest editor and reviewer of various international journals, to name a few, She has authored and co-authored important discoveries in Environmental Biotechnology in more than 20 Science Citation Index Expanded (SCIE) journals. She has guided the research work of 50 UG and PG students. She has been honoured by various awards including “Research Excellence Award 2020 by INSc for outstanding research work on vermifiltration technology, Dainik Bhaskar “Green Parrot Award” in 2018 for her contribution to Indigenous water treatment, Best Poster Award at IWA conference on Global Challenges: Sustainable Wastewater Treatment and Resource Recovery in Kathmandu in 2014, and Young Scientist Award at 8th Uttarakhand State Science and Technology Congress in Dehradun in 2013. She is also been invited to give Expert Lectures at a number of national and international venues, conferences, and workshops.

Ashwani Kumar was born (1946) to Mr. Swami Dayal Tewari and Mrs. Shanti Devi Tewari in Bandikui, Rajasthan, India. He received his B.Sc. at Agra University and his M.Sc. (Botany) at the University of Rajasthan. He was awarded a gold medal for standing first in the order of merit. His Ph.D. (1971) was under the supervision of Professor H.C. Arya and postdoctoral with Professor Dr. K-H. Neumann, and later on with Professor Dr. Sven Schubert at Justus Liebig Universitat, Giessen, Germany, as Alexander von

Humboldt Fellow. His botanist father Professor Swami Dayal Tewari (M.Sc. in Botany) was his first teacher. Professor Ashwani Kumar was also selected for Indian Administrative Services (IAS: IPS) (1972) but he opted a career in botany, which being his family subject. His wife Mrs Vijay R. Kumar has also been a Professor of Botany at the University of Rajasthan in her own stead. He was appointed as Asst. Professor in 1969, Associate Professor in 1985 and Full Professor from 1996 to 2007. Then he was an Adjunct Professor until 2016. He along with members of COC introduced Integrated Biotechnology, a 5-year course in Rajasthan. In recognition of his research contributions, Dr. Kumar was awarded the Alexander von Humboldt Fellowship for 1977–1979, with several resumption of fellowships until 2017, British Council Visitorship, UK (1986); Visiting Professorship at Toyama Medical and Pharmaceutical University in Japan (1999–2000); Toyama Prefectural University, Japan (2011); and INSA-DFG visiting Professorship in Germany (1997). He holds a diploma in German language and has a certificate in French language. His area of research includes photosynthesis in vitro and in vivo, biotic and abiotic resistance, ethno botany, bioenergy and presently understanding salinity resistance in maize. He has also carried out research projects granted by UGC, USDA-ICAR, MNES, CSIR, DST, DBT and FACT. He attended a large number of national and international conferences as an invited speaker and served as chair or co-chair of the International Botanical Conference, Berlin, and EU Biomass conferences. He has published 220 research papers in national and international journals and 23 books, of which 10 books are authored and 13 are edited from reputed publishers such as Springer and IK. He is a member of the editorial board of *Current Trends in Biotechnology and Pharmacy*. He has guided 39 research students to Ph.D. at the University of Rajasthan, Jaipur, India. He is an elected Fellow of Botanical Society, Fellow of Phytopathological Society, Fellow of the Indian Society of Mycology and Plant Pathology, Fellow of Mendelian Association, Fellow of Association of Biotechnology and Pharmacology and Fellow of Indian Fern Society. He received V. Puri Medal as Botanist and Teacher's Excellence award of CEE in 2015. He has been a consultant in a World Bank Project sanctioned to SPRI-HPPI, President of Commonwealth Human Ecology Council (India chapter) and presently President of Indian Botanical Society and President of the Society for Promotion of Plant Science 2021–2022.

Shinjiro Ogita has over two decades of experience in the field of plant biotechnology. In 1992, he started his research career as a master's student at the United Graduate School of Agriculture, Tokyo University of Agriculture and Technology (TUAT), Japan, and in 1997, he received his Ph.D. in agriculture (subject: sciences of resources and environment). He is an expert in the field of cell and tissue culture and transformation technologies for higher plants. He has worked at the following institutes based on projects worked: embryogenic capacity of elite coniferous trees at the Laboratory of Cell Manipulation, Division of Bio-resources Technology, Forestry and Forest Products Research Institute, Ministry of Agriculture, Forestry and Fisheries, Japan (October 1997–September 2000).

- To establish genetically modified decaffeinated coffee plants at the Laboratory of Plant Molecular Breeding, Research and Education Centre for Genetic Information, Nara Institute of Science and Technology (NAIST), Japan (October 2000–March 2003).
- To teach plant biotechnology, microbiology and molecular biology as assistant professor (2003–2006), lecturer (2006–2010) and associate professor (2010–2015) at the Laboratory of Plant and Cell Engineering, Biotechnology Research Centre and Department of Biotechnology, Toyama Prefectural University (TPU), Japan (April 2003–March 2015). He presently works as a full professor at the Faculty of Life and Environmental Sciences, Department of Life Sciences, Prefectural University of Hiroshima (PUH), Japan (April 2015).



Yuan-Yeu Yau obtained his master's and Ph.D. from the University of Wisconsin-Madison, USA. He worked as a postdoc and a specialist at the University of California-Berkeley and Plant Gene Expression Centre (USDA-ARS) in Albany, California, working in the areas of plant biotechnology, plant breeding, plant biochemistry and plant physiology. Dr. Yau worked on projects with grants supported by the NFS (National Science Foundation), NIH (National Institutes of Health), USDA, Cotton Incorporated, California Fresh Carrot Advisory Board and Northeastern State University.

These projects include carrot breeding for fresh market, cottonseed gossypol (a toxic compound) removal and the development of clean-gene technology and of stroke drug using molecular farming. Dr. Yau joined Dr. David W. Ow's team (UC-Berkeley) in developing an operation system for precision transgene integration, stacking (at same locus) and deletion (e.g. removal of SMG) using microbial site-specific recombination (SSR) systems. Professor Yau has authored and co-authored important discoveries in several Science Citation Index (SCI) and Science Citation Index Expanded (SCIE) journals. Dr. Yau also serves as a reviewer for several international scientific journals. He also served as a reviewer for several book proposals for Springer Nature. He is also an editor of several Springer Nature books. He joined the Chinese Academy of Sciences as a professor in 2010 and then joined Northeastern State University of Oklahoma (USA) as a research scientist and adjunct professor in 2012. Dr. Yau in an active member of *Research Gate*. He mentors numerous students from all over the world on *Research Gate*.

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Part I

Environmental Sustainability and Green Technology

1 The Use of Environmental Biotechnology: A Tool to Progress Towards Sustainable Development Goals

Sonika Saxena, Sudipti Arora, and Sutaria Devanshi

Abstract

Environmental biotechnology is the use of science and technical knowledge to remediate and rebuild the environment after it has been harmed. Environmental biotechnology grew in significance and breadth in the 1980s, as a result of the establishment of industry guidelines, regulation of conformity, and the introduction of laws for environmental protection. Biotechnology for the environment is not a modern field of research. It has been around for decades, and we are associated with some of the older innovations, such as wastewater treatment and compositing. In addition, novel approaches to harnessing the promise of biotechnological methods continue to gain traction in practice. This chapter examines the state-of-the-art and prospects of environmental biotechnology, as well as the many fields that it encompasses, as well as the challenges and consequences that come with them. The role of some bioprocesses and biosciences for environmental protection, regulation, and health centered on the use of living organisms is examined in light of the numerous issues that describe and expand the field of environmental biotechnology. Innovative new techniques that advance the use of various approaches like molecular ecology, biomarker, etc. will be taken into account. In order to increase their effectiveness, productivity, and versatility, these approaches will enhance the understanding of current biological processes. The contribution of environmental biotechnology to the creation of a more sustainable society is also significant.

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Environmental biotechnology · Bioremediation · Sustainable development

1.1 Introduction

The environment can be a complex combination of several factors, such as both the physical and biological surroundings, as well as their relationships. Each organism is affected by environmental issues such as ozone depletion, heating, overpopulation, decrease of natural resources, habitat destruction, and so on. We are vulnerable to floods and tragedies as a result of current environmental issues, both now and in the future. Only by comprehending relationships between different living species as well as physical and chemical phenomena will the threatened status of environmental health be modified. Environment pollution and climate change influence each other through complex interactions on Earth. It is essential to study the relevant areas and contribute new knowledge in the fields of the environment and climate. The major reason for climate change is greenhouse gases. Greenhouse gases emission has increased dramatically in recent years due to human activity and natural factors like volcanic eruptions. These gases accumulate in the atmosphere and causing concentrations to increase within time (Kumar 2020a, b). Environmental biotechnology is frequently regarded as a driving force for integrated environmental conservation that leads to long-term sustainability. Sustainable development is described as change in human well-being that will last for several decades rather than just a few years. In order to integrate environmental policy and growth strategies in a global context, a structure is needed. These fields are broadly divided into two categories: environmental sciences and biotechnology. Environmental biotechnology is sustainable because it has a wide capacity to lead to the reduction, identification, and remediation of environmental contamination and waste depletion. It does so by creating renewable processes and materials that are less toxic and have a lower impact on the environment than their predecessors. Its position is critical in the manufacturing, agroforestry, food, raw material, and mineral sectors when it comes to green technology options. Environmental biotechnology is the branch of biotechnology that addresses environmental problems, like the removal of pollution, renewable energy generation, or biomass production, by exploiting biological processes. Environmental biotechnology is often applied to detect, prevent, and remediate the emission of pollutants into the environment in a number of ways. Replacing chemical materials and processes with biological technologies can reduce environmental damage. The International Society for Environmental Biotechnology defines environmental biotechnology as the event, use, and regulation of biological systems for remediation of contaminated environments (land, air, water) and for environment-friendly processes (green manufacturing technologies and sustainable development).

1.2 Role of Biotechnology in Development and Sustainability

"The introduction of a biological organism, device, or processor to manufacturing and service industries" is how biotechnology is characterized. Biotechnology is described as "the application of science and engineering principles to the production of materials by biological agents to provide products and services" by the Organisation for Economic Co-operation and Development (OECD). Modern biotechnology enables us to fight detrimental and unusual diseases; reduce our environmental impact; feed the poor; use fewer and cleaner resources; and create better, cleaner, and more productive modern production processes.

The responsible use of biotechnology to promote economic, social, and environmental benefits is inherently appealing, and it has resulted in a spectacular evolution in research from traditional fermentation technologies to modern techniques to provide efficient synthesis of low toxicity products, renewable bioenergy, and the need for alternative chemicals and feedstocks fielding new techniques for environmental protection. Figure 1.1 depicts six major biotechnology subfields in relation to these application domains.

- Green biotechnology
- Red biotechnology
- White biotechnology
- Blue biotechnology
- Golden biotechnology
- Grey biotechnology

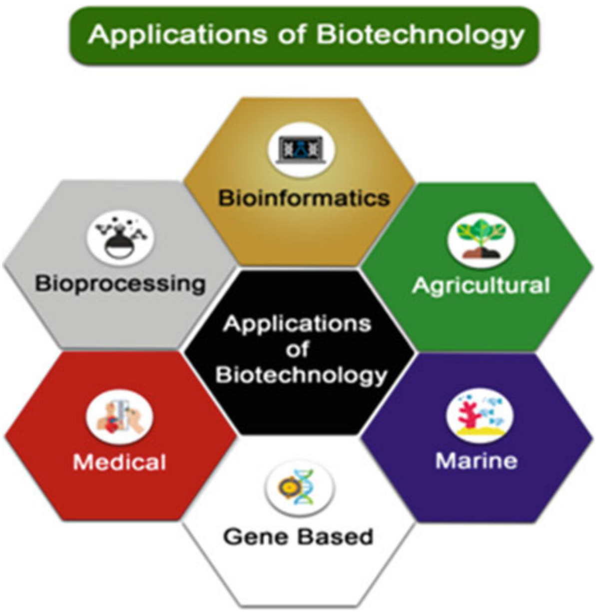

Fig. 1.1 Application of biotechnology in divergent field

This chapter focuses on the developments of biotechnological processes for environmental protection and control, as well as growth expectations and new developments in the field, taking into account the possibilities for environmental biotechnology to contribute with innovative solutions and directions in reclamation and monitoring of contaminated environments, as well as minimizing future waste release and pollution creation.

1.3 Objectives of Environmental Biotechnology (According to Agenda 21)

Environmental biotechnology aims at avoiding, stopping, and restoring environmental degradation by the appropriate use of biotechnology in conjunction with other innovations, while at the same time endorsing protection practices as a key component of the program-relevant goals, which are as follows:

1. Recycling biomass, recovering energy, and minimizing waste generation to implement production methods that allow optimum use of natural resources.
2. Fostering the use of biotechnological methods with an emphasis on bioremediation of land and water, waste treatment, soil conservation, reforestation, afforestation, and land rehabilitation.
3. Applying biotechnological processes and their products to preserve the quality of the ecosystem with a view to long-term environmental protection.

Animals, fungi, bacteria, and other living organisms absorb nutrients to survive and create waste as a by-product. Different types of nutrients are required by various organisms. Some bacteria may tolerate the chemical components of waste materials. Many microorganisms eat substances that are poisonous to others. Recombination research has opened up new avenues for environmental protection and promises to expand bioconversion in the future. Figure 1.2 depicts the different causes of emissions in the area.

1.4 Implementation of Environmental Biotechnology

Protection of the planet is an integral element of sustainable development. Every day, the world is endangered by man's actions. If the use of chemicals, oil, and nonrenewable resources continues to grow as the global population grows, the related environmental concerns are also growing. Despite increasing efforts to avoid the accumulation of waste and encourage recycling, the amount of environmental damage caused by overconsumption is created by the quantity of waste. To some extent, the remedy can be achieved by applying environmental biotechnology techniques which use living organisms in the treatment of hazardous waste and in the control of pollution. A wide variety of uses are covered by environmental biotechnology, such as bioremediation, prevention, identification and tracking, genetic

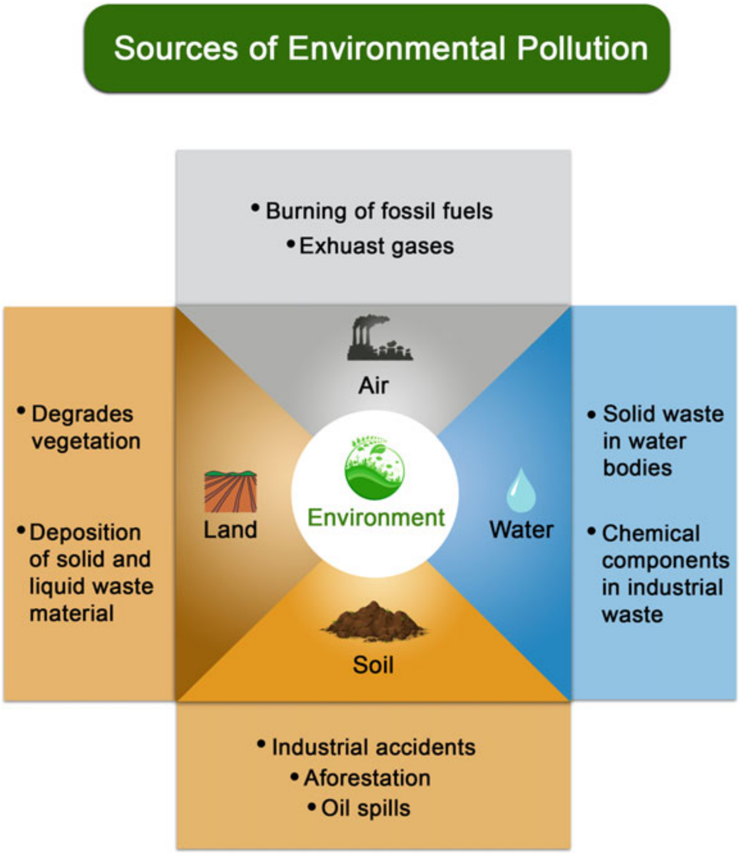

Fig. 1.2 Sources of environmental pollution

modification for sustainable development and enhanced quality of life as shown in Fig. 1.3.

1. Bioremediation
2. Biomarker
3. Biotransformation
4. Bioenergy
5. Molecular ecology
6. Biosensor

1.4.1 Bioremediation

Bioremediation is the process of using microorganisms to degrade toxins that are harmful to the atmosphere and humans. Bioremediation processes typically involve

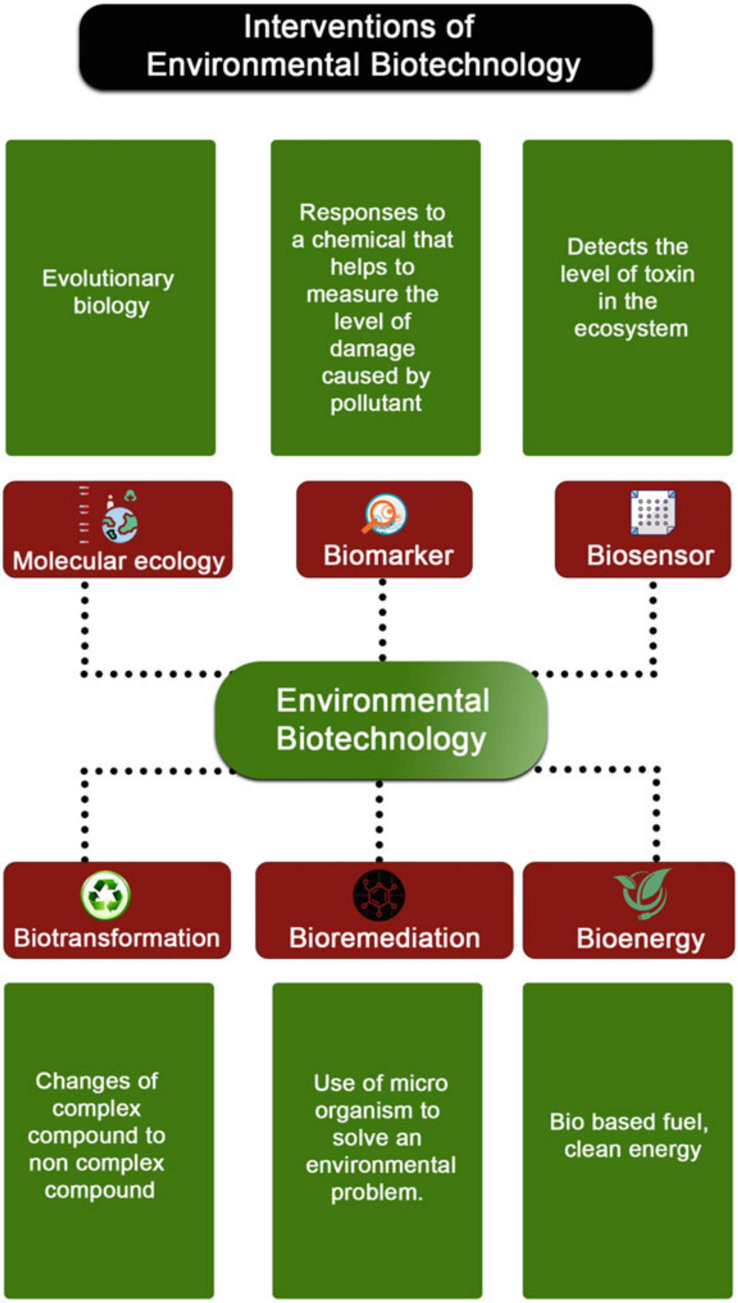

Fig. 1.3 Implementation of environmental biotechnology

the actions of several separate microbes operating in parallel or sequence to complete the degradation process. Both in situ (in place) and ex situ (out of place) remediation techniques are used (removal and treatment in another location). The versatility of microbes to degrade a wide range of contaminants renders bioremediation a technology that can be used to degrade pollutants that face environmental and human risks. Different microbes operating in tandem or sequence are alluded to in bioremediation. Changes in pH, moisture, aeration, or the incorporation of electron donors, electron acceptors, or nutrients may all be used to stimulate biostimulation. Bioaugmentation is a bioremediation technique in which microbes with high oxidation abilities are used to inoculate the polluted site. These two methods are not mutually exclusive; they can both be used at the same time.

Microorganisms use a series of mechanisms to transform chemicals in their system. In some cases, contaminants function as carbon and energy sources for microbial development, whereas in others pollutants serve as terminal electron acceptors. This is manifested in microbes' diverse ability to transform and degrade poisonous molecules. The phases and explanations of microorganism behavior are described below.

1.4.1.1 Factors Affecting Rates of Biodegradation

Temperature, pH, precipitation, carbon inputs, soil composition, aerobic versus anaerobic environments, the number of substituent, and the pollutant concentration can all affect biodegradation. However, making a broad generalization regarding the best universal conditions for biodegradation is difficult. In aerobic conditions, more substituent means slower degradation, whereas in anaerobic environments more substituent means quicker degradation.

1.4.1.2 Primary Substrate Utilization

Primary substrate usage happens when a microbe both converts and uses a substrate as an energy or carbon source. An electron acceptor is required for these transformations. It can be anaerobic or aerobic, with the presence of oxygen seeming to speed up reactions. This form of biodegradation is known to break down petroleum compounds and certain pesticides.

1.4.1.3 Co-metabolism (Utilization of Secondary Substrates)

Co-metabolism necessitates an organism's fortuitous conversion of a compound where the organism's main source of energy or carbon is a different substance. During the actual reaction that degrades the substance, the organism tends to gain little net carbon or energy production, and can even produce a chemical that is toxic to the cell. Co-metabolism is exemplified by fortuitous metabolism in the oxidation of trichloroethylene.

1.4.1.4 Bioremediation Techniques

The degradation of toxins needs ample evidence for bioremediation to be effective. Bioremediation effectiveness is determined by a combination of abiotic and biotic factors. Current laboratory procedures consider the disappearance of toxins and their

Table 1.1 Types of bioremediation and its mechanism

Technology	Types	Mechanism
In situ	Biostimulating	The alteration of the atmosphere to stimulate existing bacteria capable of bioremediation requires biostimulation
	Biosparging	It is the method of introducing the atmospheric oxygen to site through aquifers
	Bioaugmentation	Introduction of genetically modified organism to the contaminated site to support remediation
	Phytoremediation	Process that uses variety of plants to remove, transfer, stabilize, extract, or destroy contaminant in soil
	Bioventing	It helps with in situ remediation of pollutants by providing enough supply of oxygen to microbes
Ex situ	Biopile	Hybrid of landfarming and composting, used for particularly surface contamination.
	Bioreactor	A container used for the condition of raw material to a specific product through a chain of biological reactions.
	Composting	Compost is organic matter that has been decomposed. This approach recycles different organic materials that are otherwise considered waste products and creates a soil conditioner.
	Windrow	Windrow composting is the processing of compost, such as animal manure and crop residues, by piling organic matter or biodegradable waste

degradation products to legal levels regulated by toxicity tests, which are normally performed on single animals or plants to ensure no caused changes that could result in residual toxicity. The concern with these screening methods is that the measurement of pollutants will result in an inaccurate measure of residual toxicity. Rather, it could be a more detailed residual toxicity measure than a single species to research the microbial community response. There are two forms of remediation: ex situ, which is performed by extracting the polluted soil or water and treating it outside the source, and in situ, which is performed inside the contaminated area. There are several types of treatment that can be either ex situ or in situ, as mentioned in Table 1.1.

1.4.2 Biomarker

Chemical toxins in environmental matrices have accumulated dramatically in recent years as a consequence of anthropogenic activity, creating harmful environments for living organisms, like humans. Contaminants have adverse impacts at different levels of biological organization and on different time scales. First, toxins can have effects on the molecular, cellular, and physiological levels until they have more integrated effects at higher levels. Over longer time scales, ecosystem contamination has significant consequences such as habitat erosion and destruction, habitat loss, and natural resource improvements. Pollution is the leading cause of human illness and death in the world today, accounting for an estimated nine million premature deaths (Landrigan et al. 2018). Air pollution is the leading cause of human disease and death, followed by water pollution and occupational chemical exposure (Landrigan et al. 2018). Because of the increasing concern about the negative effects of chemical pollutants on ecology and human health, early warning methods to detect, assess, and analyze the threats posed to the atmosphere by chemical pollutant discharges are becoming more common. In recent years, it has been identified that chemical evidence alone of pollutant concentrations in environmental matrices (air, water, sediments, and soil) is insufficient to accurately determine the possible risks of exposure to living organisms and human health (Lionetto et al. 2019). Furthermore, a number of factors complicate the risk assessment of chemical contaminants for species and habitats, including (a) the chemical diversity and toxicity of pollutants; (b) the simultaneous presence of a number of pollutants in a mixture that may have additive/synergistic effects on organisms; and (c) the bioavailability of pollutants, which is influenced by a number of environmental factors; (d) the different sensitivity of the organisms to pollutant exposure and effects (Connon et al. 2012).

1.4.2.1 Pollution Biomarker

Pollution biomarkers are quantitative measures of shifts in the biological system in comparison to its natural state as a result of pollution exposure (Dagnino et al. 2008). Contamination at lower levels is generally acknowledged to have a quicker onset than pollution at higher levels (e.g., population effects). Molecular and cellular biomarkers may also offer a subtle early indicator of more integrated toxicological symptoms that could occur in communities in the future (Hook et al. 2014). In addition to the calculation of toxins in environmental matrices, biomarkers offer biologically valuable knowledge on exposure to biologically active pollutants and the potential effects of pollutants on the protection of the exposed organisms. Biomarkers for detecting exposure and adverse biological responses to toxins are meeting the growing need for early warning. This explains the rapid growth of this research area in recent years, both in environmental sciences and in human health surveillance. Biomarkers may be used to determine the type and duration of an exposure, to detect changes in an organism, and to determine underlying susceptibility of an organism. They will aid in the better understanding of the mechanisms by which a chemical is consumed and converted within an organism, as well as the cellular and molecular changes that contribute to toxic effects. Biomarkers are categorized as biomarkers of exposure, biomarkers of impact, and sensitivity based on the particular biological reaction used as a biomarker and the point on the spectrum from exposure to toxicity where the measured biomarker originates.

1.4.2.2 Potential Biomarkers

The biomarkers are listed as follows: biomarkers of exposure and biomarkers of impact. Biomarkers of exposure are the responses of an organism to exposure to a chemical compound or group of chemical compounds at its various levels of structural organization. These biomarkers do not, however, provide any information on the toxicological effects of the studied organism and are instead used as an early warning device in relation to a polluting event. For example, plasma esterase (butyrylcholinesterase (BchE) and carboxylesterase (CbE)) inhibition caused by organophosphorus insecticides is among these biomarkers. No adverse effects on the entire organism are caused by the inhibition of these enzymes. Biomarkers of effects are responses that are associated with both exposure to a contaminant and its toxic impact. For example, the effect of insecticides and carbamates (CBs) from organophosphorus (OPs) activates acetylcholinesterase (AchE) inhibition that causes severe acetylcholinesterase (AchE) inhibition. This causes significant harm to the functioning of many species in the central nervous system. It's also possible to identify biomarkers as general and precise. All those responses of an organism at different levels (genetic, biochemical, cellular, physiological, and behavioral) that are not triggered solely by one class of pollutants are general biomarkers. The stress condition of the species in the environment studied is reflected by these responses. The molecular and biochemical responses observable in species as a consequence of exposure to a given class of pollutants are specific biomarkers.

1.4.2.3 Environmental Biomonitoring

Environmental biomonitoring may use measurements of biomarker responses in vulnerable organisms (sentinel species) as an early warning of population-level modification with the aim of measuring environmental quality and assessing environmental changes.

The use of biomarkers in field surveys of polluted environments has grown substantially in recent years. This is due to the fact that biomarkers can be beneficial for decision-making in a variety of environmental insurance activities, such as ecological system and habitat conservation, or the execution of remediation practices, as instruments for detecting pollutant contamination and impact assessment. In addition to chemical characterization on environmental matrices, relevant molecular and cellular biomarker suites are widely used in this context to measure the impact of environmental chemical stress on bioindicator organisms. A number of biomarker responses have been observed in selected bioindicator organisms, especially in invertebrates (Hagger et al. 2008).

1.4.3 Bioenergy

Bioenergy refers to renewable energy made available from biological materials. In the form of chemical energy, biomass is any organic material which has stored sunlight. As a fuel, a number of agricultural processes can include wood, wood waste, straw, manure, sugarcane, and many other by-products. In order to optimize human well-being in the current framework without undermining the potential of future generations, the value of sustainable development has been greatly established as a way of incorporating the environmental, social, and economic goals of society. Negative natural, social, and economic impacts will ultimately result from growth that is not sustainable. In the latest scenario, biotechnology is globally recognized as a fast-growing and far-reaching technology and is appropriately defined as the technology of hope for its promising food, health, and environmental sustainability. Within the bioenergy sector, biotechnology, especially genetic engineering, has the potential for applications to agricultural output; to optimize the biomass productivity of first and second energy crops; to raise the limit of the potential yield per hectare; to change crops to increase their consumption to fuel; and to transform biomass, for example, through the application of biofuels. However, it remains to be seen if genetic engineering will deliver on its commitments, as well as when and how much the different innovations will cost.

1.4.3.1 Bioenergy and Biofuels

The cornerstone of the bio-based sector is a plentiful supply of biomass. Biofuels are oils produced from biomass. The most widely used liquid biofuels are bioethanol and biodiesel. Ethanol is an alcohol that can be used straight in cars built to run on ethanol or blended with gasoline to make "gasohol." Ethanol can be used as an octane-raising, pollution-reducing substitute in unleaded oil, eliminating chemicals like methyl tertiary-butyl ether (MTBE). Biodiesel is a synthetic fuel that resembles gasoline. It may be used as a standalone fuel or in combination with petroleum diesel. Though biofuels for transportation receive a lot of attention, the use of biofuels for cooking is a potentially huge global use, particularly in rural areas of developing countries. Cooking with biofuels will produce far less toxins than solid-fuel cooking. As a result, biofuels have the potential to improve the health of billions of people. Biofuels are primarily divided into two categories: "first generation" and "second generation" oils. The feedstock is the main distinction between these terms, despite the lack of strict technical meanings. A first-generation fuel is one made from sugars, grains, or seeds; it uses only a portion (often edible) of the biomass supplied by a plant above ground; and it is produced using a relatively simple process. First-generation fuels are currently being produced in significant commercial quantities in a number of countries. The second generation of fuels is traditionally made from nonedible lignocellulosic materials, such as nonedible food crop residues (e.g., corn stalks or rice husks) or nonedible biomass production (e.g., grasses or trees grown specifically for energy use). The conversion of lignocellulosic biomass to fuel is a complicated procedure. Enzymatic hydrolysis (which produces so-called biological second-generation biofuels) and gasification (which produces so-called thermo-chemical second-generation biofuels) are the two methods for processing second-generation biofuels. A biofuel derived by a biological method is cellulosic ethanol.

1.4.3.2 Type of Biological Resources for Bioenergy

Microalgae Biomass

Microalgae are single-cell, complex plant biomasses of varying capacity for liquid transportation fuel processing. Microalgal species can be grown in freshwater and saturated saline (or both) with a higher contribution to global carbon fixation if CO_2 from our atmosphere is used productively (up to 40%). These algal biomass can be generated very rapidly (double cycles 6–24 h), depending on the genus or species normally capable of generating energy-rich oils in their overall dry cell biomass. Spp. *Botryococcus* has stored up to 50% of the dry cell mass of long chain hydrocarbons oil. Scientists have various choices for distinguishing new microalgae strains with their genetic information for biosynthesis due to the possible existence of millions of variants in algal organisms. The reduction of nitrates and phosphates as effluents before reuse of municipal wastewater treatment has the ability to exclude microalgal species from sewage systems. The biosynthesis ability of algal biofuels would reduce the usage of fertile lands in comparison to biofuel biosynthetic pathway from higher terrestrial plants. These microalgae activities have provided an acceptable platform with greater potential for the production of biofuels at a lower cost (Hannon et al. 2010).

Since microalgae require less arable land than plant-based feedstocks, microalgal oils are another appealing choice for biodiesel production. Microalgae are being seen as a viable option for the long-term processing of edible oils. Microalgae contain plentiful polyunsaturated fatty acids (PUFA) and have high photosynthetic activity and oil productivity, making them superior to terrestrial oleaginous crops in terms of edible oil production. Since microalgae are grown in water, oil extraction methods for microalgae must account for the effects of water.

Agricultural Crop

Agricultural wastes are left in the farm sector, which helps to mitigate soil degradation by recycling nutrients back into the soil. Biomass residues from crops have been used to synthesize energy compounds thereby preserving soil fertility. Another source of sustainable material residue is milk whey sugar (leftover from cheese manufacturing industries) and manure organic compounds (synthesized from livestock operations centers), which have yielded the most benefits from bioenergy processing through lowering disposal costs and reducing emission speed effective use of biogenic by-products compounds, leftover bits, and waste material has provided alternate carbon sources for renewable energy sources. From conversion technologies through synthesis of final-used energy bioproducts, biomethane, liquid nature biofuels compounds, electric power, and heat energy synthesis has been documented. Energy shortages can be avoided by developing alternative energy supplies and optimizing the energy market system, resulting in energy savings and a decrease in GHG emissions.

Biotechnology applied to the energy sector will provide opportunities for both developed and developing nations if an adequate regulatory system is in place and cautious strategies are developed. It allows the former to use their technological capabilities to ensure national energy stability and avoid major social and economic disruptions caused by changes in fossil fuel supplies and prices. A growing number of countries will be able to reduce their dependence on oil imports, is less vulnerable to oil price fluctuations, and reduce the environmental consequences of fossil fuel combustion.

1.4.4 Use of Synthetic Biology in Biofuel Production

Synthetic biology aims to construct increasingly complex biological systems out of standard interchangeable components. The perfect microorganism for biofuel production will produce a single fermentation product with high substratum utilization and processing capacities. In addition to fast and deregulated sugar transport pathways, some microorganisms have good resistance to inhibitors and products with high metabolic flow rates. Recent findings that heterologous hosts can reestablish plant metabolic processes and that crop plant metabolites can be tailored to increase biofuel productivity have brought molecular biological approaches to enhancing food and biofuel products new hope.

1.4.4.1 Biotransformation

The oxidation of naturally occurring or industrially manufactured organic compounds is one of the most essential functions of microorganisms in nature. Both living species are subject to a large number of xenobiotics, many of which are potentially toxic. The presence of xenobiotics in a living organism will disturb the living body by suppressing its development or interacting with one or more components or chemical reactions on which it relies. The number of mechanisms by which a xenobiotic (pesticide) is subjected to chemical modifications in living organisms is known as biotransformation. Biotransformation reactions (phase I or phase II) are needed to understand the metabolism of endogenous (endobiotic) or exogenous (xenobiotic) molecules, and their role is to increase the defensive mechanisms produced in relation to cells or biological fluids. The toxicity of a cell, tissue, or organism is determined by the equilibrium between the concentrations of parent pesticides, intermediate bio-transformers, and conjugates.

1.4.4.2 Enzymatic Stages of Biotransformation

Biotransformations, including enzymes and whole microbial cells, have been used to make foods and drinks for decades. However, methods for understanding and optimizing the stability and efficacy of biocatalysts have only been established in the last decade. Enzyme innovation and guided evolution have improved enzyme function and expanded the number of biotransformation products available. The use of microbial cells in nonaqueous biocatalytic systems has also enabled researchers to better understand how cells respond to their surroundings. The enzymatically driven biotransformation of organic xenobiotics still proceeds through the measures outlined below. In phase I reactions, the molecule is normally oxidized to increase polarity and provide more reactive groups for further transformation. If the material is highly lipophilic, phase I can entail several oxidative steps. This is the case for the oxidation of polycyclic aromatic hydrocarbons (PAHs). The restricting reaction for the ultimate removal process is the first oxidation of organo-halogenated substances.

1.4.4.3 In Situ and Ex Situ Methods

For both polluted soil and water, biotransformation processes may be used as a cleanup tool and the applications of this technology fall into two different categories:

in situ or ex situ. In situ biodegradation processes treat the polluted soil or groundwater at the site where it has been detected, whereas ex situ biodegradation processes need contaminated soil excavation or groundwater pumping before they can be treated. In situ biotransformation is used where the pollutants cannot be fully eliminated through physical and chemical remediation techniques, leaving residual amounts that are beyond regulatory guidelines.

The applications for biotransformation fall under two broad categories: (1) in situ or (2) ex situ. Processes of in situ biotransformation are used to treat the polluted soil or groundwater in the place where it is located. Until they can be treated, ex situ biotransformation processes involve excavation of polluted soil or pumping of groundwater. In situ techniques do not require polluted soil excavation, so they may be less costly, produce less dust, and cause less contaminant release than ex situ techniques. Often, a huge amount of soil can be handled at once. However, in situ techniques can be slower than ex situ techniques, difficult to handle, and are most successful at permeable soil sites. The natural biodegradation processes that take place in the water-soaked underground area below the water table are intensified by the in situ biotransformation process applied to groundwater. One disadvantage of this technology is that differences in layering and density of underground soil which cause some preferred flow paths to be followed by reinjected conditioned groundwater. On the other hand, ex situ techniques can be quicker, easier to monitor and used than in situ techniques to handle a broader variety of pollutants and soil types.

1.4.5 Biosensors

Biosensors have an excellent analytical method for measuring environmental contaminants with high precision and sensitivity. They are a cheap and enticing alternative to traditional analytical approaches that are capable of providing online tracking in real time. Varieties of biosensors have been developed and are still in development because of the complex effects of these contaminants on the biological system. The main issue is the identification of heavy metals, phenolic compounds, mercury, organophosphorus, and carbamate pesticides among toxic compounds, considering their significant contribution to elevated levels of contaminants. In contrary to direct monitoring, indirect monitoring of pollutants is gaining popularity due to its high vulnerability and hence rapid growth of its market. Due to their accuracy, rapid response times, low cost, portability, ease of use, and continuous real-time signal, biosensors can provide distinct advantages in some situations. Because of their biological foundation, they are ideal for toxicological measurements in safety and health applications. Over the last 3–4 years, the number of publications on biosensors for remote sensing has increased, especially in the field of pesticide analysis.

1.4.5.1 Biosensors for Monitoring Biochemical Oxygen Demand

The requirement for biochemical oxygen (BOD) is the amount of oxygen absorbed by microorganisms during the decomposition of organic matter at a certain temperature under aerobic (oxygen-rich) conditions. The biochemical oxygen requirement test is one of the most widely used and accepted methods for calculating organic pollutants (BOD). Classical BOD tests require lengthy incubation times of up to 5 days. Biosensors have had a significant commercial impact on environmental surveillance in this region. Many commercial BOD biosensors with short reaction times and automatic sampling have emerged as a result of research that began in the 1970s in Japan and Germany.

1.4.5.2 Biosensors for Monitoring Pesticides

Pesticides have become valuable tools in the agricultural sector, as soil is the foundation. Grass, mosquito, and fungal management that is effective and does not damaging crops. Pesticide bioactive compounds are reported in over 600 different ways in the United States alone, with a worldwide demand of more than $20 billion. Insecticides, fungicides, and herbicides may all be divided into three classes. Insecticides are typically organophosphorus (such as parathion), organochlorine (such as DDT), or carbamate (such as carbofuran) substances. Fungicides are made up of sulfur, zinc, or chemical-based compounds, whereas herbicides are made up of organic or inorganic compounds. Pesticides may be applied as a vapor, as dust or granules, or more often as, or in the presence of, liquid (usually water or oil).

1.4.5.3 Biosensors for Monitoring Phenols

Pesticides, pharmaceuticals, plasticizers, bombs, and surfactants are only a few of the things that are processed with phenols. The phenol itself is one of the most essential. Chemicals that are widely used account for about 1.56 million tons of annual US supply and have been identified as a significant organic chemical with the ability to pollute the atmosphere. Biosensors have been successfully used to detect phenols using quinone reduction.

1.4.6 Molecular Ecology

Instead of being propagated as a monoculture in an optimized, controlled environment with nutrients in excess, the recombinant organism is introduced into a community of diverse organisms where it must establish itself interact with other members of the community in unknown ways, and face a multitude of poorly controllable external factors, some of which place it under considerable stress. Some environmental situations, such as those encountered in bioremediation, are patently hostile for the recombinant organism. Thus, whereas contained applications are mainly based on a few well-characterized microorganisms, such as *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and some cell lines which perform well in bioreactors, open applications are based on a more diverse range of organisms able to survive and perform in natural communities in the environment, such as Pseudomonas, Rhizobium, Salmonella, etc. Microbial ecosystems that provide society with services are regulated by environmental biotechnology.

Prominent and emerging services include the removal of water, wastewater, sludge, sediment, or soil pollutants; the processing of useful goods from renewable resources (e.g., biomass), in particular from energy carriers, but also from nutrients, metals, and water; the identification of contaminants or pathogens in the atmosphere or, maybe, in humans; and the protection of the public from harmful exposure to pathogens. In order for modern human society to be clean, prosperous, and stable, these services are necessary. Properly controlled, microbiological communities can provide such services efficiently, continuously, economically, and without creating any other hazards. The new name reflects that environmental biotechnology, as well as other developments in science and technology, is adapting and benefiting from modern molecular biology methods. Based on the plethora of new molecular tools and conceptual insights, environmental biotechnology and molecular ecology should come to terms and evolve approaches that allow transparent and effective management. Molecular ecology is the theoretical bedrock of environmental biotechnology. This will help society to understand the multitude of services rendered by microorganisms to the quality of our world in particular and to our “environmental spectrum” in particular.

1.5 Conclusion

To achieve the objectives of sustainability, a major role will have to be played in the food production fields, green raw resources, biofuel, waste reduction, and bioremediation, along with a wide variety of technologies with the potential to biotechnology. Clean processes and products are a sustainable way to evolve, less harmful, and have a lower impact on the environment, as environmental biotechnology has proven to have a tremendous opportunity to enhance to the reduction, identification, and awareness of environmental waste remediation and depletion. This role is demonstrated with regard to industrial, agroforestry, renewable energy options, food, raw materials, and minerals industries. In terms of genetics, since certain modern approaches require use of changed species, regulation to ensure the safe use of fresh or modified species in the field is essential. Biological methods of all kinds are still in use. However, new technologies are designed to track emission incidents and to continuously control pollutants. The environmental and economic advantages of biotechnology in processing control and waste disposal, as well as technical and economic management, are in line with issues that still need to be resolved. All is being done in a way that has less-negative impacts on the environment and is more sustainable. It has the potential to contribute to the aforementioned primary goal areas and serve as a valuable tool for assessing environmental sustainability.

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Environment Sustainability and Role of Biotechnology

2

Mahender Aileni

Abstract

Sustainability is the development which meets the needs of the present without compromising the ability of future generations to fulfill their needs. Environmental sustainability respects and cares for all kinds of life forms existence without affecting the sustenance of natural resources. The best method of sustaining the environment is paying back all the components of ecosystem services in a recyclable mode. Where in biotic and abiotic harmony of environment restores aesthetic values and ecosystem services of the nature. This in turn maintains intricate equilibrium required for resurrecting the natural ecosystems. Environmental biotechnology is the branch of biotechnology that addresses environmental issues removal of pollutants, renewable energy generation or biomass production, by involving biological entities and their process. Environmental biotechnology has its greatest contribution to agriculture, especially by improving crop yields for environment sustenance. It offers opportunities to create designer crops of specific environments and to make crops more efficient producers of food and energy. Thus, biotechnology can manipulate primary energy flows; it can also reduce fossil-fuel energy inputs into agricultural systems. Moreover, it contributes to the mitigation of environmental problems such as deforestation and soil erosion. Green energy methods/biofuels are urgently needed to replace fossil fuels in order to battle pollution and the threat of global warming. Biotechnology constitutes a vehicle for the improved manipulation of biogeochemical cycles, wherein bioremediation and biodegradation alleviate conditions of polluted soil and degraded water ecosystems. Industrial biotechnology aims to alter the manufacturing process by reducing wastes

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generation-conserving natural resources, trimming costs, and speeding new "greener" market products. Emerging biotechnologies having low-input techniques involving microbes, plants and animals offering novel approaches (genetic manipulation or 'engineering') for striking a balance between developmental needs and environmental conservation. This chapter reviews the issues relating to the use of biotechnological methods vis-à-vis biotools in solving the problems of environmental degradation and sustainable development.

Keywords

Biotechnology · Development · Environment · Genetic engineering · Natural resources · Sustainability

2.1 Introduction

For centuries, humans are in hope that soil, water, and air components of the environment are sufficient enough to sink and assimilate wastes generated by population centers, industry, and farming. While we now know that it's not true. Pollution occurs as a result of improper management of domestic, municipal, and industrial wastes/xenoibiotics (unnatural or synthetic; from the Greek xenons, meaning "foreign) and their rapid accumulation in the environment beyond carrying/assimilating limits of natural ecosystem causes hazard and/or nuisance to mankind and environment (Adriano 1986; Alloway 1990; Raskin et al. 1997). Anthropogenic activities due to urbanization, unsafe agricultural practices, rampant industrialization, mining, and exploration are at the forefront of global environmental pollution (Samant et al. 2018; Dharupaneedi et al. 2019). Environmental pollution is not a new phenomenon, yet it remains the world's greatest problem facing by mankind, and the leading environmental causes of morbidity and mortality.

Currently, human life and environment is greatly affected by three problems: Food security, health problems due to pollutants, and environmental deterioration. Food security and health problems are directly or indirectly linked with the sustenance of clean and safe environment/ecosystem services. These problems need to be attended with eco-friendly approaches so that the biosphere retain, assimilate, restore itself within the carrying capacity, the so-called resilience power of environment. Environment/ecological resilience is the capacity of damage that an ecosystem could withstand without altering its intricate processes and components of functioning. Both developed and developing nations share this burden together, though awareness and imposing stricter laws have contributed to a larger extent in protecting their environment. Today the abuse and overuse of natural resources that result from the pressures of development, population, and poverty have reached a point where the problems of how to sustain the human environment has become a major concern of most governments. The antipollution regulations instituted by most governments remain unforced. However, governments have been enforcing the three Rs: Reduce, Reuse, and Recycle a means to prevent further environmental deterioration. In spite of the global attention to check pollution, the impact is still being felt due to its severe long-term consequences (Arthur et al. 2005). In spite of governments enforcing laws, it is foremost important responsibility lies on mankind in protecting and maintaining the environment in long way.

Sustainability of environment is vital as human progresses into the future. *"Sustainability relies on the principle that meeting the requirements of the present needs without compromising the trend of passing the current available resources to future generations."* Magnification of environmental problem can be reduced greatly by the use of alternative (renewable) energy resources (biofuels/pesticides/herbicides) along with the reclamation of degraded natural ecosystems (Khan and Fu 2020; Pande et al. 2020). It is very crucial to conserve diverse life forms (species) existing on the biosphere (biodiversity), which maintains intricate relations operating at each tropic level of different ecosystems, as loss of single biotic or abiotic factors will drastically affect the biogeochemical cycling/ecological functioning required for environmental sustainability. Biodiversity is the pool from which human race derives food, fodder, fuel, fiber, shelter, medicine, and raw materials for industrial goods required for the ever-increasing aspirations of humans. Biodiversity somehow controls and maintains the stability of physical and chemical factors of the environment. Both food and fuel energy resources need to be served in a sustainable way. Resource recovery and recycling, and hazardous waste disposal, are environmentally-beneficial facets of biotechnology. Anthropogenic activities are accounting for loss of species, habitat destruction, and fragmentation, which are the part of biggest issues faced by the mankind on the road map to environmental sustainability. Environmental sustainability broadly deals with the sustenance (support and maintenance) of the environment to continue life on earth in a normal way, where human development and environmental maintenance go hand in hand.

The need of hour is to address these issues through modern technologies unlike traditional approaches to overcome agriculture/food, health, and environmental issues through breeding, traditional medicines, and pollutants degradation. Biotechnology is an interdisciplinary science and technology whose principles are based on the use of biological entities or its processes for the generation of reliable products and services which are being environmental benign (Haq et al. 2020). Biotechnology concepts centered on biodiversity and its gene pool availability, conservation and later controlled redesign, manipulation, and services offered for the environment and mankind's well-being. Its approaches have implications to cut short the use of fossil fuels and greenhouse emissions by the generation novel means of biofuels and thus produce biodegradable products, recycle wastes, thus making manufacturing process "*greener.*" The extraordinary achievements of biotechnology over past three decades enormous to use its modern tools and approaches in many crucial areas of agriculture, food, health care (medicines, vaccines, diagnostics, gene therapy), bioenergy, environmental protection (Yan et al. 2020; Tang et al. 2020; Peng et al. 2020; Pande et al. 2020; Khan and Fu 2020). The current chapter summarized biotechnology latest trends (Fig. 2.1) in agriculture for global food security, conservation, restoration of RET (Rare Endangered Threatened) and endemic plant species, mitigation of environmental pollutants by bioremediation and phytoremediation, cleaner

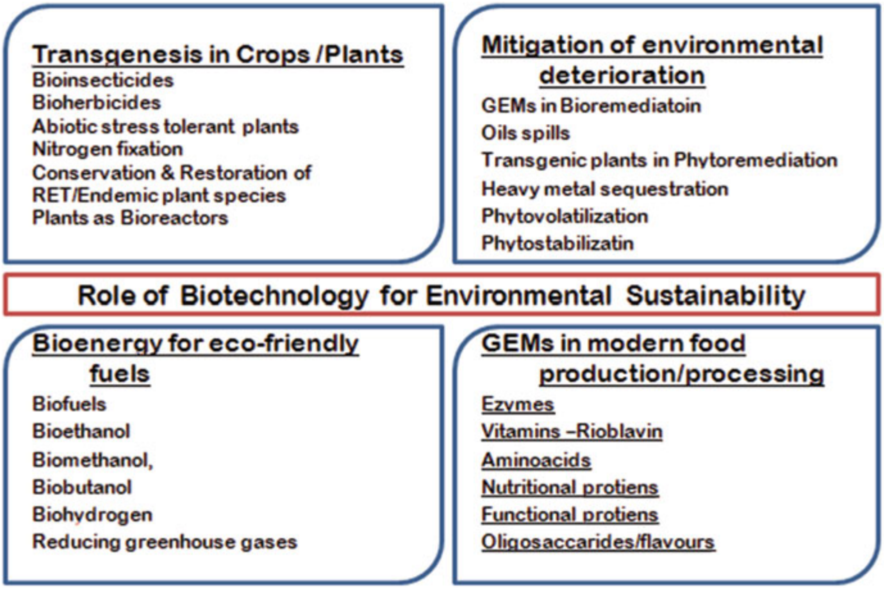

Fig. 2.1 Biotechnology for environmental sustainability

bioenergy/biofuels production by microbes and microbes in modern food production and processing. In line with this, the fears and limitations of biotechnology approaches toward attaining safe environment and possible future directions surmount limitations via the advances mentioned in this chapter.

The low-input biotechnological strategies use biological entities/organisms (microbes, animals, and plants) or bioprocesses aimed at cleaner and more effective ways to maintain the natural and esthetic value of environment thus catering to resurrect the ecosystems. The bioprocesses of the biological organisms are designed, manipulated to produce: (1) Biological alternatives (Bioinsecticides, herbicides, etc.) to replace ruthless use of harmful chemicals in agro-ecosystems. (2) GMOs (Genetically modified organisms) in bioremediation (Kumar et al. 2013; Janssen Dick and Gerhard 2020) and phytoremediation (Ilker et al. 2020) process to alleviate adverse effects of polluted soil and water bodies and for their restoration. (3) Production of Bio-fuels replaces the use fossil fuels thus overcoming environmental pollution & global warming (Tang et al. 2020; Khan and Fu 2020). (4) Genetically engineered microorganisms (GEMs) improved strains of microorganisms having high efficacy and efficiency for modern nutritive (biofortified) food production and process (Zhang et al. 2019; Hanlon and Sewalt 2020). Aforesaid are emerging, reliable, and effective strategies put forward by biotechnology to maintain human health, development, and environment sustenance.

2.2 Applications of Biotechnology

2.2.1 Transgenic Crops/Plants

Climate change and food crisis are pressing problems to scientists and policy makers worldwide. Crop production as of now is extremely vulnerable to climate change leading to low yields and diseases, making difficult to guaranty the food security by 2050. Food crisis due to low crop yields/diseases is increasing at an alarming rate due to adverse effects of biotic and abiotic environmental factors and it is difficult to keep pace of current agriculture production to the demands of raising world population. According to United Nations (UN) Food and Agricultural Organization (FAO) estimate, the world population occupancy by 2050 is expected to reach ten billion people (FAO 2018). If we continue to consume energy and food resources at the current pace, we may need more than 3.5 times the current energy supply and 1.7 times the current food supply in 2050 (Kim and Kwak 2020). Exacerbation of plant growth and productivity due to a wide range of stresses has been significantly affecting future global food security needs. In order to bridge the gap between the supply and demand of the ever-increasing global population, it is indispensable to foster new breeds of stress-tolerant crops with traits conferring higher yields in spite of several environmental abiotic and biotic stresses. Thus, understanding how and to what extent climate change will affect agricultural productivity is crucial. Agricultural biotechnology has been addressing the challenge of the food security and nutraceuticals to increasing population without effecting environmental harmony (Van Montagu 2020). Genetically modified (GM) crops represent the most rapidly adopted technology in the history of agriculture, having now reached 25 years of commercial production (Smyth 2019). These crops grown by millions of farmers, many in developing countries, the technology is providing significant economic and environmental benefits, such as reduction in chemical use of 37%, increased yields of 22% and improved farm profits of 68% (Klumper and Qaimm 2014). This area of research has contributed environmentally friendly crops such as insect resistant, herbicide resistant species; oxidative, salt stress as well crops that can fix nitrogen available in the environment (Bloch et al. 2020).

2.2.1.1 Crops Tolerant to Biotic Stresses

The goals of breeding genetically modified plants correspond to those of conventional plant breeding: on the one hand quantitative (increase in yield) and qualitative improvements (taste, color of the blooms, shelf-life, raw materials), and, on the other hand, developing crop resistance against biotic (fungi, pests, viruses, bacteria, nematode worms) and abiotic stress factors (cold, heat, wet, drought, salt content) of the environment. Plants being sessile, vulnerable to various biotic and abiotic stresses, torment them at each level (morphological, physiological, biochemical, and molecular) that intricate severe repercussions on their growth and productivity.

Agriculture biotechnology aims at sustainable agriculture by solving issues related to food production in an ecological way. Genetic engineering is an effective tool to develop climate-smart crops that can grow in adverse environmental conditions with sustainable yield and productivity (Dhankher and Foyer 2018). One of the early successes of biotechnology is insertion of genes from a naturally occurring soil bacterium, *Bacillus thuringiensis* (*Bt*), into maize, cotton, and other crops to impart internal protection from insect feeding. For many farmers, *Bt* crops are proving to be viable tool for integrated pest management programs by giving growers new pest control choices. In the recent past, several promising microbial insecticides were developed as environmentally friendly biological substitutes replaced the use of noxious chemical pesticides (Samada and Tambunan 2020). Several subspecies of the bacterium *B. thuringiensis* produce parasporal crystals that after ingestion, kills specific insects. In the gut region of targeted insect, conversion of insecticidal pro-toxin to toxin mediated by the alkaline pH and digestive proteases. The death of the insect is the consequence of the formation of membrane channels in the gut cells. Thus lead to futile use of ATP, in turn lead to decreased cellular metabolism, cessation of feeding, dehydration, and eventually insect dies. The *B. thuringiensis* toxins are found to be highly specific for a limited number of insect species, non-toxic to non-target insects and biodegradable in nature. Consequently, they are highly unlikely to cause significant biological selection to develop resistant forms under normal conditions (Azizoglu et al. 2020).

In order to control mosquitoes, *B. thuringiensis* toxin genes were cloned into various microorganisms that live near pond surfaces and thus made to engulf and target mosquito larvae (Laurence et al. 2011). This strategy appears to be an effective means of delivering the *B. thuringiensis* toxin to the targeted insect/pest. Apart from *Cry* genes, several other genes such as trypsin inhibitor, protease inhibitors, and cysteine inhibitors have also been cloned in various crop plants to enhance their tolerance against several insect pest diseases (Tanpure et al. 2017). In another study rhizosphere bacteria has been engineered with *B. thuringiensis* toxin genes lessen the damage caused by insects that attack the roots of plants (Azizoglu et al. 2020). In addition, applied potential of chitinases of *B. thuringiensis* subsp. *Isralensis* cryt1A protein has been studied, a means of gene stacking to confer both fungal and pest resistance (Martínez-Zavala et al. 2020).

Root-knot nematode (*Meloidogyne incognita*) is another group of plant pests that commonly affects the growth and yield of many horticultural crops. Transgenic plants overexpressing several proteinase inhibitor genes (*Cysteine proteinase)* have been considered as the most effective means of controlling yield losses as they block the metabolic process of *Meloidogyne incognita* by activating the proteolytic activity of several other important proteinases (Zhang et al. 2015a). Resistance against root-knot nematode has been developed in transgenic brinjal, tomato, and potato wherein 70–80% less nematode lesions noticed when compared to their non-transgenic plants (Seow-Neng et al. 2017). Chitinases are another major class of genes imparting disease resistance they catalyze the hydrolysis of chitin, an important insoluble constituent of the cell wall of fungi (Chen et al. 2018). Similarly, overexpression of other non-plant antimicrobial genes such as phytoalexins, defensin in transgenic plants was expressed to confer resistance against several fungal diseases (Levy et al. 2018).

The plant viruses do significantly affect crop growth and productivity, and till date conventional breeding has failed to generate crop plants resistant viral diseases (Trębicki et al. 2015; Bakhsh and Hussain 2015). Transgenic papaya plants cloned with the mutated replicase gene resulted in the improved resistance against Papaya Ringspot Virus (PRSV) disease under field condition when compared to wild plants (Fragoso et al. 2017). Moreover, transgenic banana plants overexpressing replicase-associated gene exhibited improved tolerance to the *Banana bunchy top virus* (BBTV); on the contrary, non-transgenic banana plants showed severe bunchy top symptoms (Ghag et al. 2015). Advanced genome engineering technologies, RNA interference and CRISPR Cas9, have received much attention owing to their simplicity and high reproducibility. RNA interference has been reported to play a significant role in tailoring plants with enhanced virus resistance by facilitating the formation of self-complementary hairpin RNA structure under the control of *rolC* promoter, thereby controlling the systemic spread of viral disease (Leus 2018). Plant-dependent RNA interference (RNAi) plays a pivotal role in improving plant tolerance against various biotic stresses (Fang and Qi 2016). In a study, overexpression of dsRNAs in transgenic tobacco plants conferred resistance against *H. armigera* (Tanpure et al. 2017). RNAi in conjunction with transgenic technology has been widely employed to enhance the resistance of crop plants against various stresses (Mamta and Rajam 2018). Furthermore, overexpression of *CYP6AE14* and cysteine protease gene in *Gossypium hirsutum* (*35GhCP1*) transgenic cotton enhanced their resistance against cotton bollworm disease (Mamta and Rajam 2018).

Baculoviruses are pathogenic to many different species of insects, but each strain of baculoviruses is specific to narrow range of insect species. Although baculoviruses kills their host (insect) organisms, the process is usually considered to be too slow in controlling insects that attack crop plants. However, when certain genes are cloned into different strains of baculovirus, the virus can act as a delivery system for a gene that produces an insecticidal protein during the viral life cycle. Several tests of this strategy have been successful in laboratory trails. Recently, when a gene for a neurotoxin that kills insects was cloned into a baculovirus, the construct worked effectively under field trails (Pazmiño-Ibarra et al. 2019).

2.2.1.2 Crops Tolerant to Abiotic Stresses

The world agriculture is in the midst of developing high yielding, disease resistant eco-friendly crop plants and during this changing era of global climate. Environmental stresses such as water logging, drought, salinity, high and low temperature (abiotic stresses), and elevated CO_2 levels affect plant development and pose threat to sustainable agriculture. For sustainable agriculture, it is important to critically investigate abiotic stress physiology and differential gene expression (functional genomics) studies. Plant biotechnology tools are viable enough to maximize plant productivity by introducing stress tolerance genes and key metabolic genes from wild germplasm into adapted cultivars (Kwak 2019). Environmental stresses have become a matter of contention due to concerns about the outcomes of climate change and its negative effects on plant resources, genetic diversity, and ultimately on world food safety (Trębicki et al. 2015). Plant responses to these stress factors are highly intricate and show modifications at the cellular, molecular, and genetic levels. Now, it has been scientifically proven that plant responds differently to multiple stresses as compared to individual stresses. As the changing climate will expose the plants to interactive effects simultaneously, therefore there is a dire need for further research in plant developmental responses to these stress factors; otherwise, this will have a negative effect on sustainable agriculture.

As plants are immobile, they have to tolerate fluctuating environmental conditions in order to survive. Naturally, plants are endowed with the ability to sense climate change and adapt accordingly. With changing environment, plants do evolve and develop precise molecular and cellular mechanisms that enable them to survive under harsh conditions. Unfortunately, little to no research done on how plants cope up under such circumstances, this knowledge gap should be minimized to develop the plant species, which can tolerate individual to multiple stresses (Ahuja et al. 2010). Luckily, several genes encoding stress-tolerant compounds, metabolites, and antioxidants have been identified in related distantly related plants that being exploited for engineering-sensitive plants for multiple stress tolerance. Since conventional breeding approaches which mainly involve varietal cross, mutation breeding and transfer of undesirable genes with desirable genes are supreme limitations. In conclusion, application of transgenic technology is the only viable option to engineer abiotic stress-tolerant plants by altering the expression levels of various genes of stress defense pathway. Recent studies "omics" approach led to better understanding of transcriptome, proteome, and metabolomics of plants by linking with stress perceptions and responses. These approaches are also studied in other crop and woody plant species besides a model plant *Arabidopsis* (Coolen et al. 2016; Varoquaux et al. 2019; Razzaq et al. 2019; Zhang et al. 2019; You et al. 2019).

Biotechnology concepts centred on biodiversity and its gene pool availability, conservation and later controlled redesign, manipulation, and services offered for the environment and mankind's well-being (Zhu 2016). Overexpression of ROS-scavenging enzymes in plants is an effective way to overcome heat stress-induced oxidative damages in plants. In transgenic apple plants, overexpression of cytosolic ascorbate peroxidase (*cAPX*) developed heat tolerance by reducing the membrane damage and improved the photosynthetic efficiency (Zandalinas et al. 2018). Recently, genetic engineering approaches for the development of genotypes with enhanced tolerance to drought stress have been reviewed by Shinwari et al. (2020). Moreover, Ali and Yun (2020) reviewed the role of *Arabidopsis* HOS15 in negatively regulating abscisic acid (ABA) signaling and drought stress tolerance.

Agricultural land salinization due to overuse of fertilizers, receiving scanty annual rainfall altered the soil compositions and pH. Thus, soil salinity is increasingly becoming the most important abiotic stress factor influencing plant growth, development, and agricultural productivity worldwide (Bless et al. 2018). Bulle et al. (2016) demonstrated overexpression of Na+/H+ antiporter gene (*TaNHX2*) in chili as well as in tomato plants wherein resulted increased ability of transgenic chili and tomato plants to adverse effect of salinity stress, improved plant water contents, enhanced accumulation of osmolytes like proline and glycine betaine, and affected the expression of other downstream stress-responsive genes. Akyol et al. (2020)

reviewed novel insights into the root microbiome of halophytes to improve salinity tolerance of crops. Li et al. (2020) reviewed biotechnology perspectives on dryland agriculture environment for sustainable productivity in China. In addition, Paeng et al. (2020) demonstrated the function of the molecular chaperone NPR1 in protecting *Arabidopsis* plants from heat stress.

Moreover, the expression of heterologous genes in plant systems allows enhanced production of a wide range of valuable compounds/metabolites of high commercial value. Plants as bioreactors, it is reported that plants in this context on large scale produce monoclonal antibodies and antibody fragments for cancer treatment (Mu et al. 2020); the polymer polyhydroxyalkanoates (PHAs) to make a biodegradable plastic-like material (Dobrogojski et al. 2018). Many biopharmaceuticals including recombinant vaccine antigens, monoclonal antibodies, and other commercially viable proteins are produced in plants, some of which are in the pre-clinical and clinical pipeline (Shanmugaraj et al. 2020).

Environmental Safety Plant stresses due to global climate changes threatening the crop productivity and food security. In view of global food demand, breeders and genetic engineers have to aim up to improve the yield and quality of major crops plants without neglecting the environmental safety. Several countries, including the USA, have adapted the principle of "substantial equivalence" when evaluating the safety of genetically engineered foods. This implies that genetically modified plant or animal food products must be similar in composition to the corresponding conventional food. Levels of nutrients, anti-nutrients, and natural toxins must not be different, and animal-feeding trials must not show differences in the development, health or performance of the animals that would indicate reduced nutritional or increased toxicity or allergenicity of the genetically modified food. If the genetically engineered product is not substantially different from the conventional product, labeling them as genetically engineered is not required.

With the introduction of (genetically modified)GM technology, particularly Bt cotton, have resulted in significant reductions in pesticide poisoning cases due to reduced applications and reduced levels of insecticide exposure (Smyth 2019). Reductions in farmer pesticide poisonings have been quantified in China, India, Pakistan, and South Africa. In South Africa, farmers reduced pesticide applications from 11.2 per year to 3.8%, in addition reported cases of pesticide poisoning declining from over 50 per year to <10 over the first 4 years of Bt cotton adoption (Bennet et al. 2003). It is reported in China that one-third of non-Bt cotton farmers got pesticide poisoning, while this is very less (9%) in comparison to Bt cotton producing farmers (Hossain et al. 2004). Following an assessment of the health effects in India, it was discovered that there was a 2.4–9 million case reduction in pesticide poisoning every year (Kouser and Qaim 2011). Cumulatively, since 2003, when Bt cotton was first commercialized in India, a minimum of 38 million fewer instances of pesticide poisoning have occurred, with an upper potential of 144 million. Farmers in Pakistan growing non-Bt cotton reported up to seven instances of pesticide poisoning in the growing season with 35% reporting no instances, versus Bt cotton farmers reporting up to six poisonings with 45% reporting none (Kouser and Qaim 2011).

In fact, GM crops with traits for insect resistance and herbicide tolerance have contributed to reduce agriculture's environmental footprint by facilitating environmentally friendly farming practices. The adoption of GM insect resistant and herbicide tolerant technology has reduced pesticide spraying by 775.4 million kg (8.3%) and, as a result, decreased the environmental impact associated with herbicide and insecticide use (Brookes and Barfoot 2020). Klumper and Qaimm (2014) conducted a meta-analysis based on primary data from farm surveys or field trials in different regions worldwide. This comprehensive study demonstrates that GM insect resistant (IR) traits have reduced pesticide usage by 36.9% on average. According to a medical evaluation of Chinese farmers that included health indicators, fungicides used in non-Bt cotton production were linked to liver dysfunction, while insecticides used in non-Bt cotton production were linked to severe nerve damage (Zhang et al. 2016).

The release of insect resistant crop varieties in the fields has begun to have a noticeable potential to improve human health by the reduction in cancer rates. Prior to the commercialization of Bt crops, chemical spray to control insect crop damage in maize increased the potential for the development of harmful health effects. With existing food security challenges in many developing countries, corn containing mycotoxins are consumed as part of the household diet due to the lack of any other option. A meta-analysis after 21 years of maize production quantified that Bt maize has lower concentrations of mycotoxins (29%), fumonisins (31%), and thricotecens (37%) (Pellegrino et al. 2018). Mycotoxins are toxic and carcinogenic to humans and animals as well. Fumonisins are correlated to being the cause of higher rates of neural tube defects in high maize-based diets (Missmer et al. 2006). Several crop plants which are grown at large scale are engineered to resistant to weeds, thus the crop productivity has been increased dramatically by reducing the agronomic competition for nutrients and space in between main crop and weeds. This approach has become most successful and is the basis for the largest number of transgenic plants that are used in the field (Gesine et al. 2017).

Genetically modified crops have made significant contributions to address the United Nations Sustainable Development Goals, in particular goals in reducing poverty and hunger. Moreover, increased yields have contributed to higher household incomes, reduce poverty; the increased yields witnessed household food security (Smyth 2019). In many developing countries, plant-based nutrient intake accounts for 100% of an individual's nutrient diet, thus highlighting the importance of nutritionally enhanced crop-derived foods. Nutritionally fortified foods improve an individual's nutrient intake, preventing leading causes of death due to morbidities (cancer, diabetes, cardiovascular disease, and hypertension). Biofortified GM crops have been adopted for increasing micronutrient availability in human nutrition (Hefferon 2015).

Production of transgenic plants, at large scale happens via transformation and expression of transgenes in the nuclear genomes. Among the ecological concerns raised about genetically engineered organisms is that transgenes could move ("transgene flow," the process of containing transgene by recurrent hybridization) via pollen from the crop and into related wild varieties growing in natural or seminatural communities. Such concerns are addressed by new field of transgene containment called as Transplastomic technology or Plastid transformation technology. Since plastids are inherited maternally in the majority of angiosperm species, they would therefore not be found in pollen grains of corps. Insertion of transgenes, therefore, into the plastid genome has the potential of preventing gene flow via pollen. Hence, the plastid transformation technology is considered as environmentally friendly method because plastid and their genetic information are maternally inherited in many species due to consequent lack of transmission of plastid DNA by pollen (Wani et al. 2010). In addition, it is important to ease public concern and increase public acceptance production of marker free transplastomic plants. As chloroplast genome is capable of expressing more than 120 foreign genes originated from different organisms (bacteria, animals, viruses, fungi, and humans), addressing other barriers will make chloroplast genome very attractive site to avail biotechnological applications with incredible impact on human life and environment (Srinivas et al. 2016; Adem et al. 2017).

Genome editing is the alteration of the genomic DNA of an organism with high accuracy and efficiency using site-specific genome targeting methods. Unlike conventional mutagenesis methods, Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR) CRISPR-associated protein 9 (CRISPR-Cas9), emerged as the popular strategy currently employed for genome editing. The CRISPR-Cas9 principle based on the adaptive immune response in archaea/bacteria which protect themselves from invading foreign DNA by catalyzing sequence-specific cleavage of nucleic acids which result in the generation of double-stranded breaks (DSBs). Once generated, these DSBs are repaired either by nonhomologous end-joining (NHEJ) method or by homologous recombination (HR), thus causing modification of target site within the plant genome, and uses three types of engineered nuclease, viz., zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALENS), and CRISPR-Cas, for directing site-specific cleavage (Bortesi and Fischer 2015). CRISPR-Cas9 has been widely used in a variety of agricultural crops such as in tomato, citrus, grape, etc. for activation or repression of certain target genes. Numerous investigations have reported that CRISPR-Cas9 is extremely helpful in developing virus-resistant crops for, e.g., researchers have successfully developed virus resistance cucumber plants with nonfunctional eukaryotic translation initiation factor 4E (*eIF4E*) using CRISPR-Cas9 technology. The developed transgenic cucumber plants exhibited enhanced tolerance to *Cucumber vein yellowing virus* and *Papaya ring spot mosaic virus* (Chandrasekaran et al. 2016) as well. In another study, transgenic grape cultivar in which ribonucleoproteins were mutated using CRISPR-Cas9 approach exhibited enhanced tolerance to powdery mildew disease, and transgenic apple cultivar mutated for the same gene using CRISPR-Cas9 showed enhanced resistance against fire blight disease (Malnoy et al. 2016). Genome editing system (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9) has revolutionized crop improvement by enabling robust and precise targeted genome modifications for the development of transgene-free disease resistant crops (Ahmad et al. 2020; Chen et al. 2019) In Fig. 2.2 genome editing with CRISPR-Cas9 for transgene-free disease resistant crops has been depicted.

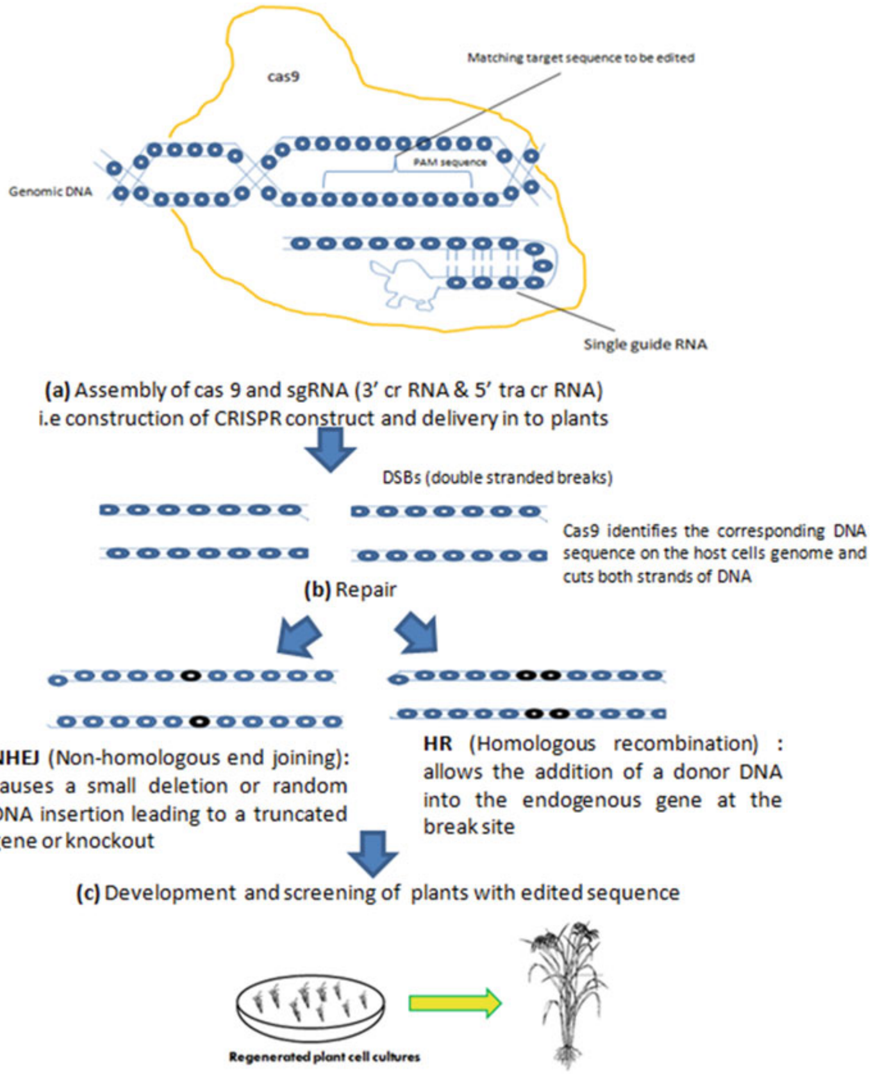

Fig. 2.2 Genome editing with CRISPR-Cas9 for transgene-free disease resistant crops: Plant trait improvement through crispr-cas9 system of genome editing found to be efficient, robust, time saving, less laborious, and cost-effective. Construct with sgRNA and cas9 are assembled and when transformed to generate crops plants tolerant to biotic and abiotic stresses could help to meet the food security in a sustainable mode

Concluding Remarks Millions of farmers producing Bt cotton are less likely to be poisoned by pesticides; worldwide, 17 million farmers growing GM crops have reduced chemical exposures (Smyth 2019). Environmental stresses are responsible for reversible and irreversible changes which minimize plant plasticity leading to reduced growth and agricultural productivity. To overcome the food shortage, it is very important to generate more climate-resilient plants in years to come. Currently holistic approaches are needed from several biological kinds of research to study individual and multiple stress conditions effecting crop plants performance. Nevertheless, the integration of OMICS approaches could help to identify stress related genes and regulators, which aids in manipulation of candidate genes for the development of climate-resilient plants. Genome editing using artificial nucleases particularly,(CRISPR), CRISPR-associated protein 9 (Cas9), has revolutionized basic as well as applied research including plant breeding by accelerating the editing of target genome in precise and predictable manner. Genome editing will greatly facilitate the engineering of complex traits, such as stacked disease tolerance (stacked traits are insect resistance and herbicide tolerance as these traits may lead to lesser use of pesticides, higher yield, and efficient control of weeds), resilience to abiotic stress, as well as nutritional and organoleptic properties (Halewood et al. 2018). For example, the de novo domestication of wild tomato, a display on how to leverage the genetic variety of wild plants (Zsogon et al. 2018), demonstrates the promise of precision genome engineering for agricultural enhancement (Khanday et al. 2018).

The hallmarks of CRISPR/Cas9 are the development of transgene-free disease resistant crops. The production of transgene-free plants (e.g. in *A. thaliana*, rice, tobacco, lettuce, wheat, potato, etc.) via this technology may avoid the plants from GMO label. Thus, powerful genome editing tool—CRISPR/Cas system in conjunction with conventional and modern breeding technologies should be utilized to cope with environmental stresses and to secure the world food security by 2050. Genetic engineering and genome editing are ready to be deployed at the earliest to improve crop breeding and build a more sustainable agriculture.

2.2.1.3 Nitrogen Fixation in Cereal/Non-legumes

Nitrous oxide responsible for global warming potential of 296 implies that it has a global warming potential (GWP) about 300 times greater than carbon dioxide—this means that 1 lb of nitrous oxide is counted as 296 lb of CO_2. Nitrous oxides persist in the atmosphere for more than 100 years. The formation and release of N_2O from agricultural fields happens when excess nitrogen fertilizer applied to crops interacts with common soil bacteria. It is estimated that nitrogen fertilizer accounts for one-third of the GHGs (greenhouse gases) produced by agriculture, reduced fertilizer reduces nitrogen pollution of ground and surface waters.

Bacterial genus *Rhizobium* fixes up to five times more nitrogen globally per year, than the amount of nitrogen fixed industrial Haber-Bosch process. Deployment of synthetic nitrogen fuels in modern agriculture and its production is energy-intensive, need massive expansion in land use and its application leads to aquatic pollution and greenhouse gas emissions (Bloch et al. 2020). Sustainable intensification of agriculture to provide food for humans, feedstock's for biobased fuels and materials requires alternative options for nitrogen management. One of the greatest challenges in agriculture is supplying sufficient plant-available nitrogen to cereal crops, which provide 45% of the world's dietary energy consumed directly by humans (Zhang et al. 2015b). For nearly 50 years, nitrogen fixation in cereal crops has been pursued to address this challenge. Engineering the capacity to fix nitrogen in cereals, either by themselves or in symbiosis with nitrogen-fixing microbes, represent attractive future options that, nevertheless, require more intensive and internationally coordinated research efforts. Biological nitrogen fixation might be a viable option for long-term agriculture (Soumare et al. 2020). Biotechnology techniques have been trying to transfer nitrogen-fixing (nif) genes from *Rhizobium* strains to other organisms. Recent study reports on transferring nitrogen fixation (*nif*) genes to non-diazotrophic hosts. These advances have laid the groundwork to enable cereal crops to "fix" nitrogen themselves to sustain their growth and yield. Transfer of *nif* genes into rhizosphere microorganisms another avenue needs further research and field testing, particularly under stress conditions that impede the effective use of *Rhizobium* strains. Efforts to engineer plants for nitrogen fixation have made strides through eukaryotic nitrogenase expression and a deepened understanding of root nodulation pathways, but deployment of transgenic nitrogen-fixing cereals may be outpaced by population growth. By contrast, a root associated bacterium that can fix and supply nitrogen to cereals could offer a sustainable solution for nitrogen management on a shorter timescale (Bloch et al. 2020). While, research is also being carried out on nitrogen-fixing bacteria (Frankia & Azospirillum species) which has symbiotic association with trees or crops.

Conservation and Restoration of Plant Species Due to anthropogenic and natural causes, endemic plant species are usually more vulnerable to higher rate of extinction risk. Unfortunately, many of these plants are under the threat of extinction due to habitat depletion, habitat destruction, and overexploitation, for which conservation efforts are required to ensure their long-term availability. In addition to climate change, invasion of alien/exotic species, biomagnifications of pollutants pushed native species to the verge of extinction. At global contest for preservation of such species in wild status, in situ conservation alone will not assure their conservation and restoration. Moreover, several active phytochemicals from different parts of rare, endemic, vulnerable medicinal plants were isolated and used as drugs either alone or in combination with other compounds. Moreover, ex situ conservation strategies must be undertaken for conservation of these species, for which establishment of seed banks are the more efficient and cost-effective methods. However, when seed banking is not an option, alternative approaches should be considered. Biotechnological tools are more viable and complementary option for plant conservation including short-, medium-, and long-term strategies, and their application for plant species conservation has increased considerably in the last years (Corlett 2017).

Biotechnology largely contributed to plant conservation and restoration biology this is not to replace the traditional conservation methods but to complement and improve the methods established (Engelmann 2011). For conservation, shoot tips and seeds are the most preferred explants, however, micropropagation/ clonal propagation using various explants is the most common in vitro culture technique for conservation of RET (Rare, Endangered, Threatened) and Endemic plant species. This involves the production and rapid multiplication of true to type plants using various explants under controlled and aseptic condition (Yarra et al. 2010; Aileni et al. 2011; Kokkirala et al. 2012). Plant conservation biotechnology comprises not only the conservation of plant genetic resources but also its management, characterization, and application (sustainable use) (Coelho et al. 2020). In particular, in vitro conservation, which is the maintenance of plant germplasm in culture collections using tissue culture technologies, can provide easy access for the evaluation, utilization, and safe exchange of plant material (Deepa and Dennis Thomas 2020). Because fast-growing trees are more efficient at exploiting CO_2 for photosynthesis, micropropagation and synthetic seed generation using plant tissue culture techniques are recommended. The major problem is the continuous deforestation that is associated with a possible increase in atmospheric $CO_{2.}$ The conservation of 33% of forests and plantations coverage is the need of hour. Certain biotechnological approaches have also been made to improve the CO_2 utilization for enhancing photosynthetic efficiency. The enzyme ribulose-bisphosphate carboxylase (RUBPcase) is closely associated with plants CO_2 assimilation and fixation. In line with this attempts have been made to genetically manipulate this enzyme for increasing the biomass and photosynthetic efficiency. Recently, research was conducted to improve the photosynthetic capabilities of several C_3 crops, either by the introduction of C_4 genes or through the overexpression of C_3 genes (Ashraf and Harris 2013).

Cryopreservation, when long-term (several years), uses liquid nitrogen at ultra-low temperature (−196aniC) to store plant materials, wherein cells metabolic process is slowed down almost completely. Retaining the genetic viability and timely retrieval of cell culture are very crucial during conservation studies. Cryopreservation of plant genetic resources of crop or economically important species is being carried out and similar conservation studies are applied from RET and endemic plant species (Haque and Ghosh 2016). A medium-term (1–15 years) in vitro conservation technique is slow growth culture, which is maintained at low temperature and low light intensity to reduce the growth of plants. Synthetic seed technology for the production of artificial seed using somatic embryos, meristems or adventitious shoots is another viable in vitro conservation technique in several plant species (Rihan et al. 2017). Low survival rate and genetic variations including epigenetic changes in cryopreserved plant materials are problems associated with ex situ conservation strategies. There is an urgent need to expand conservation research and particularly transfer of this academic knowledge to the actual implementation of conservation strategies and their restoration.

2.2.2 Mitigation of Environmental Deterioration

Over the past 20–30 years, large scale of industrialization and traditional agricultural practices contributed diverse and harmful loads of pollutants (organic halogen compounds, metal and sulphur containing compounds, inorganic and organic acids) to environment which has resulted in tremendous pollution of air, water, soil leading to their intervention in natural ecological process of organisms and environment. The problem is worldwide and possibly causing threat to both the environment and human health (Samant et al. 2018). The use of pesticides and herbicides helps to increase agricultural productivity; however, unremitting application of these noxious chemicals causes a huge loss of biodiversity and contamination of agricultural land, enter the food web, whereby get biomagnified (Pande et al. 2019). Such pollutants are prime causes of life-threatening human degenerative diseases and morbidity (cancer, Alzheimer's disease, atherosclerosis, Parkinson's disease, etc.). Conventionally, incineration and chemical treatment have been used to breakdown many toxic chemicals, but these methods are expensive and often create new environmental problems (Arthur et al. 2005).

2.2.2.1 Bioremediation

Environmental pollution is the major obstacle in the past several decades owing due to increasing human activities on natural reservoirs, unsafe agricultural practices and rapid industrialization. Bioremediation is the process involves plants and microbes natural capacity of cleaning the environment by degradation, detoxification, and sequestration of contaminants present in water and soil (Adriano 1986; Alloway 1990; Ojuederie and Babalola 2017). Bioremediation facilitates the restoration of contaminated and degraded habitat by removing up hazardous wastes is an innovative, environmentally safe, noninvasive process and results in safe and recyclable end forms (Shah 2011; Ojuederie and Babalola 2017). Bioremediation can be carried in situ or ex situ methods. In the in situ bioremediation, the contaminants are treated directly at the site, whereas in ex situ bioremediation the contaminants are collected from the site and are treated elsewhere. Till now little number of microbes (culturable microbes) have been exploited and a huge microbial biodiversity is still unexplored. In addition, to enhance the metabolic potential of the microbes for ecological restoration and degradation of recalcitrant pollutants, various forms of bioremediation approaches such as bioaccumulation, biosorption, biotransformation, biomineralization chemotaxis, biostimulation, bioaugmentation, biofilm formation, application of genetically engineered microorganisms (GEM) with improved catabolic activities, advanced omics have been widely employed (Girma 2015; Pande et al. 2020). In the past few years, the metabolic potential of microbes is being used for efficient degradation and remediation of environmental wastes (Janssen Dick and Gerhard 2020). Using bioremediation methods, highly toxic pollutants were converted into less non-toxic forms using the different metabolic potentials of microbes such as transformation, mineralization, and immobilization. However, xenobiotic compounds such as highly halogenated, nitrated aromatic compounds, and pesticides are not investigated on their microbial degradation till date (Gangola et al. 2019). It is tricky that the efficiency of microbial degradation relies on different factors, i.e. concentration, chemical nature of pollutants, physiological features of the environment, and nutritional requirements of microorganisms.

It is known that microbial genus *Pseudomonas* is the predominant group of soil microorganisms used to degrade xenobiotic compounds. Biochemical assays showed that various *Pseudomonas* strains capable break down and as a consequence, detoxify hundreds of different organic compounds. In many cases, one strain can use any of several different related compounds as its sole carbon source. The biodegradation of complex organic molecules generally requires the concerted efforts of several different enzymes. In some organisms the genes that contribute to the biodegradative pathway are found in both chromosomal and plasmid DNA as well. In general, degradative bacteria in most cases, enzymatically convert xenobiotic, nonhalogenated aromatic compounds to either catechol or protocatechuate. Later, through a series of oxidative cleavage reactions, catechol and protocatechuate are processed to yield either acetyl coenzyme A (acetyl-coA) and succinate or pyruvate and acetaldehyde, compounds that are readily metabolized by almost all organisms. Despite the ability of many naturally occurring microorganisms to degrade different xenobiotic chemicals, there are certain limitations to the biological treatment of those waste materials. For example, (1) no single microorganism can degrade all organic wastes; (2) high concentration of some organic compounds can inhibit the activity or growth of degradative microorganisms; (3) most contaminated sites contain mixtures of chemicals, and an organism that can degrade one or more of the components of the mixture may be inhibited by other components; (4) Many nonpolar compounds adsorb on to particular matter in soils or sediments and become less available to degrative microorganisms; and (5) microbial biodegradation of organic compounds is often quite slow.

One way to address these problems is to transfer plasmid genes carrying different degradative pathways by conjugation into a single bacterium. Way back, Chakrabarty and his coworkers for the first time genetically engineered bacterium (*Pseudomonas*) carrying plasmid genes of different pathway encoded genes involved to degrade four different organic molecules (camphor, octane, salicylate, and naphthalene). The first genetically engineered microbe was created by Chakrabarty group in 1971. The patent was approved in 1980 by the United States Supreme Court, later, variant of the genus *Pseudomonas* capable of degrading crude oil constituents has been investigated (Ezezika and Singer 2010). Azad et al. (2014) narrated a comprehensive review of the use of genetically engineered bacteria and plants on the bioremediation of contaminated soils having heavy metals and other organic pollutants.

Because native microorganisms are not capable of carrying out pollutant cleanup, the use of genetically modified microorganisms (GEMs) for in-situ bioremediation of damaged habitats is being studied. The aim of genetic engineering for bioremediation is to modify plants, microorganisms, and enzymes so that they would be useful tools for degradation of harmful substances (WolejKo et al. 2016). GEMs have been demonstrated successfully as they have better genetic makeup to lessen the effect of harmful pollutants in the surrounding environment (Azad et al. 2014; Shah and Pathak 2019). Dixit et al. (2015) reviewed that several engineered microbes are competent strains for bioremediation of contaminated environment as they have enhanced ability to breakdown a variety of contaminants. Some of the most widely used genes in bioremediation process include t*merA* gene for Hg up take, phenol catabolic genes (*pheA, pheB, pheC, pheD, and pheR*) (Marconi et al. 1997) and the ArsM gene for the removal of As from contaminated soils (Liu et al. 2011). It is also reported by Dixit et al. (2015) that expression of *mer* operon (genes) from

Escherichia coli encoding Hg^{2+} reduction into genetically engineered bacterium *Deinococcus geothermalis* gave that microorganism ability to reduce Hg contamination at high temperatures. In a study, site directed mutagenesis and rational design tools of biotechnology has been used in manipulations of enzymes involved to degrade organic compounds (Kumar et al. 2013). Moreover, deployment of GEMs in bioremediation process is found to be indispensable tools in the war to degrade noxious substances of polluted sites. In the same process, it is very important to check stability of GEMS to perform consistently under field conditions (Ghosal et al. 2016).

Bioremediation process necessitates the blending of diverse multifaceted variables that will provide us a better understanding and prediction of pollutant's fate. Maintenance of controlled environmental factors and with standing opposition from indigenous microbes is the prerequisites need to be worked out for successful application of GEMs during bioremediation process (Dixit et al. 2015). In addition, novel molecular techniques should be explored to screen and isolate microorganisms for use in heavy metal bioremediation. With advancements in the areas of modern techniques of genetics and omics (genomics, proteomics, and metabolomics), it has enabled scientists to investigate and understand the physiology, ecology, and biochemistry of polycyclic aromatic hydrocarbon (PAH)-degrading microorganisms (Ghosal et al. 2016). These approaches also help us to assemble information on biodegrading genes, proteins, and metabolites. Moreover, with the advent of NGS (next-generation sequencing) and in silico techniques in this area facilitated to use metagenomic, proteomic, and bioinformatic studies of diverse eco-friendly microbes which render an exceptional understanding of major pathways for biodegradation.

Metagenomics studies proved to be significant in analyzing the functional range of the microbial consortia. Currently, several investigations on metagenomics have given key concepts aid to enhance bioremediation (Duarte et al. 2017; Tripathi et al. 2018). Furthermore, the use of GEMs in the bioremediation process has been discovered to be essential instruments in the battle to decompose noxious compounds at contaminated locations (Jaiswal et al. 2019; Malla et al. 2018). Metatranscriptomic investigations have profound interest in environmental restoration, as these are used to verify the gene activity within a specified environmental condition. The combined effects of omics based practices with environmental proteomics can help to give much better outcomes. Moreover, Niu et al. (2018) studies have explored the significance and success of comparative transcriptomic and proteomic analysis for microbial-mediated bioremediation.

Researchers (Bargiela et al. 2015; Malla et al. 2018) used metaproteomic and metabolomic approaches for the bioremediation of environmental sites which were severely contaminated with petroleum hydrocarbons. With the deployment of omics studies novel genes, transcripts, or enzymes engaged in xenobiotic bioremediation can be identified (Fig. 2.3).

Fig. 2.3 Various approaches for enhanced in situ bioremediation

Environmental Safety Due to modernization (rampant industrialization), habitat degradation and related anthropogenic activities, it is the only bioremediation tool to clean and sustain the earth planet from the short term to long terms effects of noxious pollutants. The ability of the microorganisms used in bioremediation to compete with indigenous microbial population is essential for the success of bioremediation. Environmental constraints such as temperature and low nutrient concentrations, as well as other factors that are difficult to manage, impede their usage and the bioremediation process efficacy. GEMs are used in bioremediation of affected environmental sites by their detoxification and degradation but their sustainance is poorly understood in the processes of their action**.** In addition, the use of antibiotic genes as selectable markers should be discontinued and replaced with other scorable markers to avoid antibiotic resistance genes being unintentionally transferred to other soil microorganisms.

Bioremediation is eco-friendly and economical in restoring the biological and physicochemical properties of the degraded soil (Arora 2018), however, still these in situ techniques need to be improvised and advanced research and ethical issues need to be addressed for the use of efficient and safe GEM's. Moreover, the development of pollutant degrading microbial consortium requires more studies to determine the catabolic potential of microbes both alone and in combination (Arora and Panosyan 2019). Thus, it may strengthen our understanding of microbial consortia-mediated remediation of contaminated sites. For utilizing the full potential of known as well as novel species, it is very essential to expand our knowledge to know the interaction between microbial communities and the polluted environment.

Indigenous microorganisms are found to be inefficient to remove noxious and refractory pollutants, which is a major challenge to environmental biotechnologists. Moreover, the developed GEMs In situ application found to be impactful but associated with the risk of horizontal gene transfer, which causes unrestrained propagation (of the GEMs) in the environment (Naik and Duraphe 2012). The impacts of these bacteria on the ability of indigenous microbes in the environment before being released into hazardous areas, would need to be researched. Nevertheless, for any microbial-based bioremediation process, it is crucial to monitor implanted recombinant strains of bacteria and design strategies to program cell death once the biocatalyst had completed its task (Jan et al. 2014). Induction of controlled suicidal gene system operates just after engineered microbes have completed the remediation of contaminated sites, an alternative to prevent horizontal gene transfer. In addition, horizontal gene transfer can also be prevented by the use of antisense technology which involves inserting antisense RNA-regulated plasmids and protein plasmids into the microbe which terminate or degrade after carrying out their work in remediation (Azad et al. 2014). Moreover, the use of GEMs in the ex situ bioremediation process in bioreactors is considered to be the best choice, as there is no competition with indigenous microorganisms and also they are maintained with controlled temperature and growth conditions (Urgun-Demirtas et al. 2006). Furthermore, field studies are important to check the efficiency and associated risk factors of GEM after incorporating into the natural ecosystems. Significant development of computational tools and omics based investigation has concepts to be gained to unravel microbial-mediated bioremediation pathways. Undoubtedly, the methodical application of microbial consortia and knowledge of well-defined molecular and biochemical mechanisms will facilitate successful implementation of bioremediation techniques. The operations of these strategies are still in nascent stage; however, application of omics strategies in the investigation of microbial molecular action would assist in trailing the desired organism and also effectively eradicating the environmental pollutants.

Conclusion Remarks Once bioremediation process is optimized, it is considered safe, inexpensive, and environmentally amicable to eradicate the pollutants by speeding up the natural process of biodegradation and detoxification. Therefore, urgent need for better understanding the microbial genetics to widen the knowledge of microbial communities and their reaction toward the environmental pollutants will enhance pollutant degrading potential of a given microbe. Undoubtedly, bioremediation is rendering a pathway for a better pollution free planet leading to its sustainability. To investigate the functional makeup of microbial communities inside contaminated locations for metal resistance genes that may be exploited to improve particular heavy metal breakdown strains of microorganisms, metagenomic techniques and metabolic studies should be applied. Public perception of the use of gene technology for bioremediation will also need to change for its effective utilization; this will require cooperation between researchers and environmentalist.

2.2.2.2 Phytoremediation of Polluted Soils/Water Ecosystems

Rapid industrialization resulted in drastic rise in the waste discharge into the environment and led to the accumulation of major contaminants such as heavy metals, hazardous wastes, explosives, and petroleum products (Adriano 1986; Alloway 1990). Heavy metal (arsenic, cadmium, copper, chromium, mercury, zinc, selenium, nickel, and lead) accumulation in soil has been increased largely due to various natural catastrophes and human interventions (Suman et al. 2018; Ashraf et al. 2019). As heavy metals are non-biodegradable, they persist in the environment, have potential to enter the food chain via plant systems, and eventually may accumulate in large quantities (biomagnification) in human body as he is major tertiary consumers. Owing to their noxious nature, heavy metal contamination posed a serious threat to human health and environmental ecosystems (Yan et al. 2020). The total impact of degradation by heavy metals is an irreparable and irreversible threat for the environment with negative consequences. Using plants for bioremediation (called phytoremediation) is an effective in situ technology used in restoration of polluted water and soil (Pajević et al. 2016).

Phytoremediation is an eco-friendly approach that could be a successful mitigation measure to revegetate heavy metal-polluted soil in a cost-effective way. Phytoremediation has good public acceptance and shows a variety of advantages compared with other physicochemical techniques (Suman et al. 2018; DalCorso et al. 2019). There are a number of phytoremediation strategies that are applicable for the remediation of heavy metal-contaminated soils, including (1) phytostabilization—using plants to reduce heavy metal bioavailability in soil

(Gerhardt et al. 2017; Burges et al. 2018), (2) phytoextraction—using plants to extract and remove heavy metals from soil (Sarwar et al. 2017), (3) phytovolatilization—using plants to absorb heavy metal from soil and release into the atmosphere as volatile compounds (Mahar et al. 2016), and (4) phytofiltration—using hydroponically cultured plants to absorb or adsorb heavy metal ions from groundwater and aqueous waste (Dhanwal et al. 2017).

Increasing levels of toxic metals and metalloids in the environment have led to identification of hyperaccumulator plants as they accumulate very high heavy metals concentrations in their aboveground tissues in their natural habitats. It is also known that plants are useful sensors to identify environmental contamination and potential exposures to pollutants (Henry et al. 2013). The application of heavy metal hyperaccumulators is the most straightforward approach for phytoremediation, and hundreds of hyperaccumulator plants have been identified so far (Yan et al. 2020). However, phytoremediation with these natural hyperaccumulators still suffers from a few limitations, as it is a time-consuming process, which takes a very long time to cleanup heavy metal-contaminated soil, particularly in moderately and highly contaminated sites. This may partially be due to slow growth rate and low biomass production of these hyperaccumulators. In order to enhance efficiency of phytoremediation, a better understanding of the mechanisms underlying heavy metal accumulation and tolerance in plant is indispensable. Fortunately, recombinant DNA approaches have been emerging as a powerful tool to modify plants with desired traits such as fast grow, high biomass production, high heavy metal tolerance and accumulation, and good adaption to various climatic and geological conditions. Hence, better understanding of the mechanisms of heavy metal uptake, translocation, and detoxification in plants, and identification and characterization of different molecules and signaling pathway, will be of great importance for the design of ideal plant species for phytoremediation via genetic engineering (Wu et al. 2010; Shah and Pathak 2019).

Notably, apart from exploiting of transgenic approach using functional genes, defined promoters were over expressed to encode accumulation/detoxification mechanisms for heavy metals remediation (Ilker et al. 2020). In study, transgenic canola (*Brassica napus* L. cv. Westar) developed using a rice gene, *OsMyb4*, under the control of *Arabidopsis thaliana COR15a* stress-inducible promoter (Raldugina et al. 2018). When cultivated under high levels of Cu (as 150 M $CuSO_4$) and Zn (as 5000 M $ZnSO_4$), such transgenic canola plants showed greater tolerance (more than 15 days) than natural kinds. This study has demonstrated that *OsMyb4* is positive regulator of phenylpropanoid pathway and proline synthesis and may also have potential in phytoremediation. Zhang et al. (2014) reported that lignin biosynthesis was enhanced Jute *CCoAOMT1* gene was over expressed in to *A. thaliana*. Ectopic expression of *Vicia Sativa* Caffeoyl-CoA *O* methyltransferase (*VsCCoAOMT*) increased the uptake and tolerance of cadmium in *A. thaliana* (Xia et al. 2018). In another study Wang et al. (2018) exploited heavy metal ATPases activity by its expression in model plant tobacco, wherein it has showed improved tolerance against cadium. With data available from *Populus trichocarpa* genome, its heavy metal ATPase gene, *PtoHMA5* was cloned into *N. tobaccum*. Following which, the transgenic *N. tabacum* leaves exhibited increased Cd accumulation (25.05%) and tolerance (Wang et al. 2018). Transgenic plants carrying the genes from endophytic bacteria has significance of remediating heavy metals (Zn, Pb, Cd) from soils. In one of the study when *CUP* and *bph C* genes under control of cauliflower mosaic virus 35 promoter introduced into the stinging nettle plants (Urtica *dioica*) increased heavy metal accumulation for polychlorinated biphenyls (PCBs) in contaminated soils has been noticed. This finding claim that remediation by stinging nettle could have much wider range of applications than previously thought (Viktorova et al. 2017).

In a report transgenic sugar beets demonstrated to have dominant ability to retain Cd, Cu, and Zn ions and showed enhanced glutathione and phytochelatin activities under the applications of heavy metal stresses compared with wildtype sugar beets (Liu et al. 2015). In another study, bacterial γ-glutamylcysteine synthetase has been overexpressed which enhanced the cd detoxification in *Populus tremula* × *P. alba* (He et al. 2015). Notably, transgenic plants expressing bacterial reductases were reported to enhance volatilization of mercury, selenium, and arsenic accumulations in plant shoots (Mosa et al. 2012). Sun et al. (2018) investigated that poplar (genus *Populus*) were effectively used in remediation of Hg from soils using a class of ATP-binding cassette transporter gene. This was achieved by the transformation of ATP-binding cassette transporter gene (*PtABCC1*) from *Populus trichocarpa* to wildtype *Arabidopsis*.

Moreover, apart from metal transporter proteins, functional gene Arsenic reductase 2 gene from *A. thaliana* was cloned in tobacco to study its arsenic phytoremedial potential (Nahar et al. 2017). Recently, Basharat et al. (2018) reviewed that the simplicity, inexpensiveness, and capabilities of gene editing (CRISPR–mediated) technique could soon be used to enhance plants and bacteria involved in phytotechnologies, such as phytostabilization, phytoextraction, phytomining, phytovolatilization, and bioenergy generation.

Phylloremediation is a kind of phytoremediation where plant leaves and leaf associate microbes are engaged to clean the air in the environment to get rid of toxic pollutants (Wei et al. 2017). The ability of removal of heavy metal in the environment can be improved by genetically modified crops engaged in absorption and accumulation of heavy metals from both the soil and air. It is reported that genetically modified crops for the absorption of heavy metals from both air and soil carried out in agricultural areas by intercropping or mixed cropping practicing to increase the effectiveness of phytoremediation (Vamerali et al. 2010). Using genetically engineered organisms with appropriate distances in agricultural farming areas may reduce heavy metal entry into the food chain and will help to remove heavy metals from soil. Eventually, transgenic species are not suppose to competitors of agricultural plants and should be easily removed from the farming land after their tasks of phytoremediation attained. Moreover, there is an increasing worldwide economic interest and scientific focus on developing transgenic biofuel//bioenergy crops with increased biomass and high heavy metal accumulation ability to make phytoremediation feasible as well (Barbosa et al. 2015). Shah and Pathak (2019) reviewed various strategies to increase phytoremediation potential of

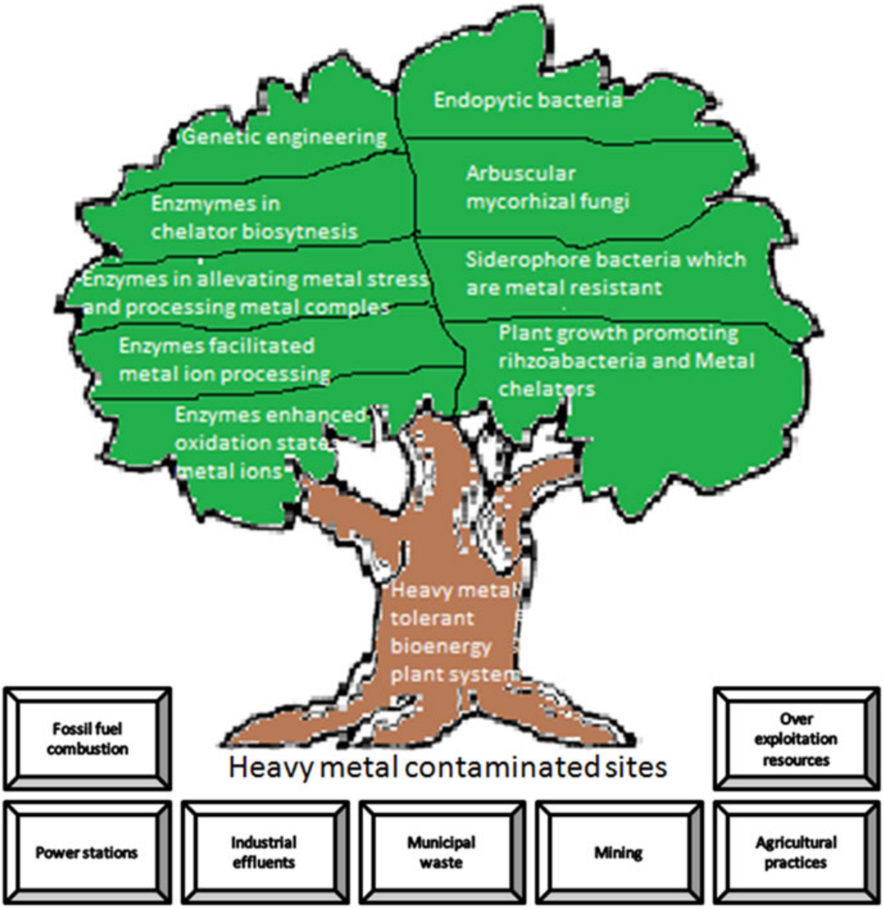

Fig. 2.4 Methodology to increase phytoremediation efficiency of hyperaccumulator plant rich energy

hyperaccumulator plants whose biomass has high calorific value. In Fig. 2.4 explains the Methodologies to increase phytoremediation efficiency of hyperaccumulator plants rich in energy depicted.

Environmental Safety The ability of removal noxious chemicals form environment achieved with identification of candidate wild plant species or by developing genetically modified crops for enhanced reclamation of pollutants from the environment. It is now alarming situation to maintain the environment resilience potency for the earth plant for its sustainability. Anh et al. (2017) reported that *Pteris vittata*, *Pityrogramma calomelanos*, and *Vetiveria zizanioides* were employed to remove heavy metals from soil in Vietnam under field conditions. In another study, pollutants from wastewater were successfully carried out using agricultural species including *Medicago sativa*, *Zea mays*, *Helianthus annus,* and *Sorghum bicolor* (Atia et al. 2019). Cameselle et al. (2019) showed good results with large-scale phytoremedial field treatments utilising *Brassica rapa* subsp. *rapa* and *Lolium perenne*. Phytoremedial experiments utilising Elodea canadensis for Co removal from wastewater and *Plantago lanceolate* for Cd and As removal from polluted locations were also conducted (Mosoarca et al. 2018). In addition, phytoremedial studies were performed using *Elodea canadensis* for Co removal from wastewater and *Plantago lanceolate* for removal of Cd and As from the contaminated sites (Mosoarca et al. 2018). Moreover, *Salvia sclarea* (Chand et al. (2015), *Sedum plumbizincicola* (Deng et al. (2016), and *S. alfredii* and *P. vittata* (Wan et al. 2015) were used for large-scale remediation from harmful heavy metal-contaminated sites. These studies witnessed eco-friendly approaches associated with the wild-type plant species, while there is dire need to speed up the plant earth recovery from burdened pollutants with the introduction of genetic engineering methods. However, owing to the great potential for the removal of pollutants, there are some serious concerns about the environmental applications of transgenic plants. One of the serious concerns is about the gene flow from the genetically modified plants to the corresponding wild relatives (Gunarathne et al. 2019). Most important prerequisite in phytoremediation for the effectiveness relies on their field applications, no matter how good and effective a method developed with employing a genetically modified organism it remains experimental and it is the greatest limitation for it. Environmental risk assessment of those transgenic plants introduced for effectual phytoremediation must be associated with complete and long-term field testing as a critical step in research and development. The research and development process must look closely at the prevention technologies for further spreading of modified genes from transgenic plants to natural plant communities. It is well documented that transgenic plants have exceptional capacity to contribute to the revitalization process of contaminated lands through the phytoremediation process. However, the development of further mechanisms such as genetic use restriction technologies, which can be utilized to control the dispersion of transgenic plants in natural habitats, should be facilitated.

Concluding Remarks The use of hyperaccumulator plants to remediate contaminated sites depends on the quantity of metal at that site and the type of soil. Environmental factors play a major role in the success of bioremediation as the microbes used will be hampered if appropriate environmental conditions are not available. More rapidly growing plants with high phytoextraction ability should be identified for the remediation of pollutants from contaminated environmental sites. Moreover, assessment of metal stress on beneficial rhizospheric microorganisms and crops should be carried out and effective ways of enhancing the bioremediation process need to be worked out. Transgenic microbes and plants could effectively remediate contaminated sites of heavy metal and organic pollutants but its use should be subject to stringent biosafety procedures to ensure that there is no health or environmental hazards. More efficient ways of using transgenic plants and microbes should be identified that will enable effective remediation of polluted environments without horizontal transfer of recombinant plasmids or pollens to indigenous organisms, which is currently a major challenge (Gunarathne et al. 2019).

Phytoremediation is acknowledged as a cost-effective and environment-friendly alternative to traditional methods of environmental cleanup. It is a promising means of ameliorating heavy metal pollution from contaminated sites using transgenic hyperaccumulator plants. It is prioritized to consider aromatic plants, native wild plants or invasive plants as targets for creating transgenic plants for remediation of heavy metals, and also research on these targets should be carried out for identifying novel genes related to heavy metal accumulation and detoxification processes. Plants develop various cellular and molecular adaptations to tolerate the heavy metal stress. Some advancements are there in identification and understanding of metal transporters, hyperaccumulation, phytochelatins (PCs), and metallotioneins (MT) proteins that ensure heavy metal tolerance to plants. However, genes responsible for heavy metal uptake, translocation and sequestration need to be explored more to allow the development of new plant varieties that can be successfully exploited for phytoremediation. Practically, single approach is neither possible nor sufficient for effective cleanup of heavy metal-polluted soil. The combination of different approaches, including genetic engineering and microbe-assisted and chelate-assisted approaches, is essential for highly effective and exhaustive phytoremediation in the near future (Fig. 2.5).

2.2.3 Bioenergy for Eco-Friendly Fuels (Microbes and Biofuel: Toward a Brighter Future)

We are now seeing a warming earth with the increased incidence of extreme weather events, loss of habitat and species on a scale never seen before, and the problems due to overpopulated world. In the industrial era, fossil fuels are indispensable energy contributors; however, they are non-recyclable and less eco-friendly in nature as they accounting for the main cause of environmental pollution and its consequences. Current trend of population raise, overexploitation fossil fuels are increasing the demand for fuels availability and causing an upsurge in fuel prices and global warming effects (Tang et al. 2020). According to BP statistical review of world energy that fossil fuels including crude oil, coal, and natural gas are predicted to exhaust in the next 50 years (Dudley 2018). Long run reliance on fossil fuels is a major threat to world energy security, and its further mining from natural reserves poses serious environmental threats. Exploration of novel, economical and environmental benign and renewable sources of energy is very important to mitigate these concerns. Therefore, the need of the hour is equipping with an affordable, renewable, alternate, and sustainable energy source to conventional fossil fuels. In the recent times, biofuels are considered as inexhaustible and alternative source of energy. These biofuels categorized as first generation, second generation, and the third generation, corresponding to the feedstocks of food sources sugarcane, wheat, corn, soybean, potato, or sugar beet (Bringezu et al. 2007), lignocellulosic biomass

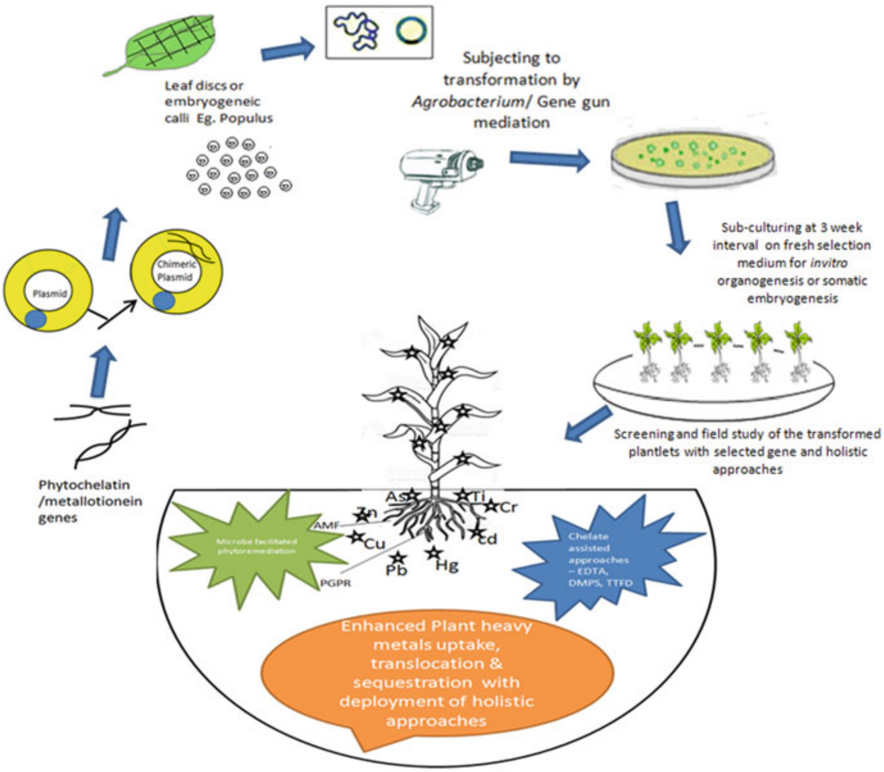

Fig. 2.5 Various strategies for enhanced phytoremediation process; *AMF* arbuscular mycorhizal fungi, *PGPR* plant growth promoting rhizobacteria

and agricultural wastes (Eisentraut 2010), and microalgae and cyanobacteria (Demirbas 2011; Khan and Fu 2020), respectively. It is expensive to convert plant material into useable ethanol. Also, there are ethical considerations. Large amounts of land must be used to produce fuel, which reduces the land available for food production, which is a problem in developing countries with large populations to feed. The amount of land needed also threatens vital ecosystems. Microalgae and cyanobacteria are considered to the best attractive source for energy due to its high biomass productivity rate (dry weight per unit time per unit area) than those of higher plants. Thus, microbial system to produce biofuel is therefore less wasteful, more ethical, and cheaper, owing to their positive effect on the environment due to less to no greenhouse gas emissions. Microalgae- and cyanobacteria-based biofuel is considered as the promising one and has attracted more and more attention in the past years, owing to the characteristics such as fast growing rate, high-efficiency photosynthesis, and high lipid content for some species (Peng et al. 2016; Khan and Fu 2020). Microalgae and cyanobacteria are the current green molecular factories found to be effective and sustainable tools to grapple the pressing problem of climate

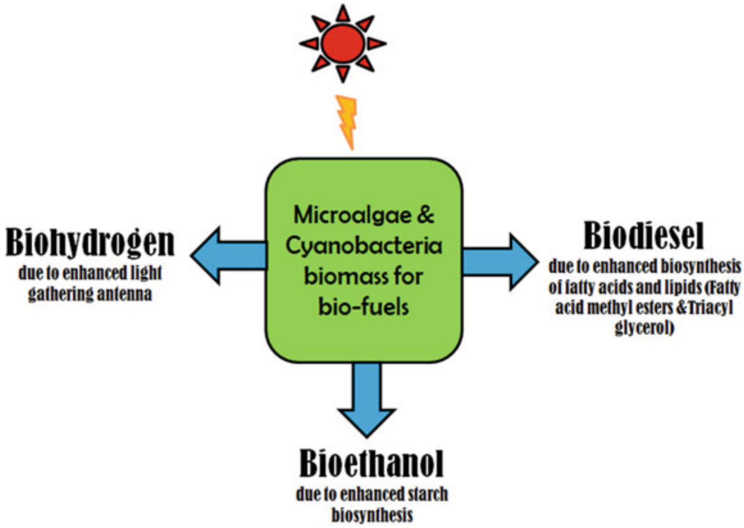

Fig. 2.6 Biotechnological perspectives for eco-friendly algal biofuels production. (1) Knock-out and overexpression of genes involved in lipid biosynthesis. (2) Overexpression of native fermentation-related genes (PDC gene encoding pyruvate decarboxylase, ADH gene encoding alcohol dehydrogenase) in starch biosynthesis. (3) Genetic engineering of enzyme hydrogenase for improved biohydrogen (H_2) photolysis, biogas (methane), and bioelectricity production

change caused due to reliance on fossil fuels and based emission of anthropogenic CO_2 (Urtubia et al. 2016; Choi et al. 2019).

Brazil is a world leader in sustainable biofuel production, as most automobiles of this country running on bioethanol or bioethanol blends. In view of this, currently it is demanding to isolation of new algae, with higher biomass production and oil accumulation. A schematic presentation of typical algal cell depicting biotechnological perspectives for boosting algal biofuel (biodiesel, bioethanol, and biohydrogen) production has been explained in Fig. 2.6.

Recent investigation on microalgal strain improvement for biofuels (biodiesel, bioethanol, biogas, and biohydrogen) production has been carried out by mutagenesis of single target gene or engineering of multiple gene pathways involved in lipid, starch or pigment biosynthesis (Shin et al. 2019). In conjunction studies on knocking-out and overexpression of genes involved in lipid biosynthesis with the aim to modify microalgae and cyanobacteria for better ability to produce fatty acids and lipids (which upon transesterification generate biodiesel) has been documented (Urtubia et al. 2016). Previously, overexpression studies of native fermentation-related genes (for example, the PDC gene coding pyruvate decarboxylase and the ADH gene coding for alcohol dehydrogenase) have been attempted for the production of bioethanol (Dexter and Fu 2009). In the recent past, biogas (methane) produced via anaerobic digestion of algal biomass is a well-studied example of bioprocess configuration (Jankowska et al. 2017). Biohydrogen manufacturing has also been carried out, as biohydrogen is an appealing and alternative fuel because it creates no carbon byproducts during burning and is preferable for bio-electricity generation via fuel cells (Ban et al. 2018). Therefore, genetic manipulation of hydrogenases in algae has improved the yield of biohydrogen (H_2) production more recently by photolysis (Yang et al. 2019), which is an attractive clean fuel helps in drastic reduction of carbon emission (Ban et al. 2018; Nagy et al. 2018). There has been an increasing interest in exploiting microalgae and cyanobacteria for the production biofuel precursors, such as triacyl-glycerol (TAG) and starch, which can be readily transformed into biodiesel and bioethanol (Rengel et al. 2018; Yunus and Jones 2018). Some species are better-suited for biofuel production as they are able to naturally biosynthesize and accumulate considerable amounts of intracellular lipids and starch which may be further improved with the advent of genetic manipulation studies (Urtubia et al. 2016). It is *Chlamydomonas reinhardtii*, *Synechocystis* sp. PCC 6803, *Phaeodactylum tricornutum*, and various *Chlorella* species are among the extensively studied engineered algal strains with regard to their biofuel producing potentials (Yang et al. 2019; Xue et al. 2017; Yunus et al. 2018).

Cyanobacteria are excellent biotechnological platforms to overproduce valuable compounds, with the advantages of comparatively simple genomes, easy transformation, diversity of suitable strains and availability of fully sequenced genomes and considered as model strains/systems (Yunus et al. 2018; Majidian et al. 2018). The biotechnological strategies used for enhancing algal biofuel production through genetic manipulation of these microorganisms are diverse, but the main themes are enhancing lipids and starch biosynthesis (Yunus and Jones 2018; Yunus et al. 2018; Takemura et al. 2019), increasing carbon-capture ability and lipids accumulation (Dinamarca et al. 2017; Gee and Niyogi 2017), and genetic engineering for biohydrogen production (Khetkorn et al. 2017; Yang et al. 2019; Bolatkhan et al. 2019). Immobilization studies of microalgae are capable of metabolizing the nutrients present in medium or wastewater into biomass and high-value cellular constituents (Bouabidi et al. 2019). The essential nutrients (required during the microalgae growth, such as salinity, nitrogen, pH, temperature, and light) are altered to promote the accumulation of lipid in microalgae under the concept of nutrient stress enhancement. Thus, lack of nitrogen, sulfur, phosphorus, and carbon will exert stress on the microalgae and result in the accumulation or enhanced production of energy storage metabolites, such as carbohydrates and lipids (Ran et al. 2019). In a study, Song et al. (2020) combined the nutrient stress approach and phytohormones fortification to enhance the microalgae lipid production, wherein it has promoted the biomass production.

Parsaeimehr et al. (2017) demonstrated the use two classes of low-cost exogenous bioactive molecules (phytohormones and antioxidants), to enhance the biomass and lipid content (alpha-linolenic acid fraction) of *Chlorella protothecoides*. Moreover, Bhuyar et al. (2020) used different types of phytohormones effect on *Chlorella* sp. within these, indole-3-butyric acid showed profound effect with enhanced outcome with regard to its biomass and lipid oil production. In a study conducted by Chen et al. (2020), within the range of phytohormones used, indoleacetic acid acted as positive stimulator and reported the enhanced coproduction of astaxanthin and lipid content in the green algae *Chromochloris zofingiensis*. Ryu et al. (2020)

had produced *Nannochloropsis salina* mutant Mut68 via insertion mutation to increase the lipid production. After Mut68 was incubated for 8 days. When compare to wild type, the fatty acid methyl ester contents and productivity in the mutant increased by 34% and 75%, respectively.

Microalgae being simpler and its unicellular structure make the organism to more amenable to conduct genetic engineering or metabolic engineering studies. Fortunately, recent developments in microalgae omics together with the unlocking of its signaling and biosynthesis pathway have paved the way for understanding gene regulation, metabolic flux, proteins activity, and interaction (Guarnieri and Pienkos 2015). These technological perspectives were used to develop genetically modified microalgae strains engaged in enhanced lipid production/accumulation (Rastogi et al. 2018). It is evident that genetic engineering works to yield improved strains of an organism by overexpressing the key genes involved in fatty acid biosynthesis or inhibiting the lipid catabolism pathway such as starch synthesis and beta-oxidation. In a study, the microalga *P. tricornutum* was engineered at metabolic level to enhance the overproduction of omega-3 long chain polyunsaturated fatty acid docosahexaenoic acid (DHA). In second iteration, co-expression of anacyl-CoA-dependent Δ6-desaturase with the Δ5-elongase further increased DHA levels. (Hamilton et al. 2014). Apart from the genetic manipulation studies in *Nannochloropsis oceania,* Poliner et al. (2018) overexpressed different fatty acid desaturase genes (FAD), Δ5 and Δ12 fad encoding sequences, to increase the eicosapentaenoic acid production in microalgae. In another study, Li et al. (2019) overexpressed a novel bZIP1 transcription factor NobZIP1N that is involved in lipid metabolism transcription regulation in *N. oceanic* for concurrent lipid overproduction and secretion with no effect on microalgae growth.

The understanding of the lipid biosynthesis pathway of the microalgae provides a pathway to induce or accumulate the lipid in the microalgae for the production of biofuel and other bioproducts through the employment of precise genetic modifications. Investigations established CRISPR/Cas9 as a cutting-edge genome-editing technology, as CRISPR technique has relatively short duration, low cost, high accuracy and enhances the efficiency compared to other existing DNA editing tools (Doudna and Charpentier 2014). The application of these genome editing technologies amenable to enhance the quality and quantity of the valuable biofuel compounds in the microalgae. Shin et al. (2019) demonstrated the use of genome editing tool CRISPR-Cas9 for enhancing biodiesel production. This study shows they have knock out phospholipase A2 gene in *Chlamydomonas reinhardtii* to increase (64.25%) the overall lipid production. Chang et al. (2020) knocked out target gene ADP-glucose pyrophosphorylase (AGP) in marine algae *Tetraselmis* sp. using CRISPR-Cas9 RNP method to inhibit carbohydrate synthesis and thus to increase the lipid production by 274% as compared to the wild type.

Environmental Safety Genetic engineering involves the modification or alteration of genes that were involved in lipid production and the effects are overwhelming and satisfactory. Genetic engineering offers the advantages by “tailor-made” microalgae species with faster maturation time and greater yield with reduced harvesting time.

Despite this, genetic engineering should be monitored and examined closely, so that it does not become a possible ecological hazard. Therefore, the genetically modified microalgae have to pass thorough regulatory affairs for open cultivation system. Mutagenesis offer to design microalgae mutants, this may reduce the production cost, but it's a chance event and tedious to identify the beneficial mutants of interest. However, this raises the concern of the scientist about the unknown side effects or outcomes by these microalgae mutants. In addition, the release of new genetically engineered microalgae may cause an imbalance in the ecology and limit the genetic diversity by disrupting the natural process of gene flow (Patra and Andrew 2015). Besides, the compounds produced from the genetically modified microalgae may pose a human health risk and additional regulations are required for the introduction of genetically modified microalgae for human consumption. In conclusion, the lipid accumulation machinery in the microalgae can be trigged by different approaches, such as stress conditions, genetic modification or the addition of exogenous stimulators, which in turn produce lipid in a large-scale and sustainable way. Biotechnology intervention for sustainable production of algal bioproducts has a great potential to produce next-generation eco-friendly biofuels because of their higher productivity, ability to grow on non-arable land and high solar energy conversion efficiency (Ban et al. 2018).

Concluding Remarks As on date, successful large-scale applications of algal-based biofuel production are rare, highlighting the scientific and applied challenges to integrate for the production of eco-friendly and sustainable biofuels. The recent development in biotechnology offers an effective CO_2 capture, higher biofuel productivities, and more diverse biofuel molecules for sustainable development. Therefore, a shift of research paradigms is needed to introduce cutting edge biotechnologies, such as synthetic biology, gene editing, and artificial intelligence to promote the industrial scale production of algal biofuels. As a whole, the challenges must be tackled comprehensively for successful industrialization, sustainable, and economical production of microalgae biofuels and bioproducts. This goal may be accomplished by continuous research and development efforts by academia-industry linkages.

GEMs in Modern Food Production Microorganisms contribution in the timeline of human history evident their remarkable role in the production/processing of food with nutritive values, even much before humans aware that these organisms accounted for the fermentation processes involved (Jay et al. 2005; Zhang et al. 2017). All enzymes are proteins that have catalytic functions at optimal conditions that are being leveraged in food processing. Natural food enzymes often have constrained for higher production due to extreme food processing conditions. Various food enzymes are commonly used in the food production to perform a number of different technical effects such as: reducing lactose content of foods (lactase), dough strengthening or starch modification in baking (amylases), vegetable oil refining (phospholipase), coffee production (mannanase), fruit- and vegetable processing (pectin esterase), conversion of starches into sugars and specialty products (carbohydrases such as amylase, glucoamylase, and transglucosidase), and hydrolysis of proteins (protease). Thus, genetically modified enzymes (gene sequence manipulation) are designed to improve the enzymological properties and to increase purity and yield after standardizing their gene expression studies. A given microorganism produces the enzyme as per the sequence of amino acid building blocks that define its properties and catalytic function. Various methods of genetic modification of microorganisms (strain improvement) are used to tailor enzyme functionality and stability via protein engineering, it also includes the controlled cultural conditions (e.g., pH and temperature) which differ from the natural conditions under which the organism develops and its improved strains are identified and commercialized.

Advances in the areas of biochemistry and molecular biology over the last several decades have led to largely use of GEMs in the production of medical and food substances, especially such processes are attributed to environmentally friendly, animal-friendly, and cost-effective means of production. For instance, insulin is currently produced using microbes rather than sacrificing pigs to harvest their pancreas, the only earlier source of insulin. Moreover, microbially-produced trypsin and chymosin are available as alternatives replacing the harvesting trypsin or rennet from animal sources such as pigs and cows. The applications of GEMs production are not only limited in replacing animal-based production methods but also extends to replace plant-based products. For example, alternatives to traditional agricultural production of plant-based substances, such as stevia extracts and vanilla, GEMs-based production of steviol glycosides (Philippe et al. 2014) and vanillin (Brochado et al. 2010) has many sustainability benefits including reduction in land usage, production of less waste, and provision of a more stable and affordable supply to meet the increasing growing population. Moreover, examples of food ingredients produced today by GEMs include vitamins, amino acids, functional proteins (e.g., texturants), nutritional proteins, oligosaccharides, flavors, and sweeteners (Adrio and Demain 2010). The industrial biosynthesis of riboflavin is one of the great success stories in biotechnology and metabolic engineering, till date around 100% of the commercially-produced riboflavin is manufactured using GEMs (Schwechheimer et al. 2016). Acevedo-Rocha Carlos et al. (2018) has review current biotechnological status of mircrobial cell factories for manufacturing of B vitamins in a sustainable way. Currently, cheese made with chymosin (the active milk protein coagulating enzyme in rennet) produced by GEMs, otherwise the earlier source is harvesting the enzyme from calf stomachs. Luckily, over the past several decades, the use of genetically modified microorganisms (GEMs) has become indispensable tools in food production and processing. Deployment of GEMs has improved food production by enhancing efficiency, reducing, resource requirements, and ultimately enabled innovations such as the cost-effective fortification of food with nutrients, vitamins, and amino acid, and delivery of tailored enzymes to achieve best food processing capabilities (Hanlon and Sewalt 2020).

Apart from genetic engineering much comprehensive understanding and integration of bioin-formatics, enzymology, biophysical chemistry, and molecular biology required for producing genetically modified food enzymes. Most genetically modified enzymes are expressed in well-established standard microbial model systems such as *Escherichia coli* and *Pichia pastoris* (Zhang et al. 2019). Briefly, following are the few important strategies employed while producing genetically modified enzymes. (1) Gene sequence optimization is commonly used for heterologous expression of recombinant enzymes to optimize codon bias of the host cell, as well as to limit mRNA secondary structure that could inhibit translation (Rosano and Ceccarelli 2014). Example: Genetically modified invertase with high transfructosylating activity was used for simple and efficient production of prebiotic fructooligosaccharides (Marin-Navarro et al. 2015). (2) Gene truncation or fusion requires knowledge of the relationship between amino acid sequence and enzyme function. (3) Site-directed mutagenesis is a rational design process based on in-depth knowledge of the target enzyme in terms of structure, catalytic mechanism, and active site. Example: Novel *Bacillus licheni*-formis α-amylases obtained using directed evolution by replacing active site domain His residues with Arg and Asp residues had higher activity at pH 4.5 than the wild type enzyme (Liu et al. 2017). In another study, the substrate specificity of *Thermotoga maritima* b-glucosidases after genetic modification was enhanced for quercetin glucosides (Sun et al. 2014). (4) Directed evolution, a non-rational design process that requires no prior knowledge of the target enzyme is an in vitro accelerated process that mimics natural evolution for generating enzymes. It consists of two key processes: genetic diversity generation for constructing combinatorial libraries and high-throughput screening or selection. Example: *Bacillus* spp. are the main producers of alkaline proteases with high thermal and pH stabilities. The cold activity at 10 °C and alkali-resistant properties of a *Bacillus alcalophilus* alkaline protease were improved through directed evolution using error-prone PCR (Liu et al. 2014) for use in cold-temperature food processing. Similarly, an alkaline serine protease from mesophilic *Bacillus pumilus* was engineered to have increased hydrolytic efficiency at 15 °C without compromising thermostability (Zhao and Feng 2018). (5) Semi-rational design involves mutations based on sequence, structure or computational models, followed by small-scale mutagenesis and screening methods (Hua et al. 2018). An example of semi-rational design is site saturation mutagenesis, in which a mutant library is created containing all possible substitutions at one or more target positions in a gene sequence (Ozgun et al. 2016). Figure 2.7 depicts various improved microbial strains methods used for sustainable food production.

2.3 Environmental Safety and Concluding Remarks

Various genetically modified food enzymes are currently produced from GEMs. Safety concerns have been raised regarding potential contamination of food with bacterial toxins or mycotoxins, allergens, or uncharacterized extraneous substances as impurities (De Santis et al. 2018). Genetic modification of enzymes may also change their allergenic properties, posing new potential health risks while processing or production of food additives/substances. For instance, type I sensitization was found in a study of 813 exposed industrial workers using genetically modified enzymes (Budnik et al. 2017). Thus, before marketing, genetically modified food

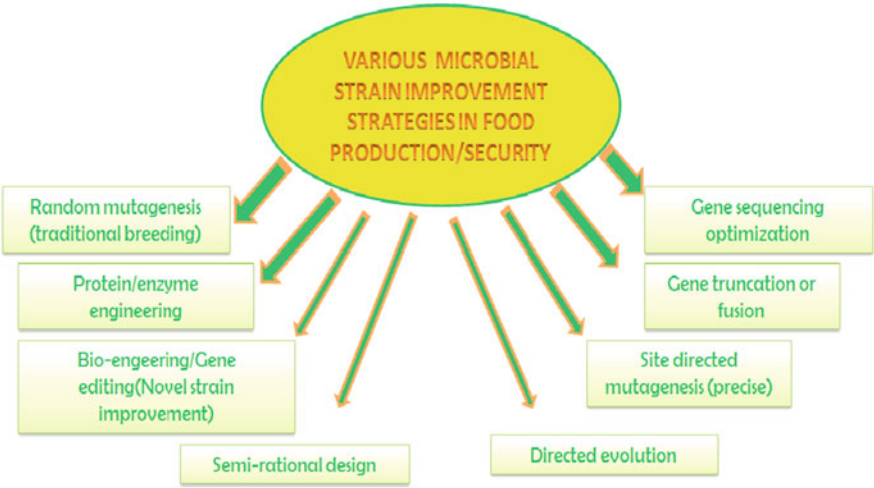

Fig. 2.7 Genetically engineered microbes are indispensable tools for sustainable food practices: diverse recombinant Proteins/Enzymes/Vitamins/additives production at large scale

enzymes need approval from various regulatory bodies such as the US Food and Drug Administration, the Association of Manufacturers and Formulators of Enzyme Products and the European Food Safety Authority, through processes that vary as per different countries guidelines. In addition, in several cultural and developing countries like India ethical and religious concerns have been raised for genetically modified enzymes. For instance, it has been suggested that raw materials or ingredients used in fermentation of microorganisms to produce enzymes should be halal (Ermis 2017). Currently, novel genetically modified food enzymes are largely used for applications in food processing involving carbohydrates, followed by lipids. Although there are challenges for safe use, genetic modification strategies for the development, whilst, novel food enzymes are highly promising for the future, and expected that more genetically modified enzymes will be introduced in various aspects of food processing.

In conclusion, most current production methods are unsustainable because they use nonrenewable sources and often generate hazardous waste. Many microorganisms produce vitamins naturally, but their corresponding metabolic pathways are tightly regulated since vitamins are needed only in catalytic amounts. Metabolic engineering is accelerating the development of microbial cell factories for vitamins/enzymes that could compete with chemical methods and they have been optimized and successful over decades, but scientific hurdles remain. Additional technological and regulatory issues need to be overcome/addressed for innovative bioprocesses to reach the market.

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Mahender Aileni has pursued Ph.D. on developing novel traits in selected medicinal plants under the supervision of emeritus **Prof. A. Sadanandam** (Kakatiya University, Warangal, India). He developed novel in vitro regeneration protocols for conservation and are prerequisite for genetic enhancement and of their medicinal values (Anti-HIV, Antiviral, Antidiabetic compounds of *Scoparia dulcis*; Memory enhancing compounds—bacoposides of *Bacopa monnerie*; Anti-cancer compounds—Woodfordin C of *Woodfordia fruticosa*). In addition, Dr. Aileni worked on molecular biology studies related to sustainable use of products from bio-energy crop plants (Cassava and sweet potato while working under the co-supervision of Prof. Zhang Peng at Institute of Plant Physiology and Ecology, SIBs, CHINA as *CAS-TWAS* fellow). His excellent skills of research and capacity evident with his recent funding sanctioned under UG Start-Up grant (2015–2017) and interdisciplinary [DST-SERB Early Career research (2016–2019)] grant. He has published more than 20 research articles to his credit in peer-reviewed national and international journals.

Global Environmental Problems: A Nexus Between Climate, Human Health and COVID 19 and Evolving Mitigation Strategies

3

Ashwani Kumar, Srishti Goel Khandelwal, and Nisha Gadhwal

Abstract

Global warming has increased the frequency and intensity of some extreme weather and climate events, and will continue to increase if no mitigation strategies are undertaken urgently. Climate change poses unprecedented threats to human health by impacts on food and water security. The increasing air pollution is promoting diseases. Water pollution and increasing secondary soil salinization is reducing agricultural production. There is a nexus between climate and COVID and there is urgent need to understand this nexus and act swiftly. Political and business leaders throughout the world have recognized that global climate change is real as evidenced by Paris Accord. However, there is urgent need of increased public engagement in adaptive and mitigative behavior. Moving the fossil fuel-based economy to decarbonization is major goal for achieving zero carbon emissions by 2050. Besides obtaining first-, second-, and third-generation biofuels, the agricultural and municipal wastes can readily be converted to bioenergy. Evolving green economies will result in human health benefits for mankind. This mini review will discuss nexus between climate change, health, and evolving mitigation strategies.

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Keywords

Climate change · Global warming · Biofuels · Agricultural productivity · Human health

3.1 Introduction

Secretary-General António Guterres in his address to the UN General Assembly in 2018 quoted World Meteorological Organization (WMO) data showing that the past two decades have included 18 of the 20 warmest years since record-keeping began in 1850. "Climate change is moving faster than we are," said Secretary-General Guterres. "We must listen to the Earth's best scientists," (IPCC 2018). It is unequivocal that human influence has warmed the atmosphere, ocean and land. Widespread and rapid changes in the atmosphere, ocean, cryosphere and biosphere have occurred (IPCC 2021). UN Framework Convention on Climate Change 26th Conference of the Parties (COP26) at Glasgow is meeting to discuss outcome of global efforts (IPCC 2021). The 2021 Lancet countdown on health and climate change report coincides with the UN Framework Convention on Climate Change 26th Conference of the Parties (COP26) (Romanello et al. 2021).

As per the National Oceanic and Atmospheric Administration (2021) and Goddard Institute for Space Studies Surface Temperature (GISS (2021) Analysis team reports (GISTEMP v$)the world is now 1•2°C warmer than in the preindustrial period (1850–1900) and past 7 years have been the hottest 7 years on record. Implementation of Paris Agreement to keep the global average temperature rise to 1•5°C requires considerable shift from fossil fuel use to "green energy" in order to generate effective climate response. To meet the Paris Agreement goals and prevent catastrophic levels of global warming, global greenhouse gas emissions must reduce by half within a decade. Romanello et al. (2021) reported Global land area affected by drought events per month in their recent report published in Lancet Countdown, (an international collaboration that independently monitors the health consequences of a changing climate) (Fig 3.1). Semenza et al. (2012) and recently Caminade et al. (2019) have reported that climate change is affecting the distribution of arthropod-borne, food-borne, and water-borne diseases. Green et al. (2019) reported that frequent droughts could limit the Earth's carbon reuptake which will again result in increased CO concentrations in the atmosphere. Romanello et al. (2021) also discussed the implications of climate change on human health including COVID-19 pandemic—a global health crisis that has claimed millions of lives. Romanello et al. (2021) listed several diseases like like dengu and Plasomodium falciperuam malaria, Vibrio cholerae causing Cholora and Vibrio bacteria have increased several fold since 1950 (Fig. 3.2).

Climate change is interrupting the provision of basic services such water and sanitation, education, energy, and transport (https://www.unenvironment.org/explore-topics/sustainable-development-goals/why-do-sustainable-development-goals-matter/goal-13). Human activities are negatively affecting ecosystem services

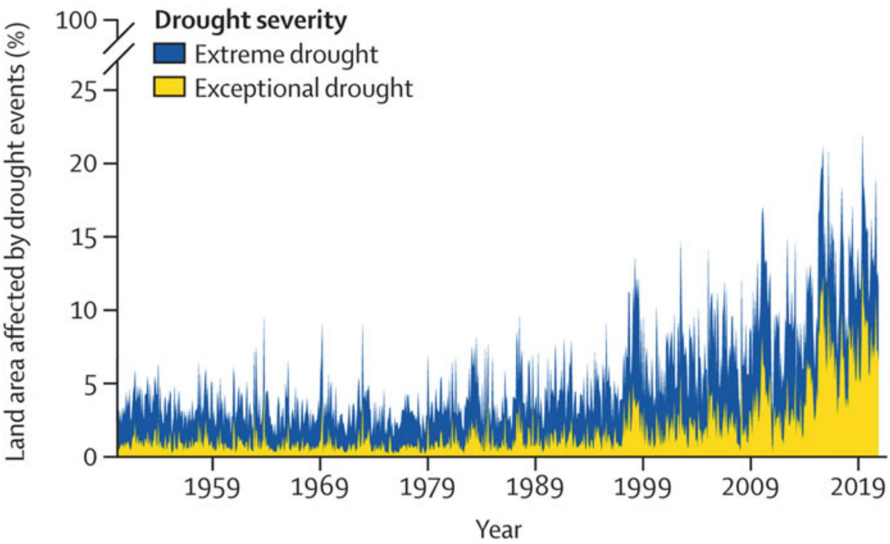

Fig. 3.1 Global land area affected by drought events per month. Extreme drought is defined by a SPEI of ≤1·6 and exceptional drought is defined by a SPEI of ≤2. SPEI=standardised precipitation-evapotranspiration index. Source: Romanello et al. (2021). The 2021 report of the Lancet Countdown on health and climate change: code red for a healthy future. The Lancet, 0(0). https://doi.org/10.1016/S0140-6736(21)01787-6. Reproduced under licence no. 5174500569873 dated 22nd October from RightsLink

and biodiversity through land degradation (Hill et al. 2009; Verner 2010; Crill and Thornton 2017; IPCC 2014a, b, 2019a, b, c; Kumar et al. 2020; Sena and Ebi 2021). Tropical cyclones are more frequent and furious (Lewis et al. 2019). A human rights-based approach to loss and damage under the climate change regime has been presented by Toussaint and Blanco (2020) (see also IEA 2021).

Michelle Bachelet, United Nations High Commissioner for Human Rights, in his Opening Statement to the 42nd session of the Human Rights Council on September 9, 2019, stated that "*Climate change is a reality that now affects every region of the world*" (Source: https://www.ohchr.org/en/ewsEvents/Pages/DisplayNews.aspx?NewsID=24956&LangID=E; see also IPBES 2018; Sena and Ebi 2021).

Intergovernmental Panel for Climate Change 2017 (O'Neill et al. 2017) stated that "*Under emissions in line with current pledges under the Paris Agreement (known as Nationally Determined Contributions, or NDCs), global warming is expected to surpass 1.5 °C above pre-industrial levels, even if these pledges are supplemented with very challenging increases in the scale and ambition of mitigation after 2030*."

The air pollution from burning fossil fuels kills 3.6 million people in countries around the world every year (Ritchie and Roser 2020). Moreover effects of global warming are also worse in low-income countries where urbanization have occurred

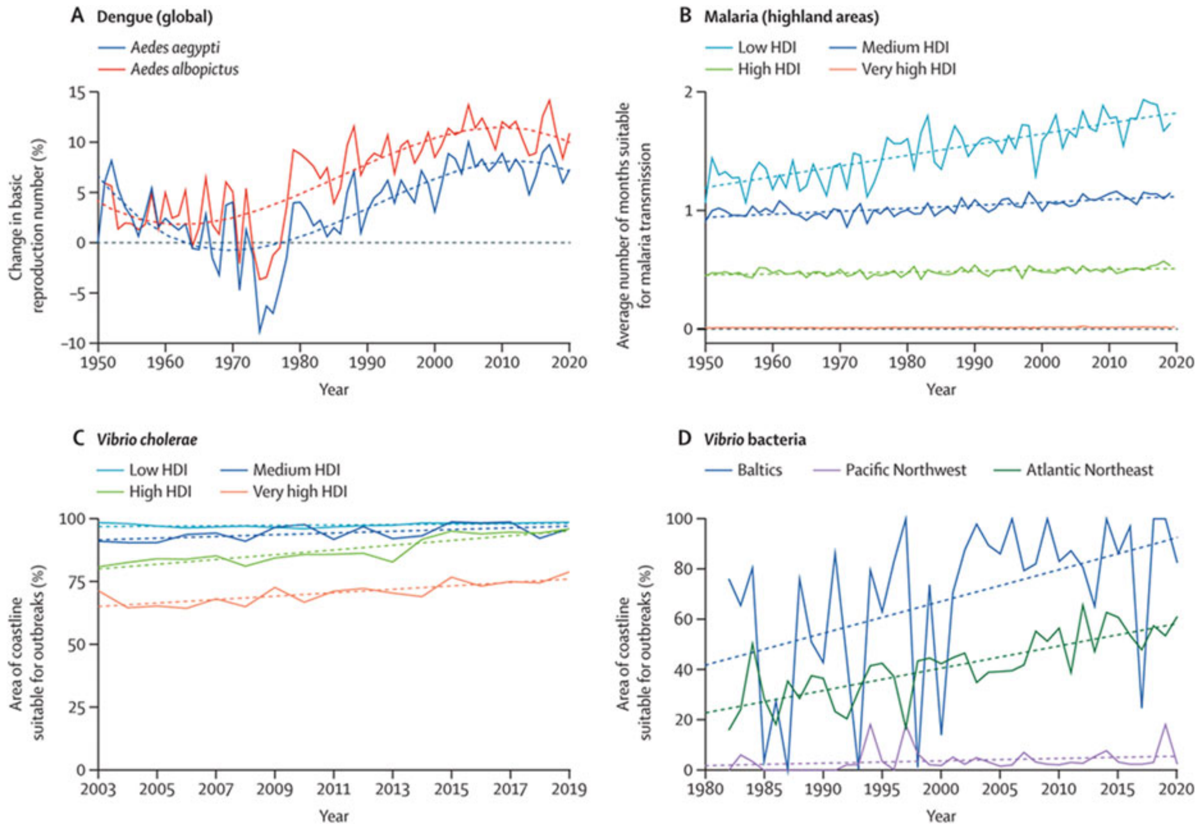

Fig. 3.2 Change in climate suitability for infectious diseases. Change in climate suitability for infectious diseases. Solid lines represent the annual change. Dashed lines represent the trend since 1950 (for dengue and malaria), 1982 (for Vibrio bacteria), and 2003 (for Vibrio cholerae). HDI=human development index. Source: Romanello et al. (2021). The 2021 report of the Lancet Countdown on health and climate change: code red for a healthy future. The Lancet, 0(0). https://doi.org/10.1016/S0140-6736(21)01787-6. Reproduced under licence no. 5174500569873 dated 22nd October from RightsLink

rapidly and without planning (Kjellstrom and McMichael 2013; Roundtable 2014; Law et al. 2018).

Recent publications highlighted the issues of global warming and climate change (Kumar 2018; Kumar et al. 2018, 2020; Wheeler and Watts 2018). Wheeler and Watts (2018) suggested that climate change poses a significant threat to human health. The object of this review paper is to review recent publications on link among climate change and health and highlight global efforts being made to mitigate global warming for human welfare.

3.2 Anthropocene

The Earth is over 4.5 G year old. The last period of time, the Quaternary, began just 2.6 Ma, and includes two epochs, the Pleistocene and the Holocene. The latter—by far the shortest in the geological time scale began only about 11,500 years ago, witnessed by changes in climate that manifest in an ice core from Greenland (Walker et al. 2009). Crutzen (2002) proposed the concept of "Anthropocene" to the present, in many ways human-dominated, geological epoch, supplementing the Holocene—the warm period of the past 10–12 millennia. The Anthropocene started in late eighteenth century and coincided with the designing of steam engine in 1784 by James Watt (Crutzen 2002).

Anthropocene defines an entirely new position for humans: from a minor element in planetary evolution to a driving force at planetary scale that is now compared in its geological significance to ice ages (Zalasiewicz et al. 2011; Lewis and Maslin 2018). Anthropogenic changes in the Earth's climate, oceans, land, and biosphere are now so great and so rapid that the concept of a new geological epoch defined by the action of humans, the Anthropocene, is widely and seriously debated (Zalasiewicz et al. 2011).

Biermann (2021) raised a question about the future of "environmental" policy in times of earth system transformations and the recognition of the "Anthropocene" as a new epoch in planetary history? Amoureux (2021) reported that "Multiple Anthropocenes" instead of "Anthropocene" is the multiplicity of experiences of extinction and visions of political and ethical response.

3.2.1 Global Warming

The human population has grown approximately ninefold since the beginning of the industrial revolution (United Nations 2015), and is predicted to exceed 11 billion by 2100 (Tilman et al. 2002; Gerland et al. 2014). The methane-producing cattle population has risen to 1.4 billion. Energy use has grown 16-fold during the twentieth century, causing 160 million tons of atmospheric sulfur dioxide emissions per year, more than twice the sum of its natural emissions (Crutzen 2002; Hill et al. 2009). Emissions grew more quickly between 2000 and 2010 than in each of the three previous decades. Recent studies by Intergovernmental Panel on Climate

Change (IPCC 2017, 2018a, b) have stated that besides carbon dioxide the evolution of methane and sulfur dioxide emissions strongly influences the chances of limiting warming to 1.5 °C.

According to IPCC (2019a, b, c), "It is certain that globally averaged land surface air temperature (LSAT) has risen faster than the global mean surface temperature (i.e., combined LSAT and sea surface temperature) from the preindustrial period (1850–1900) to the present day (1999–2018)". According to the single longest and most extensive dataset, from 1850–1900 to 2006–2015 mean land surface air temperature has increased by 1.53 °C (very likely range from 1.38 to 1.68 °C), while global mean surface temperature has increased by 0.87 °C (likely range from 0.75 to 0.99 °C) (see also https://www.epa.gov/climate-indicators/climate-change-indicators-global-greenhouse-gas-emissions). The last 5-year period has been the warmest 5 years on record. June 2020 was just 0.01 °C below the record-breaking temperatures of June 2019, driven by exceptional heat in Arctic Siberia; May 2020 was the hottest May on record (Bernstein et al. 2008; Bessou et al. 2011; Kjellstrom and McMichael 2013; Watts et al. 2015).

"Agriculture, forestry and other land use (AFOLU) is a significant net source of GHG emissions contributing to about 23% of anthropogenic emissions of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) combined as CO_2 equivalents in 2007–2016. AFOLU results in both emissions and removals of CO_2, CH_4 and N_2O to and from the atmosphere. Land is a net source of CH_4, accounting for 441% of anthropogenic CH_4 emissions for the 2006–2017. AFOLU is also the main anthropogenic source of N_2O primarily due to nitrogen application to soils" (IPCC 2019a, 2014a, b; Watts et al. 2015; IPCC 2021); different sources of greenhouse gas emissions are given by Watts et al. (2015) Past 30 have seen statistically significant increase in the number of extreme weather events .Romanello et al. (2021) indicated global land area affected by drought events per month (Fig. 3.1).

3.2.2 Energy Production and Use

The increasing energy requirement for the mankind requires use of clean, sustainable energy sources (Chang et al. 2009). Although use of bioenergy has potential to limit CO_2 levels significantly (Kumar 2013; Kumar et al. 2018, 2020), but reduction of the non-CO_2 emissions like agricultural nitrous oxide (N_2O) and methane (CH_4) hydrofluorocarbons require specific measures (IPCC 2017). Burning of fossil fuels accounts for 87% of the world's CO_2 emissions; a world run on fossil fuels is not sustainable. They endanger the lives and livelihoods of future generations and the biosphere around us (Fig. 3.3). World needs renewable energy sources mainly green energy without grossly affecting its economy. Ritchie and Roser (2020) reported that the world's energy supply largely comes from coal, oil, and gas—which presently account for 79% of the world's energy production. Framework for evaluating biomass resources and associated CHG mitigation potential in China have been presented by Kang et al. (Fig. 3.3)

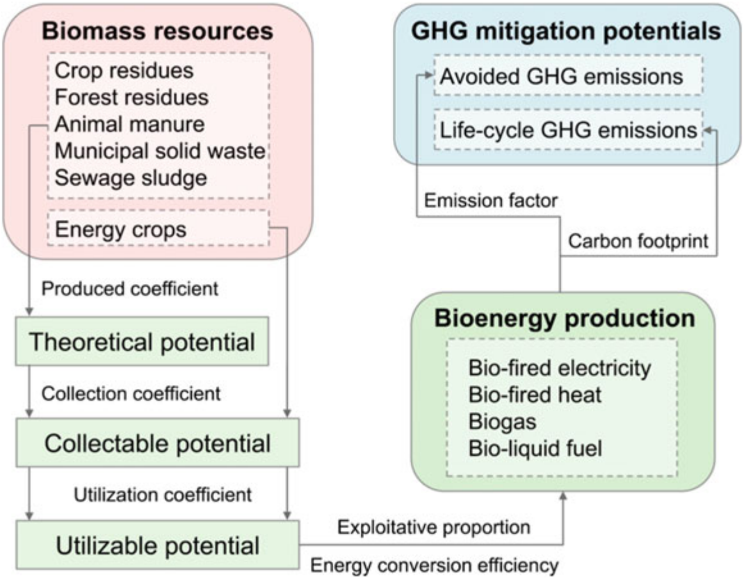

Fig. 3.3 The framework for evaluating biomass resources and associated GHG mitigation potentials in China. Source Kang Y, Yang Q, Bartocci P, Wei H, Liu SS, Wu Z, Zhou H, Yang H, Fantozzi F, Chen H. Bioenergy in China: Evaluation of domestic biomass resources and the associated greenhouse gas mitigation potentials. Renew Sustain Energy Rev. 2020 Jul;127:109842. doi: 10.1016/j.rser.2020.109842. Reproduced under RightsLink licence number 5174811141778 dated October 2021

3.2.3 Climate Change

Anthropogenic greenhouse gas emissions such as carbon dioxide, methane, and nitrous oxide in the Earth's atmosphere since mid-nineteenth century are causing increased average temperature at a much faster rate as compared to past thousand years (Cubasch et al. 2013). The use of mainly fossil fuels has increased emissions of carbon dioxide 42% over the last decades. As per 6th assessment report of IPCC "in 2019, atmospheric CO2 concentrations were higher than at any time in at least 2 million years (high confidence), and concentrations of CH_4 and N_2O were higher than at any time in at least 800,000 years (very high confidence). Since 1750, increases in CO2 (47%) and CH4 (156%) concentrations far exceed, and increases in N2O (23%) are similar to, the natural multi-millennial changes between glacial and interglacial periods over at least the past 800,000 years" (IPCC 2021) (Fig. 3.4).

Paris Agreement, which binds nations to keep global warming to 1.5 °C, is guided by the 2015 United Nations Sustainable Development Goals and is a global collective effort. The 1.5 °C emission pathways is defined as "given current knowledge of the climate response, provide a one-in-two to two-in-three chance of warming either remaining below 1.5 °C or returning to 1.5 °C by around 2100 following an overshoot." This will require either reducing net global emissions of long-lived

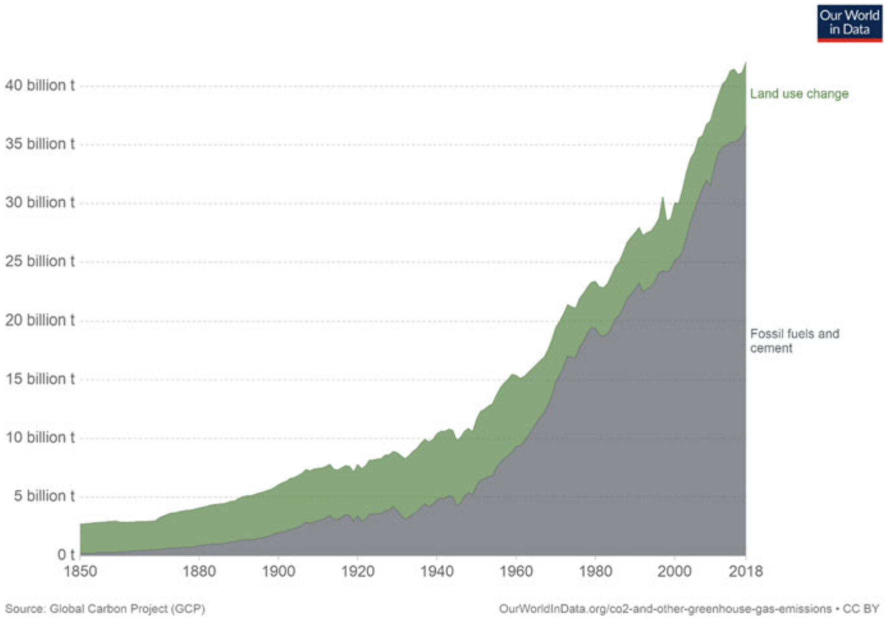

Fig. 3.4 Global CO2 emissions from fossil fuels. (Source: Ritchie H. and Roser M (2020)—"Energy." Published online at OurWorldInData.org. Retrieved from: https://ourworldindata.org/energy [Online Resource], https://ourworldindata.org/grapher/primary-sub-energy-source?country¼~OWID_WRL)

greenhouse gases to zero before the cumulative limit is reached, or net negative global emissions (anthropogenic removals) after the limit is exceeded (Allen et al. 2018) (https://www.ipcc.ch/site/assets/uploads/sites/2/2019/05/SR15_Citation.pdf).

3.3 Climate Change

Desertification, land degradation, and drought (DLDD): The fast-growing world population exerts great pressure on the land to produce enough nutritious food. It is projected that global population will be 50% greater than at present by 2050 and the demand for global grain will have doubled (Godfray et al. 2010). Climate change is affecting the degradation of terrestrial and aquatic ecosystems. It has assumed global dimensions and least 3.2 billion people worldwide are affected by this complex phenomenon (Kumar 2013; Díaz et al. 2015a; Rossati 2017; IPBES 2018; Kumar et al. 2020; Sena and Ebi 2021). Sena and Ebi (2021) studied interrelationship among desertification, dryland ecosystem services, and lower ecosystem health, including losses in biodiversity and human health effects. Land use changes expedite the climate change and desertification (Fig. 3.5). UNCCD 2018–2030 Strategic Framework aims to reduce it as it has commitment for Land Degradation Neutrality

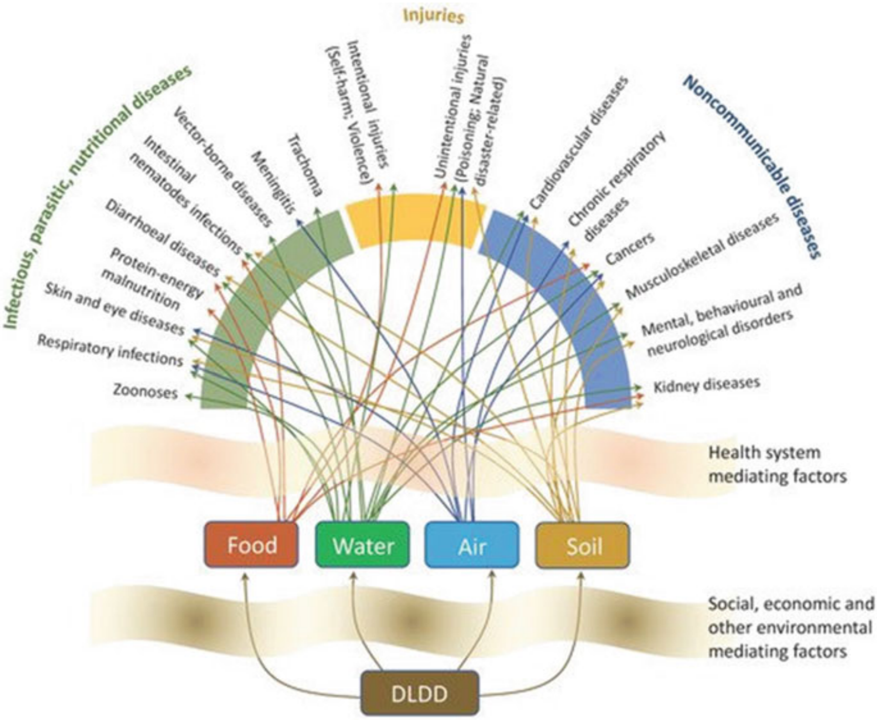

Fig. 3.5 Desertification, land degradation and drought (DLDD) pathways and health effects. (Source: Sena, A., & Ebi, K. (2021). When Land Is Under Pressure Health Is Under Stress. International Journal of Environmental Research and Public Health, 18(1). https://doi.org/10.3390/ijerph18010136. Original source: Sena, A. Land under pressure—Health under stress. In Global Land Outlook Working Paper — United Nations Convention to Combat Desertification (UNCCD): Bonn, Germany, 2019. Available online: https://knowledge.unccd.int/publication/landunder-pressure-health-under-stress (accessed on December 25, 2020). This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited)

(LDN) in order to restore the productivity of vast expanses of degraded land and to improve the livelihoods.

Global warming can accelerate and retreat as Antarctic ice sheet which is now losing 159 billion tons of ice each year. This may cause the collapse of ice shelfs. Recently more than 1600 square miles of ice which calved off the Ronne Ice Shelf into the Weddell Sea. The European Space Agency's twin Copernicus Sentinel-1 satellites spotted this enormous chunk of iceberg named it as A-76 (Fig. 3.6) (https://www.nbcnews.com/science/science-news/worlds-largest-iceberg-just-broke-antarctic-ice-shelf-rcna974; https://phys.org/news/2021-05-vast-antarctic-iceberg-drift). Sea levels are rising inexorably (McMillan et al. 2014).

Fig. 3.6 The world's largest iceberg, dubbed A-76, has calved from Antarctica. This animation shows the giant slab of ice breaking off from the Ronne Ice Shelf, lying in the Weddell Sea. (Source: U.S. National Ice Center using images taken by the European Space Agency's Sentinel 1A satellite; https://www.nbcnews.com/science/science-news/worlds-largest-iceberg-just-broke-antarctic-iceshelf-rcna974; https://phys.org/news/2021-05-vast-antarctic-iceberg-drift-ocean.htm)

3.4 Climate Change and Human Health Impacts

Climate change is considered one of the greatest threats to human health by the World Health Organization (Moro et al. 2010; WHO 2014; Woodward et al. 2014; Campbell-Lendrum et al. 2015; Caminade et al. 2019). Climate changes and human rights (2015) (https://web.law.columbia.edu/sites/default/files/microsites/climate-change/climate_change_and_human_rights.pdf) Smith et al. (2014) suggested that climate change affects human health through changing frequency and severity of heatwaves, drought, and heavy rain which are extreme events. Climate change creates habitats suitable to the transmission of human and animal pathogens (Kjellstrom and McMichael 2013; Rossati 2017; WHO 2020, https://www.who.int/globalchange/publications/reports/health_rioconventions.pdf?ua=1).

The effects of climate change are being felt today, and future projections represent an unacceptably high and potentially catastrophic risk to human health

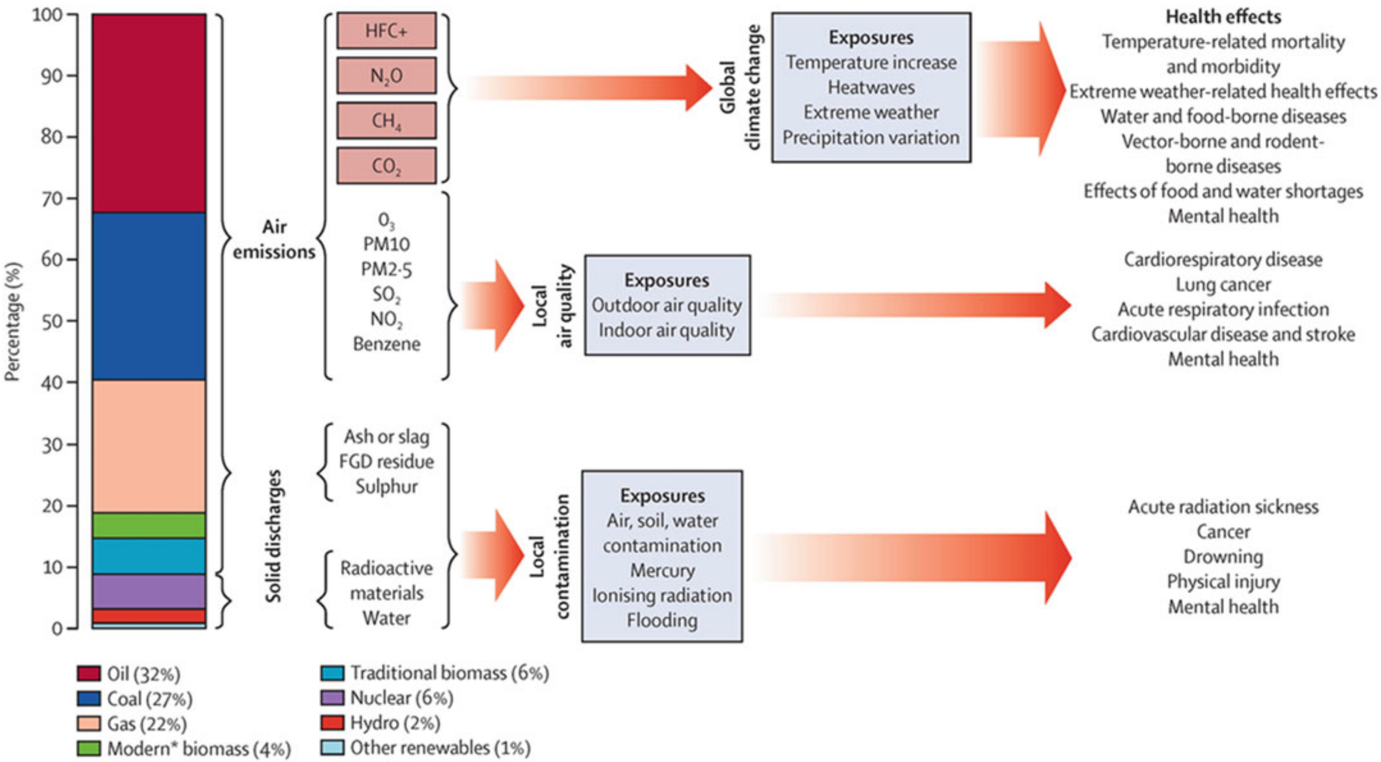

Fig. 3.7 Connections between the global energy system and health impacts. (Source: Watts N,WNeil Adger, Paolo Agnolucci, Jason Blackstock, Peter Byass, Wenjia Cai, Sarah Chaytor, Tim Colbourn, Mat Collins, Adam Cooper, PeterMCox, Joanna Depledge, Paul Drummond, Paul Ekins, Victor Galaz, Delia Grace, Hilary Graham, Michael Grubb, Andy Haines et al. (2015) The Lancet, 386: 1861–1914. Reproduced with permission from RightsLink license number 5060710880266, May 2, 2021)

(Chevalier et al. 2010; Sarkar 2011; Paz and Semenza 2013; Rezza 2016; Semenza et al. 2016; Yu and Sarfaty 2017; UNEP 2017; Watts et al. 2018; Sena and Ebi 2021; Pasztor et al. 2021). Kumar et al. (2019) and Kumar (2020a) reviewed the nexus between air pollution, green infrastructure, and human health. Watts et al. (2015) presented connections between the global energy system and health impacts (Fig. 3.7). Actions that seek to mitigate climate change have the potential to be beneficial to health, both directly and indirectly (Watts et al. 2015, 2018).

3.4.1 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the Coronavirus Disease 2019 (COVID-19)

As of May 21, 2021, the coronavirus disease 2019 (COVID-19) pandemic has caused more than 165 million infections across all ages globally, as well as more than 3.4 million deaths (WHO 2021: https://covid19.who.int/. opens in new tab).

Human Development report of UNDP (2020) says that COVID-19, 2020, has been a dark year. "*Scientists have been forewarning a pandemic like this for years, pointing to the rise in zoonotic pathogens—those that jump from animals to humans—as a reflection of the pressures people put on planet Earth. There is talk of returning to 'normal,' as if some predetermined end date exists for the many crises gripping our societies and the planet, as if going back to normal is desirable or even possible*" (https://report.hdr.undp.org).

COVID-19 disease is caused by novel coronavirus, which was earlier named as 2019-nCOV virus but later on termed as SARS-COV2 virus due to its resemblance to SARS-COV viruses. It was first reported in Wuhan city of China in 2019 and, within a short span of time, it spread like a fire, thus giving birth to pandemic which whole world is facing today. The COVID and other vector-borne and -derived diseases due to climate change have pushed mortality rate by almost 300 times in developing nations, causing health inequity by putting brakes on socioeconomic development and acting as a strain on health services leading to even total collapse of health system as witnessed recently (Campbell-Lendrum et al. 2015). Zang et al. (2021) provided excellent review on emergence of COVID-19 and intersection of the pandemic with climate change. Columbia University's Earth Institute released a State of the Planet report on *COVID-19's Long-Term Effects on Climate Change—For Better or Worse* on Global Environmental Health Day, that is, July 1, 2020 (Cho 2020). Wu et al. (2020) reported that air quality may impact severity of illness in COVID-19. They recorded that long-term exposure to fine particulate matter is associated with 15% increase in the COVID-19 death rate, even after controlling for other health conditions (see also van Doremalen et al. 2020; Zhang et al. 2020; Triggle et al. 2021).

Jowell and Barry (2020) wrote "*COVID-19 is not the first example of an emerging zoonotic infection that has devastating impacts on global human health. In recent decades, humans have altered surrounding habitats and increased deforestation to make room for growing populations, extract natural resources, and build farms*".

The ongoing COVID-19 pandemic has changed the way we experiment with life in general. Heavily treated patients who had recovered from COVID are coming back with post-COVID complications like mucormycosis, etc. These complications are waiting for all of us in large numbers after this pandemic gets over. Human behavioral change is one of the most important milestone to curb the virus from its roots. COVID and environment factors (temperature, UV index, humidity, wind speed, pollution, and population density) go hand in hand in terms of transmissibility and virus survival. So besides taking effective, timely, and appropriate measures for environment control, maintaining good personal hygiene, inculcating habit of yoga/ meditation, having balanced diet, wearing masks, and practicing social distancing will be the key to survive and prevent future such pandemics.

The current Global situation is that there are 165,772,430 confirmed cases and 3,437,545 deaths worldwide due to COVID-19 disease. Beside the highly contagious nature of virus, global travel, urbanization culminating into overcrowding and unhygienic environment, climate changes, increased human animal contact all have contributed significantly to the spread of this virus. Initially it was believed to spread through droplet nuclei on close contact but now WHO has declared that SARS-COV2 virus do have airborne transmission. SARS-COV2 virus causes Covid-19 disease. This virus has a protein envelope and +ssRNA in the core of virus. Virus undergoes rapid mutations for its survival, and this helps the virus to stay infective for prolong periods and spread of disease during pandemic period.

SARS-COV2 virus attaches to host cell ACE2 receptors and then enter into cells either via endocytosis or by membrane fusion (Perrotta et al. 2020; Fig. 3.8).

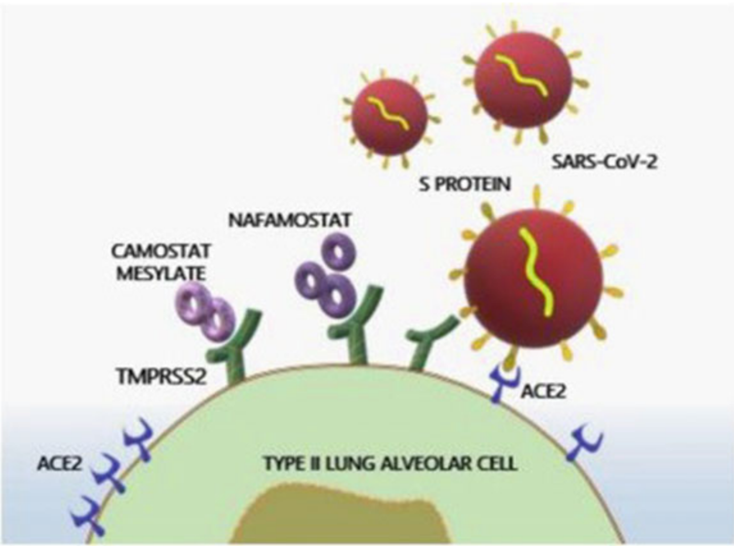

Fig. 3.8 Postulated mechanisms modulating SARS-CoV-2 attachment and fusion machinery on human host cells. (Source: Perrotta F, Matera MG, Cazzola M, Bianco A. Severe respiratory SARSCoV2 infection: Does ACE2 receptor matter? Respir Med. 2020 Jul; 168: 105996. https://doi.org/10.1016/j.rmed.2020.105996. License number 5075330204643 dated May 24, 2021)

Once inside the cells, viral mRNA uses host nucleus for replication and synthesis of viral proteins, which on maturation comes out of cells (lung pneumocytes) via exocytosis, thus causing viremia and dissemination of virus throughout the body via blood stream. In the meantime, antibodies are also being produced by the body in response to virus and thus a fight will ensue between both of them resulting in release of more and more cytokines, leading to cytokine storm-related lung injury, myocyte injury, intestinal and cardiopulmonary changes (Triggle et al. 2021; Fig. 3.9).

3.4.2 Clinical Presentation

In approximately 85–90% patients, COVID-19 disease will manifest as normal flu with mild upper respiratory tract symptoms, which will resolve within 5–6 days with symptomatic treatment, home remedies, plenty of fluids, protein rich diet, yoga, and rest. These patients just have to do continuous monitoring of their vitals and oxygen saturation levels while practicing home isolation. In the rest 10–15% cases, the symptoms either continued after 6 days or they reappear again (due to cytokine storm) like there is reemergence of fever or sudden spike in persisting fever, increase in dry cough causing difficulty in speaking or sleeping, chest heaviness, or difficulty in breathing; all these patients require investigations and further follow-up. Majority of these 10–15% patients who maintained oxygen saturation above 94 will respond to proning position, breathing exercises, chest physiotherapy along with nebulization, steroids, anticoagulants, and other symptomatic treatment. Strict monitoring of vitals and sugar levels and having continuous consultation with treating physician are keys to get cure within these patients. Only 3–4% of patients who either do not maintain oxygen saturation above 94 or shows continuously falling saturation levels need oxygen support and hospitalization.

A sharp decline in COVID-appropriate behavior such as wearing masks, practicing social distancing, and avoiding gathering and crowds was seen in beginning of this year (March–April 2021), which led to faster spread of the virus, thus bringing second wave with such ferocious intensity that it brought the whole healthcare system on its knees. Already eight new variant of SARS-COV2 are circulating worldwide, with variant B.1.617 seemingly more transmissible and more dangerous, constituting almost 50% of positive case in India and thus becoming predominant strain to cause COVID disease in this country. To rapidly curb its transmission, India needs to dramatically expands its vaccination coverage and that too on an unprecedented speed. Vaccination is the only way in today’s scenario to save lives and spark hope and optimism in millions. India’s huge immunization drive in the history of this country began on January 16, 2021, with approval of two vaccines: COVISHIELD (Oxford University–AstraZeneca) and COVAXIN (home production—Bharat Biotech–ICMR-NIV). India’s biotech companies are also expected to produce Russia Sputnik V vaccine in coming days, thus putting India in a prime position to both benefit from world vaccine need and provide for its own citizens.

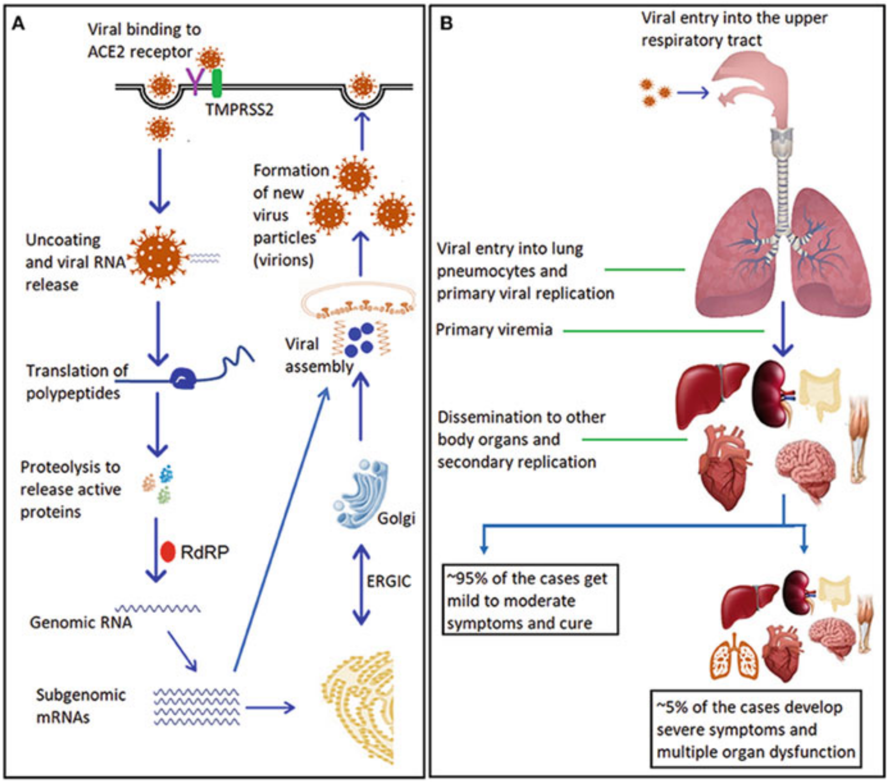

Fig. 3.9 SARS-CoV-2: mechanism of entry, replication, and dissemination. **(A)** Entry and replication of SARS-CoV-2 inside the host cells. The virus spike glycoprotein binds to the cellular receptor ACE2. This binding induces conformational changes at the receptor binding domain (RBD) that expose co-receptors to bind with transmembrane protease serine-2 (TMPRSS2), with the help of cellular endosomes it facilitates viral internalization via endocytosis. Internalization results in uncoating of viral genomic RNA into the cytoplasm. Genomic RNA binds to the host ribosome, which leads to formation of polyproteins that contain transcription complex, including the viral RNA-dependent RNA polymerase (RdRP). The RdRP generates viral genomic RNA and several subgenomic mRNAs, which will be translated into relevant viral proteins. The translated proteins translocate into endoplasmic reticulum (ER) membranes and transit through the ER-to-Golgi intermediate compartment (ERGIC), where interaction with encapsidated, newly produced genomic RNA results in budding into the lumen of secretory vesicular compartments. The newly formed viral particles (virions) are subsequently released out of the cells via exocytosis. **(B)** Viremia and dissemination into body organs: Initial replication takes place in the upper respiratory tract, followed by the migration of the virus to the lungs. A primary viremia occurs after establishment of infection and replication in the lung pneumocytes. This viremia disseminates the virus throughout the body via blood stream where another cycle of viral replication takes places and ensuing viremia leads to further dissemination (for more details and references see section SARS-CoV-2 Genome Structure: Role in Infectivity and Virulence and Replication Cycle and Pathophysiology of the review). ACE2, Angiotensin-converting enzyme 2; RdRP, RNA-dependent RNA polymerase. ER, Endoplasmic reticulum; ERGIC, ER-Golgi Intermediate Compartment. (Source: Triggle, C. R., Bansal, D., Ding, H., Islam, M. M., Farag, E., Hadi, H. A., & Sultan, A. A. (2021). A Comprehensive Review of Viral Characteristics, Transmission, Pathophysiology, Immune Response, and

A COVID-19 Pfizer–BioNTech vaccine (BNT162b2) containing nucleoside-modified messenger RNA encoding the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike glycoprotein has been found to be effective across all ages from 12 onwards (see Frenck et al. 2021). Bharat Biotech India has been approved to start clinical trials on children above 2 years with nasal variant of COVAXIN. These all will open pathways for immunization of children, thus helping to prevent any more waves of this pandemic to occur.

3.4.3 Treatment Modalities

The mechanism of action of different therapeutics against COVID-19 was provided by Triggle et al. (2021) (Fig. 3.10).

3.4.4 Diagnostics

In this pandemic, rapid and accurate identification of COVID-19 patients is critical to break the chain of infection in community. Currently, reverse transcription polymerase chain reaction (RT-PCR) is gold standard test for COVID-19 testing, with turnaround time of 6–8 h for result (Majumder and Minko 2021; Fig. 3.11). The sensitivity of RT PCR is 85–95%; thus, RT PCR negative report doesn't completely rule out COVID-19. Moreover, lots of factors contribute to RT-PCR result like sample collection, sample handling, and viral load. Lateral flow immunochromatography for detecting antigen/antibodies in nasopharyngeal swabs and serum/plasma have been approved and are widely used for point-of-care testing as they make detection rapid, simple, safe, and cost-effective. Antibody detection via lateral flow assay is also being used for seroprevalence studies thus allowing contact tracing too. Next-generation gene sequencing is being used to understand more about the pathogenesis of this virus as well as to detect novel mutations SARS-COV2 is undergoing. High-resolution computerized tomography (HRCT) scan helps in diagnosing lung lesions and grading them as mild, moderate, severe which then decides the line of treatment along with other clinical findings.

3.4.4.1 New-Generation Diagnostics

While the qPCR test is the primary tool utilized in clinical laboratories to recognize the causative agent of the CoVID-19 (e.g., SARS-CoV-2), recently, the development of clustered regularly interspaced short palindromic repeats (CRISPR)-based

Fig. 3.9 (continued) Management of SARS-CoV-2 and COVID-19 as a Basis for Controlling the Pandemic. Frontiers in Immunology, 12, 631139. https://doi.org/10.3389/fimmu.2021.631139.

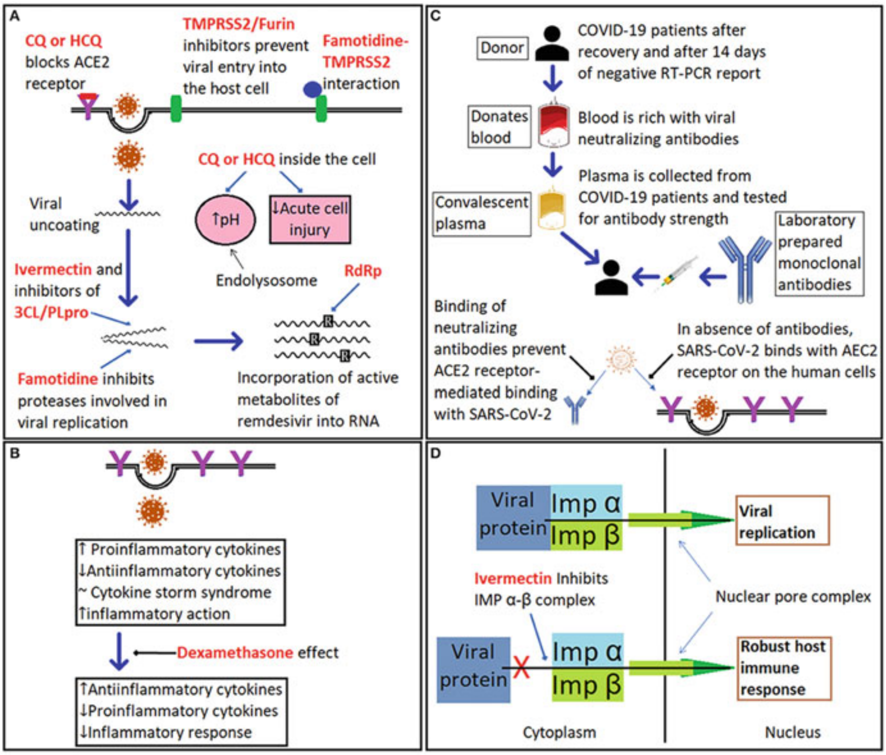

Fig. 3.10 The mechanism of action of different therapeutics against COVID-19. **(A)** illustrates the mode of action of drugs targeting COVID-19 including chloroquine (CQ) and hydroxychloroquine (HCQ), which have multiple putative sites of action: (i). ACE2 receptor for SARS-CoV-2; (ii). Increasing the pH of the endolysosome; and (iii). suppression of the immune response. Sites of action of TMPRSS2 inhibitors such as camostat, famotidine, and furin inhibitors are shown; famotidine is also a putative inhibitor of the 3CL/PLpro proteases; ivermectin is a putative TMPRSS2 inhibitor that also inhibits the importin (IMP) α-β complex and viral replication; while remdesivir inhibits viral RNA polymerase. **(B)** Dexamethasone suppresses expression of pro-inflammatory cytokines. **(C)** Summary of role of convalescent plasma and monoclonal antibody therapy. **(D)** Ivermectin inhibits the heterodimeric importin (IMP) α/β complex via binding directly to IMPα preventing nuclear import of key viral proteins (for more details and references, see section COVID-19 therapeutics). (Source: Triggle, C. R., Bansal, D., Ding, H., Islam, M. M., Farag, E., Hadi, H. A., & Sultan, A. A. (2021). A Comprehensive Review of Viral Characteristics, Transmission, Pathophysiology, Immune Response, and Management of SARS-CoV-2 and COVID-19 as a Basis for Controlling the Pandemic. Frontiers in Immunology, 12, 631139. https://doi.org/10.3389/fimmu.2021.631139. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY))

diagnostic systems (Rahimi et al. 2021; Fig. 3.12). DNA endonuclease-targeted CRISPR trans reporter (DETECTR) is another CRISPR-based diagnostic system (CRISPR/Cas12) that detects viral infections rapidly (~30 min), inexpensively, and accurately (Chen et al. 2018; Broughton et al. 2020; Rahimi et al. 2021; Perrotta

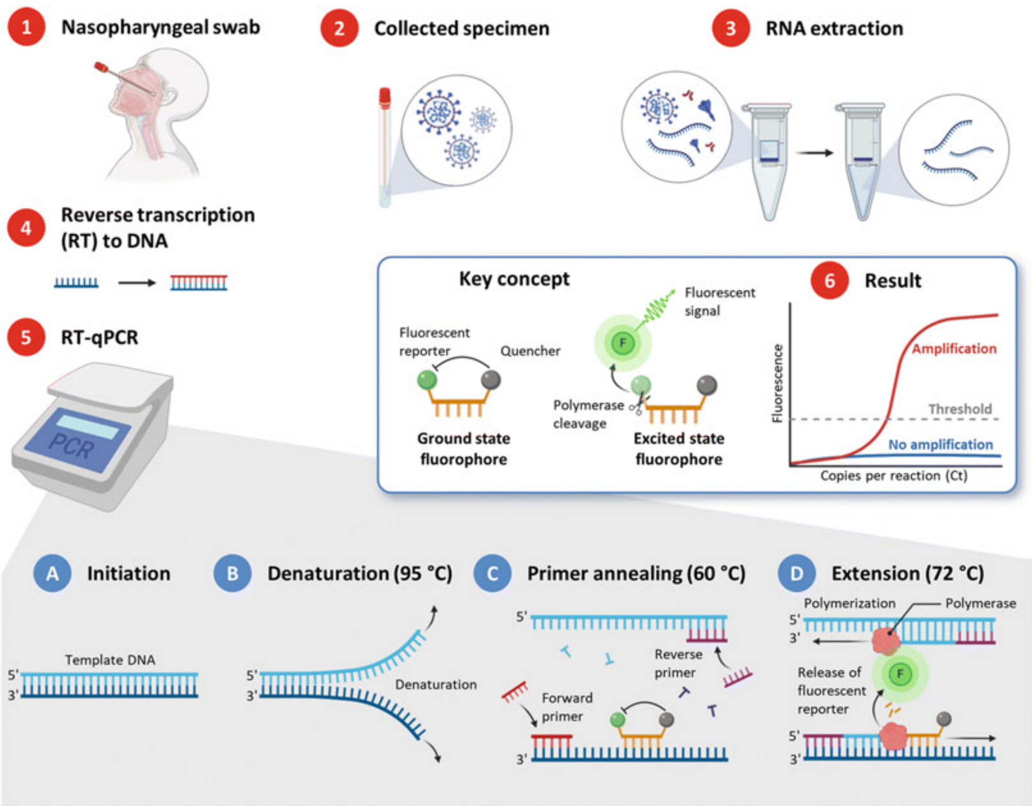

Fig. 3.11 A typical procedure of COVID-19 diagnostics through RT-PCR. The RT-PCR method involves sample collection and extraction of viral RNA sample. The extracted RNA sample is converted to its complementary DNA (cDNA) by the reverse transcription. Finally, the amplification of this DNA sample is performed in qPCR, and the viral cDNA is detected by a fluorescent signal. (Source: Majumder J, Minko T. Recent Developments on Therapeutic and

et al. 2020; Fig. 3.12) JIAO et al. (2021) reported "engineering of reprogrammed tracrRNAs that link the presence of any RNA of interest to DNA targeting with different Cas9 orthologs." JIAO et al. (2021) utilized this capability to develop as the basis for a multiplexable diagnostic platform termed LEOPARD (leveraging engineered tracrRNAs and on-target DNAs for parallel RNA detection), which allows simultaneous detection of RNAs from different viruses in one test and distinguished severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its D614G (Asp614 → Gly) variant with single-base resolution in patient samples (Jiao et al. 2021, *SCIENCE*, MAY 28, 2021: 941–948).

3.5 Climate Change Control Measures

Achieving 1.5 °C requires close interaction among mitigation, adaptation, and sustainable development under geophysical, environmental–ecological, technological, economic, sociocultural, and institutional feasibilities (Kjellstrom et al. 2009; Levy and Patz 2015; Kumar et al. 2018, 2020).

3.5.1 The Role of United Nations and Its Agencies

The international political response to climate change began at the Rio Earth Summit in 1992, where the "Rio Convention" included the adoption of the UN Framework on Climate Change (UNFCCC) on March 21, 1994. UNFCCC has a near-universal membership of 197 parties and aims at stabilizing atmospheric concentrations of greenhouse gases (GHGs) to avoid "dangerous anthropogenic interference with the climate system." UNEP facilitates the development of renewable energy and raising public awareness for transition to low-carbon societies with its 2030 Agenda. It also undertakes Global Programme of Action (GPA) for the Protection of the Marine Environment from Land-Based Activities (https://www.unenvironment.org/explore-topics/sustainable-development-goals/why-do-sustainable-development-goals-matter).

In December 2015, the 21st Session of the Conference of the Parties (COP21/CMP1) convened in Paris, France, adopted Paris Agreement on climate change a landmark for the multilateral climate change process, legally binding 196 parties together in an international treaty with the goal to limit global warming to well below 2 (and preferably 1.5 °C) compared to preindustrial levels (UNFCCC 2015; see also UNEP 2020) by cutting carbon pollution as much as possible. The 24th Conference of the Parties (COP 24) to the United Nations Framework Convention on Climate

Fig. 3.11 (continued) Diagnostic Approaches for COVID-19. AAPS J. 2021 Jan 5; 23(1): 14. https://doi.org/10.1208/s12248-020-00532-2. PMID: 33400058; PMCID: PMC7784226. http://creativecommons.org/licenses/by/4.0/)

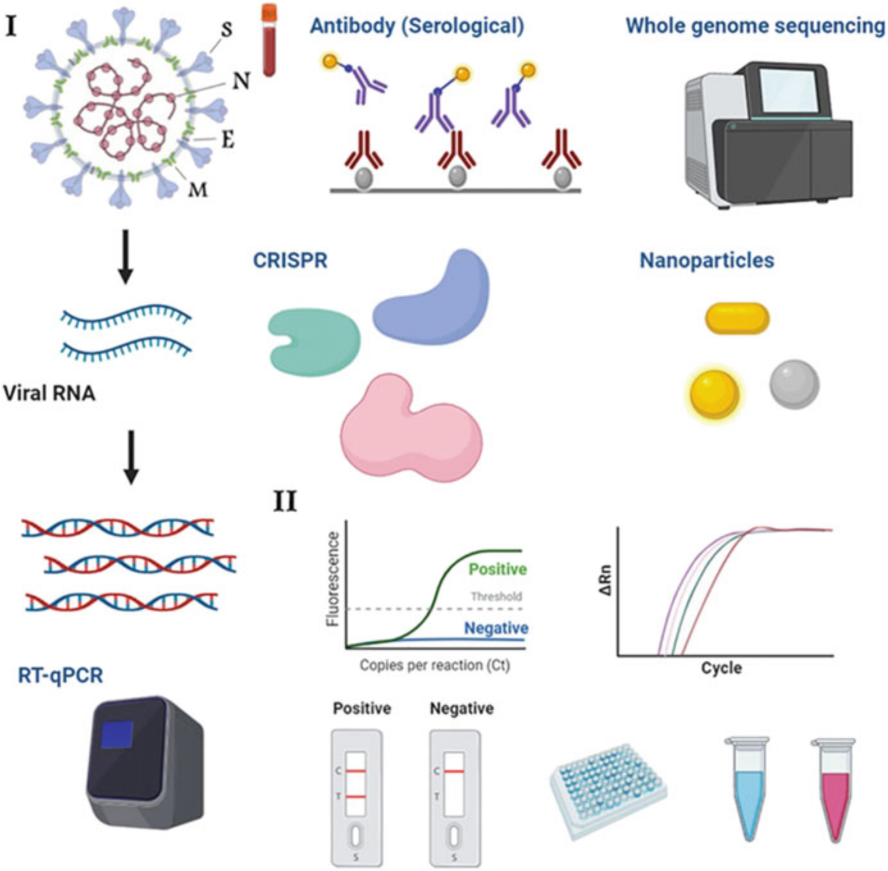

Fig. 3.12 Schematic representation of various diagnosis tools for SARS-CoV-2. I. Types of methods used to diagnose SARS-CoV-2 and II. SARS-CoV-2 diagnostic methods results' readout ways. (Rahimi, H., Salehiabar, M., Barsbay, M., Ghaffarlou, M., Kavetskyy, T., Sharafi, A., Davaran, S., Chauhan, S. C., Danafar, H., Kaboli, S., Nosrati, H., Yallapu, M. M., & Conde, J. (2021). CRISPR Systems for COVID-19 Diagnosis. ACS Sensors, 6(4), 1430–1445. https://doi.org/10.1021/acssensors.0c02312. Permission granted ACS permission granted May 27, 2021)

Change (UNFCCC) focused on adopting a package of implementing rules for the Paris Agreement—Katowice Rulebook (https://cop24.gov.pl/fileadmin/DEKLARACJE/Katowice_Ruleboo_E-BOOK_mini.pdf). During COP24 in Katowice (Poland), 44 countries signed the declaration "Driving Change Together"—Katowice Partnership for Electromobility, as well as 82 parties the "Forest Declaration" (https://unctad.org/en/pages/MeetingDetails.aspx?meetingid=2257). Next COP 26 will be held in Glasgow UK on November 1–12, 2021.

The United Nations Convention to Combat Desertification (UNCCD), established in 1994, aims at "*A future that avoids, minimizes, and reverses* *desertification/land degradation and mitigates the effects of drought in affected areas at all levels . . . to achieve a land degradation-neutral world consistent with the 2030 Agenda for Sustainable Development*" (Source: https://www.unccd.int/convention/about-convention). The UNCCD encourages the participation of local people in combating desertification and land degradation (https://www.unccd.int/sites/default/files/inline-files/ICCD_COP%2813%29_L.18-1716078E_0.pdf; https://doi.org/10.3390/ijerph18010136). UNCCD collaborates closely with Rio Conventions; the Convention on Biological Diversity (CBD) and the United Nations Framework Convention on Climate Change (UNFCCC) to meet complex challenges of dynamics of land, climate, and biodiversity (https://www.unccd.int/convention/about-convention).

Increasing awareness of the global climate emergency continues to keep the issue at or near the top of the list of concerns facing policymakers, business leaders, and citizens internationally (UN 2021; UNDP 2020, 2021; WEF 2021; Cong et al. 2021). Recently, Pasztor (2021) indicated that recent UN reports the indications warn us about continuous rise in atmospheric concentrations and keeping the earth on a trajectory to levels of warming that will precipitate further environmental, social and economic disruption and suffering on unprecedented scales (IPCC 2018; UNEP 2020; WMO 2020). The United Nations Environment Programme (UNEP) along with multilateral environment agreements (MEAs) launched Frontiers 2018/19 on March 4, 2019, prior to the fourth UN Environment Assembly in Nairobi, Kenya (https://www.unenvironment.org/explore-topics/sustainable-development-goals/why-do-sustainable-development-goals-matter/goal-13).

3.5.2 Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES)

The Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) established in (2012) makes regular and timely assessments of knowledge on biodiversity and ecosystem services and their interlinkages at the global level (Díaz et al. 2015a, b, 2019; http://www.ipbes.net) (Fig. 3.13). It works in close interaction with Conference of the Parties of the Convention on Biological Diversity (CBD) to prepare a global assessment of biodiversity and ecosystem services building, inter alia, on its own and other relevant regional, subregional, and thematic assessments, as well as on national reports (Díaz et al. 2015b; Fig. 3.13).

3.6 Sustainable Development Goals (SDGs)

The UN sustainable development goals (SDGs) include 17 sustainable development goals for 2030 (United Nations. Transforming Our World: The 2030 Agenda for Sustainable Development, Working Papers, 2015). SDS goals requires multistakeholder approach (https://www.unenvironment.org/explore-topics/sustainable-development-goals/what-we-do/global-opportunities-sustainable).

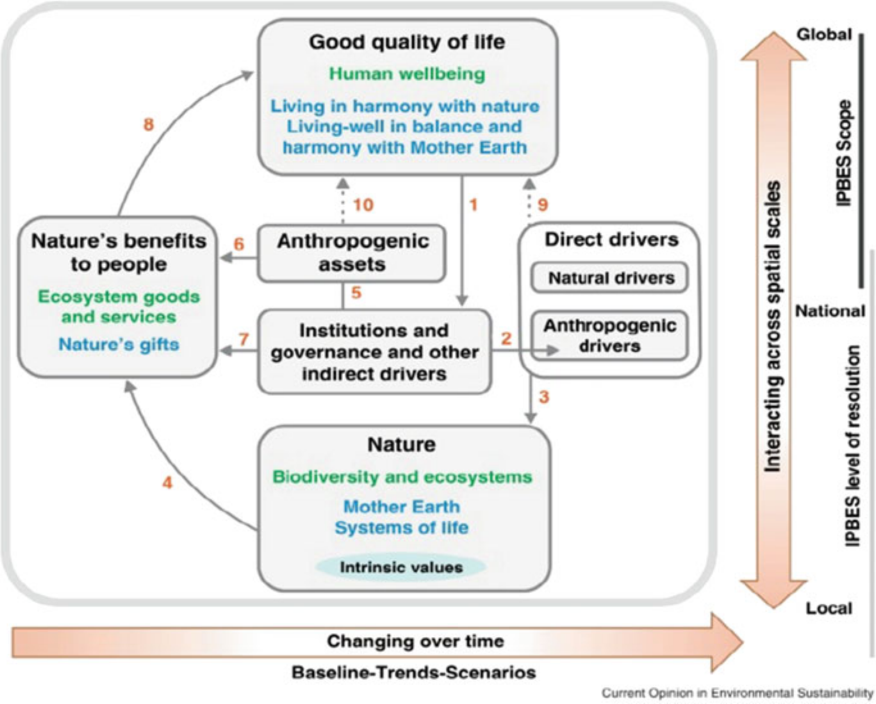

Fig. 3.13 The IPBES Conceptual Framework (CF). In the central panel, delimited in gray, boxes, and arrows denote the elements of nature and society that are at the main focus of the platform. In each of the boxes, the headlines in black are inclusive categories that should be intelligible and relevant to all stakeholders involved in IPBES and embrace the categories of western science (in green) and equivalent or similar categories according to other knowledge systems (in blue). The blue and green categories mentioned here are illustrative, not exhaustive, and are further explained in the main text. Solid arrows in the main panel denote influence between elements; the dotted arrows denote links that are acknowledged as important, but are not the main focus of the platform. Links indicated by numbered arrow are described in the main text (section on linkages among the elements, and Box 2). The anthropocentric values of nature are embedded in the nature, nature's benefits to people and good quality of life boxes, and in the arrows connecting them. The intrinsic values of nature (represented by a blue oval at the bottom of the nature box) are independent from human experience and thus do not participate in these arrows (see values section in main text for detailed explanation). The thick colored arrows below and to the right of the central panel indicate that the interactions between the elements change over time (horizontal bottom arrow) and occur at various scales in space (vertical arrow). The vertical lines to the right of the spatial scale arrow indicate that although IPBES assessments will be at the supranational–subregional to global–geographical scales (scope), they will in part build on properties and relationships acting at finer—national and subnational scales (resolution, in the sense of minimum discernible unit). The resolution line does not extend all the way to the global level because, due to the heterogeneous and spatially aggregated nature of biodiversity, even the broadest global assessments will be most useful if they retain finer resolution. This figure, modified from Ref (Díaz et al. 2015a), is a simplified version of that adopted by the Second Plenary of IPBES (UNEP 2014) and it retains all its essential elements but some of the detailed wording explaining each of the elements has been eliminated within the boxes to improve readability. (Source: Díaz, S., Demissew, S., Carabias, J., Joly, C., Lonsdale, M., Ash, N., … Zlatanova, D. (2015b). The IPBES Conceptual Framework — connecting nature and people. Current Opinion in Environmental Sustainability, 14, 1–16. https://doi.org/10.1016/j.cosust.2014.11.002. Reprinted with RightsLink license no 5067401150652, dated May 13, 2021, see also https://www.ipbes.net/)

Dakora et al. (2020) reported that they include Agriculture Green Development (AGD) focused on alleviating the deleterious effects of intensive agriculture along with excessive use of mineral fertilizers and agrochemicals. Achieving these goals will provide land degradation neutrality which will result in food security, as well as poverty reduction (IPBES 2018; IPCC 2019a, b, c). Progress toward SDG 3, SDG 7, SDG 13, and SDG 15 will also contribute to accomplishing other SDGs. SDG 7 aims at "The environment provides a series of renewable and non-renewable energy sources, i.e., solar, wind, hydropower, geothermal, biofuels, natural gas, coal, petroleum, uranium. Increased use of fossil fuels without actions to mitigate greenhouse gases will have global climate change implications. Energy efficiency and increased use of renewables contribute to climate change mitigation and disaster risk reduction" (https://www.unenvironment.org/explore-topics/sustainable-development-goals/why-do-sustainable-development-goals-matter/goal-7). SDG 13 focuses on strengthening resilience and adaptive capacity to climate-related hazards and natural disasters in all countries and integration of climate change measures into national policies, the improvement of education, awareness-raising and institutional capacity on climate change mitigation, adaptation, impact reduction, and early warnings. SDG 15 stands to protect, restore, and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, halt and reverse land degradation, and halt biodiversity loss.

3.6.1 Climate Adaptation

Energy demand for cooling in the hot tropical and subtropical areas can be reduced by using energy-efficient building structures. DARA (2012) building design reduces exposures to temperature extremes, and thus reduces energy demand for cooling or heating. World Trade Park (WTP), Jaipur, has longest glass wall structure with special glass which reduces impact of solar radiation and reduced cooling expenses of the building (personal communication). Energy-efficient electric cars could save energy.

3.6.2 Climate Mitigation

Climate change mitigation will have significant role in achieving the necessary protection of good population health for current and future generations (Kjellstrom et al. 2009; Institute of Medicine 2014; Dooley et al. 2018; Consoli 2019). The mitigation pathways are characterized by decarbonization of electricity production, electrification of energy, and deep reductions in agricultural emissions. Mitigation strategies related to land use are a key element of most modeled scenarios that provide strong mitigation, alongside emissions reduction in other sectors.

Rehabilitation and sustainable management of land matters are critical to closing the emissions gap and staying on target (https://www.unccd.int/issues/land-and-climate-change). About one-quarter of the 2030 mitigation pledged by countries in their initial Nationally Determined Contributions (NDCs) under the Paris Agreement is expected to come from land-based mitigation options (IPCC 2019a, b, c).

3.6.2.1 The Role of Carbon Dioxide Removal (CDR)

Achieving the temperature targets of the UNFCCC Paris Agreement through emissions abatement alone is currently looking increasingly infeasible as global emissions continue to rise. According to Hoegh-Guildberg (2018), most least-cost-mitigation pathways to limit peak or end-of-century warming to 1.5 °C make use of carbon dioxide removal (CDR), predominantly employing significant levels of bioenergy with carbon capture and storage (BECCS) and/or afforestation and reforestation (AR) in their portfolio of mitigation measures. The longer the delay in reducing CO_2 emissions toward zero, the larger the likelihood of exceeding 1.5 °C, and the heavier the implied reliance on net negative emissions after mid-century to return warming to 1.5 °C (Rogelj et al. 2018; IPCC 2018a). Carbon dioxide removal (CDR) focuses on carbon storage on land or sequestration in geological reservoirs. It is also intrinsically required to meet the Paris Agreement target of balancing emissions sources and sinks (UNFCCC 2015). Several mitigation response options have technical potential for >3 Gt CO_2 eq year^{-1} by 2050 through reduced emissions and CDR (IPCC 2019a, b, c; Bednar et al. 2019).

3.6.2.2 Bioenergy with Carbon Capture and Storage (BECCS)

BECCS entails burning CO_2-absorbing biomass, capturing the emissions, and storing them in long-term underground bioenergy with carbon capture and storage (BECCS) (Brack and King 2021). Substantial use of bioenergy use is required in 1.5 °C pathways with or without BECCS due to its multiple roles in decarbonizing energy use (De Coninck and Backstrand 2011; Obersteiner et al. 2018; Kumar 2020a, b).

3.7 Deforestation and Restoration Measures

Yamamoto et al. (2001) further projected that the global mature forest area will decrease by 24% between 1990 and 2100, because of growth of both population and wood biomass demand per capita in the developing regions. Some developing regions, such as Centrally Planned Asia, Middle East and North Africa, and South Asia, will suffer major loss of forests by 2100 (Yamamoto et al. 2001). This is very alarming situation. In order to combat this, an action plan Great Green Wall (GGW) has been announced.

3.7.1 Great Green Wall (GGW)

The Great Green Wall Initiative has evolved from its initial focus on tree planting toward a comprehensive rural development initiative aiming to transform the lives of Sahelian populations by creating a mosaic of green and productive landscapes across

11 countries (Senegal, Mauritania, Mali, Burkina Faso, Niger, Nigeria, Chad, Sudan, Ethiopia, Eritrea, Djibouti). The Great Green Wall multi-actor Accelerator seeks to facilitate the coordination and collaboration of donors and stakeholders involved in the Great Green Wall Initiative (source: https://www.unccd.int/actionsgreat-green-wall-initiative/great-green-wall-accelerator). The Great Green Wall Accelerator will be coordinated through the Pan Africa Agency for the Great Green Wall (PAAGGW), with initial support from the United Nations Convention to Combat Desertification (UNCCD).

3.8 Renewable Energy Sources

Popp et al. (2016) reported that in the last 35 years global energy supplies have nearly doubled but the relative contribution from renewables has increased from 13% to 19%, including about 9.3% from traditional biomass and about 9.7% from modern renewables (Gielen et al. 2019). Sustainable fuel alternatives such as alcohol, natural gas, and biodiesel is the best way to reduce NOx, CO, HC, CO_2 emissions (Thakur et al. 2017).

"In contrasts to median estimates for current unconditional NDCs of 52–-58 Gt CO_2 eq year^{-1} in 2030 the available pathways that aim for no or limited (less than 0.1 °C) overshoot of 1.5 °C keep GHG emissions in 2030 to 25–-30 Gt CO_2 eq year^{-1} reaching net zero around 2050 (2045–2055 interquartile range)" (Bernstein et al. 2008). This will require large-scale deployment of carbon dioxide removal (CDR) measures. A transition away from fossil fuels to low-carbon solutions will play an essential role, as energy-related carbon dioxide (CO_2) emissions represent two-thirds of all greenhouse gases (GHG) (IPCC 2014a, b). Breakdown of energy-related CO_2 emissions by technology in the Renewable Energy Map Case compared to the Reference Case was presented by Gielen et al. (2019) (Fig. 3.14). Adaptation and mitigation measures must also be planned to protect human rights, promote social justice, and avoid creating new problems or exacerbating existing problems for vulnerable populations.

Gielen (2019) provided breakdown of renewables use in total final energy consumption terms (Fig. 3.15). Yamamoto et al. (2001) projected that the ultimate bioenergy supply potential of biomass residues will be 265 EJ year^{-1} in the world in 2100. The practical potential of biomass residues in the world will be 114 EJ year^{-1}, which is almost equal to one-third of the commercial energy consumption in the world in 1990. Primary energy supplied by bioenergy ranges from 40 to 310 EJ year^{-1} in 2050 (Fig. 3.13) (https://www.unenvironment.org/resources/frontiers-201819-emerging-issues-environmental-concern). "Large-scale implementation of mitigation response options that limit warming to 1.5 or 2 °C would require conversion of large areas of land for afforestation/reforestation and bioenergy crops, which could lead to short-term carbon losses" (https://www.ipcc.ch/site/assets/uploads/sites/4/2019/11/03_Technical-Summary-TS.pdf). Around 4 million square kilometers of farmland will be needed to grow bioenergy crops for climate action which is over ten times the amount of land involved in biofuels

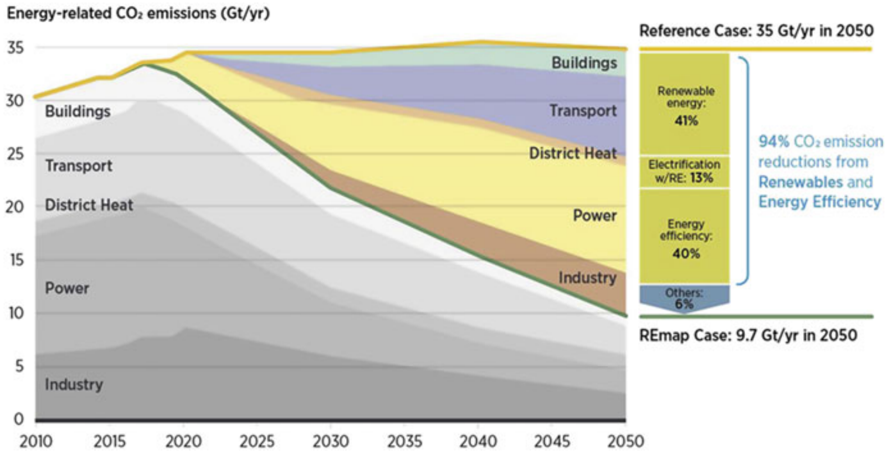

Fig. 3.14 Figure shows the breakdown of energy-related CO_2 emissions by technology in the REmap Case compared to the Reference Case. The figure excludes emissions from non-energy use (feedstocks). (Source: Gielen, D., Boshell, F., Saygin, D., Bazilian, M. D., Wagner, N., & Gorini, R. (2019). The role of renewable energy in the global energy transformation. Energy Strategy Reviews, 24, 38–50. Reproduced with license number 5065640898563, dated May 10, 2021. Based on IRENA Global Energy Transformation: A Roadmap to 2050 IRENA, Abu Dhabi (2018))

production today (IPCC 2014a, b). This is about 0.5% of total world farmland. By 2050, renewables (including bioenergy, hydro, wind, and solar, with direct-equivalence method) supply a share of 52–67% of primary energy in 1.5 °C pathways while coal decreases to 1–7%. However, large fraction of this coal use will be combined with carbon capture and storage (CCS) technology.

3.8.1 Biomass, Bioenergy, and Biofuels

The biomass feedstocks include plant oils, starchy materials, sugar crops like sugarcane and sugar beets, cereals, switchgrass, hydrocarbon-yielding plants, non-edible oil-yielding plants, and organic waste (Hill et al. 2009; Kumar and Roy 2018; Dugar and Stephanopoulos 2011; Caspeta and Nielsen 2013; Kumar 2013; Kumar et al. 2018, 2020; Kumar 2018, 2020a, b; Kumar and Jaiman 2021, this volume). One of the great benefits of using biomass for energy production is its potential to reduce the greenhouse gas emissions associated with fossil fuels (http://www.financialexpress.com/news/second-generationbiofuels/271495; https://www.un.org/en/ecosoc/docs/pdfs/biofuels.pdf). Challenges will remain until we are able to rely on first-generation technologies for biofuel production. The associated risks, however, include potential effects on land use, agricultural production systems, habitats, biodiversity, land, air and water qualities (Shaik and Kumar 2014). Details of biofuel production and improvement have been presented earlier (Kumar and Kumar 2002;

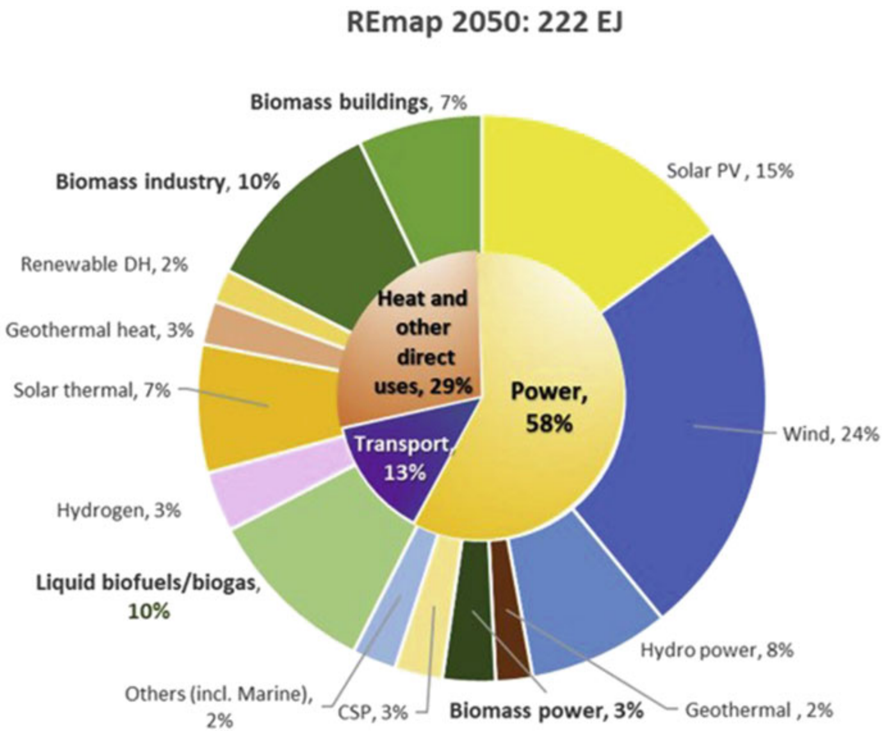

Fig. 3.15 Breakdown of renewables use in total final energy consumption terms, REmap 2050. (Source: Gielen, D., Boshell, F., Saygin, D., Bazilian, M. D., Wagner, N., and Gorini, R. (2019). The role of renewable energy in the global energy transformation. Energy Strategy Reviews, 24, 38–50; https://doi.org/10.1016/j.esr.2019.01.006. Reproduced with license number 5065640898563, dated May 10, 2021. Based on IRENA Global Energy Transformation: A Roadmap to 2050 IRENA, Abu Dhabi (2018))

Kumar 2008, 2011, 2013; 2020a, b; Kumar and Roy 2018; Kumar et al. 2018, 2020; Kumar and Jaiman 2021, this volume).

Depending on the biofuel and the blend mix ethanol and biodiesel are better combustibles reducing the emission of pollutants such as CO, hydrocarbons (HC), sulfur oxide, and particulates by up to half of the emissions from fossil fuels (Erdiwansyah Mamat et al. 2019). Bioenergy is renewable energy from biological sources (see FAO 2008a, b; see also FAO 2019, 2020). Abanades et al. (2015) suggested that in 2050, biofuels will still be as important as electromobility in the displacement of carbon-emitting fossil fuels (ASTM 2008; Moser and Vaughn 2010; Moser 2011). With synthetic biology, it may be possible to produce fuel from various sources of carbon and energy (Bhansali and Kumar 2018; Datta et al. 2020; Liang et al. 2020; Milne et al. 2020). Kumar and Gupta (2018) studied potential of lignocellulosic materials for production of ethanol.

Second-generation technologies, such as production of fuels from microbes and lignocellulosic materials, may offer some respite, but not in the immediate future (Kumar 2018; Kumar et al. 2018, 2020). Because cellulosic ethanol can offer health benefits from $PM_{2.5}$ reduction that are of comparable importance to its climate-change benefits from GHG reduction, a shift from gasoline to cellulosic ethanol has greater advantages than previously recognized. These advantages are critically dependent on the source of land used to produce biomass for biofuels, on the magnitude of any indirect land use that may result, and on other as yet unmeasured environmental impacts of biofuels (Hill et al. 2009; Roy and Kumar 2013). Fermentation, station biofuels, application, source and influence of air pollution on the environment has been studied in detail by Erdiwansyah et al. (2019).

Several biofuels such as biodiesel (Kumar et al. 2018), bioethanol (Behera et al. 2014), bio-oil (Shuping et al. 2010), biogas (Singh et al. 2020; Saini et al. 2021; Esquivel-Patiño and Nápoles-Rivera 2021), and biohydrogen (Han et al. 2015) are in the process of full development and in use. Anaerobic microorganisms break down the carbohydrate-rich in dark fermentation processes and produce biohydrogen (Ghimire et al. 2015). Marques Thiago et al. (2019) presented elaborate review on biohydrogen production. Third-generation advanced biofuels include algal biofuels (Randhir et al. 2018).

de Coninck and Benson (2014) reported that nearly all of the climate measures assessed for limiting global warming to below 1.5 °C require large-scale bioenergy programs to succeed. Presently plant biomass provides 10% of global primary energy mainly as ethanol and biodiesel production are both expected to expand to reach, respectively, almost 135 billion L and 39 billion L by 2024 (5OECD/FAO. Agricultural Outlook, 2015, available online: https://doi.org/10.1787/data-00736-en) (Popp et al. 2016). In 2040, the share of biofuels in road transport fuels would range—depending on policies—from 5% to 18% globally, from 11% to 31% in the European Union and from 11% to 29% in the United States (International Energy Agency. World Energy Outlook 2015) (Bopp et al. 2016). Sustainable and renewable alternative sources have gained growing interest (Fig. 3.16)) (Vasić et al. 2021).

3.9 Future Perspectives

"It is unequivocal that human influence has warmed the atmosphere, ocean and land. Widespread and rapid changes in the atmosphere, ocean, cryosphere and biosphere have occurred" (IPCC 2021). Article 2 of the United Nations Framework Convention on Climate Change (UNFCCC) set goals are to develop climate-resilient pathways are development trajectories of combined mitigation and adaptation to realize the goal of sustainable development that help avoid "dangerous anthropogenic interference with the climate system" (Denton et al. 2014).

Possibility of synthetic or semisynthetic biofuel production using microorganism and synthetic pathways have been reviewed (Bhansali and Kumar 2018). Petroleum-derived liquid fuels currently provide around 95% of transport energy, and roughly

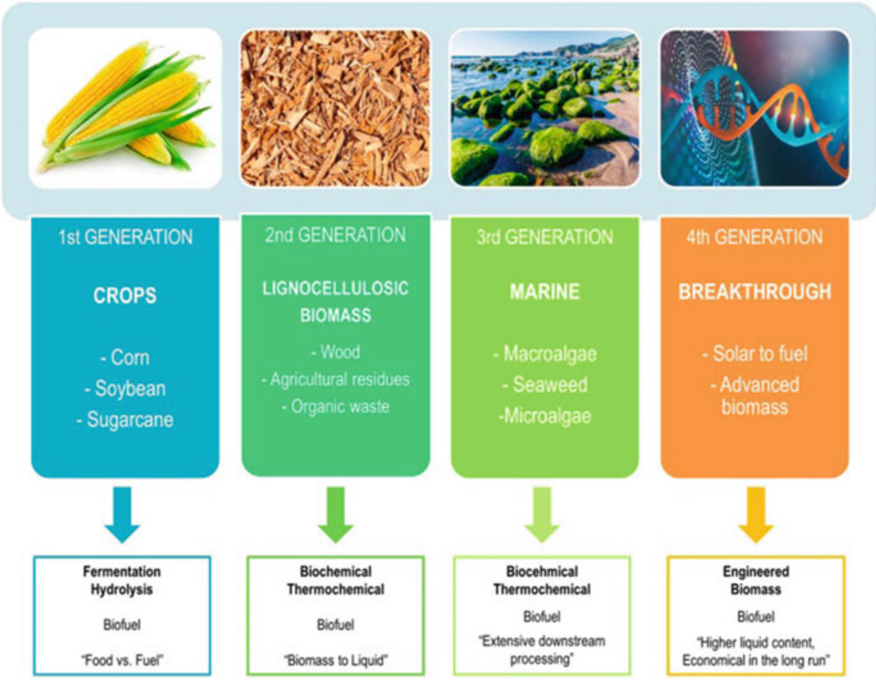

Fig. 3.16 Generations of biofuels. Source: Vasić K, Knez Ž, Leitgeb M. Bioethanol Production by Enzymatic Hydrolysis from Different Lignocellulosic Sources. Molecules. 2021 Feb 1;26(3):753. doi: 10.3390/molecules26030753. Reproduced under articles published in an open access Creative Common CC BY license any part of the article may be reused without permission provided that the original article is clearly cited. Reuse of an article does not imply endorsement by the authors or MDPI. RightsLink licence

60% of crude oil produced is used to make transport fuels (EIA 2016). The demand for transport fuels across the world is very large, at around 4.9 billion L each of gasoline and diesel and 1.3 billion L of jet fuel each day (Kalghatgi 2018). Transport accounts for about 20% of all energy used and around 23% of global carbon dioxide (CO_2) emissions (Sims et al. 2014). However, transport contributes only around 14% of global greenhouse gas (GHG) emissions—an amount that is comparable to the share of livestock farming for meat and dairy products if gases such as methane are included (Grossi et al. 2019). The carbon dioxide equivalent is a standard unit used to account for the global warming potential (IPCC 2013). Grossi et al. (2019) reported that the most important greenhouse gases from animal agriculture are methane and nitrous oxide. Methane, has an effect on global warming 28 times higher than carbon dioxide and nitrous oxide, arising from manure storage and the use of organic/inorganic fertilizers, is with a global warming potential 265 times higher than carbon dioxide.

Significant initiatives with varying motivations are taking place to develop the battery electric vehicle (BEV) (Yao et al. 2020). However, the greenhouse gas

(GHG) impact of battery electric vehicles (BEVs) would be worse than that of conventional vehicles if electricity generation and the energy used for battery production are not sufficiently decarbonized (Kalghatgi 2018). Kalghatgi (2018) further states that if coal continues to be a part of the energy mix, as it will in China and India, and if power generation is near urban centers, even local urban air quality in terms of particulates, nitrogen oxides, and sulfur dioxide would get worse. However, as the energy system is decarbonized and battery technology improves, there will be an increasing role for BEVs and hydrogen which could replace liquid hydrocarbons in transport and the required infrastructure will evolve in next decades (Yao et al. 2020). Meanwhile, there will certainly be increasing electrification, particularly of LDVs in the form of hybridization to improve ICEs (Yao et al. 2020).

3.10 Discussion

The global prosperity is increasing and so is the demand for energy which is largely derived from fossil fuels (Kumar 2018; Morrow et al. 2018). Anthropocene-induced climate change threatens to disrupt the life support systems on which humans depend such as food, air, water, shelter, security (Pattberg and Zelli 2016; Delanty and Mota 2017), while acknowledging the differentiated responsibility and opportunity to limit global warming and invest in prospects for climate-resilient sustainable development (Harrington 2016; Biermann et al. 2016). Is it an indication that we are living in (and causing) "the sixth mass extinction." The global environmental changes threaten our survival in Anthropocene. Anthropocene can be employed as a "boundary concept" (Brondizio et al. 2016).

Climate change is a threat to human health and survival, but adaptations and mitigation provide climate solutions which offer opportunities for advancing health and equity (Hulme 2014; Gould and Rudolph 2015). IPCC (2019a, b, c) reported "Implications of climate change, variability and extremes for land systems and historical changes in anthropogenic land cover have resulted in a mean annual global warming of surface air from biogeochemical effects." Climate change already endangers human health and well-being in numerous ways (Luber et al. 2014). The environmental and health consequences of climate change will profoundly affect human rights and social justice (Mathur and Kumar 2013, 2016; Levy and Patz 2015). Global climate change is causing miseries all over world but it disproportionately impacts poor and marginalized populations thereby increasing health inequities (Shonkoff et al. 2011; NRC 2007; Roundtable 2014). Robinson and Shine (2018) concluded that to limit global temperature rise to 1.5 °C above preindustrial levels and to minimize the adverse impacts of climate change on people and their human rights it is vital to pursue a pathway to zero carbon emissions by 2050.

In the next years the engagement of the health sector would be working to develop prevention and adaptation programs in order to reduce the costs and burden of climate change (Rossati 2017). The low-income countries have far less capability to adapt to climate change than high-income countries (DARA 2012; Levy and Patz 2015; Sanz et al. 2017). Gould and Rudolph (2015) have rightly said that the political controversy about the science of climate change appears to significantly influence the willingness of public health professionals to speak publicly about climate change as a significant public health issue.

Climate change is leading to desertification on one hand and melting of glaciers on the other. National Statement on Ethical Conduct in Human Research (2007) earlier suggested that "an appropriate institutional structure that fosters multidisciplinary intramural and extramural research is needed" to take full advantage of the "revolutions in biology and biotechnology" and development of "innovations in environmental biotechnology." United Nations Convention to Combat Desertification (UNCCD), land degradation in arid, semiarid, and dry subhumid areas suggested that various factors, including climatic variations and human activities, are the major causes of desertification (UN 2012).

Global efforts are underway for keeping global temperature well below 2 °C while pursuing efforts toward and adapting to a 1.5 °C warmer world. However, high-end emissions projection scenarios show global average warming of 2.6–4.8 °C by the end of the century, with all their regional amplification and attendant impacts (Watts et al. 2015). The ideal condition would be to keep global average temperature rise to less than 2 °C to avoid the risk of potentially catastrophic climate change impacts.

Pathways consistent with 1.5 °C of warming above preindustrial levels require total anthropogenic carbon dioxide (CO_2) emissions to be kept below 2900 billion tons ($GtCO_2$) by the end of the century. Limiting warming to 1.5 °C implies reaching net-zero CO_2 emissions globally around 2050 and concurrent deep reductions in emissions of non-CO_2 forcers, particularly methane. There is no single answer to the question of whether it is feasible to limit warming to 1.5 °C and adapt to the consequences. However, as of 2011, total emissions since 1870 were a little over half of this and with current trends it is expected to exceed 2900 Gt CO_2 in the next 15–30 years. Whether or not humanity can reduce greenhouse gas emissions quickly enough to slow climate change to a rate that will allow societies to successfully adapt is not yet known (Barrett et al. 2015).

Rapid rate of deforestation is leading to reduction in carbon sinks. Global desertification is increasing. One of the global responses is the UNCCD Strategic Framework for 2018–2030 aims to reduce the impacts of drought in affected areas, restore the productivity of degraded land, and to achieve a land degradation-neutral world. This is consistent with the 2030 Agenda for Sustainable Development, specifically with Sustainable Development Goal (SDG) 15, "Life on Land," and with the objectives of the UNCCD (UN 2017). UN laid goals of social development SDGs promotes use of more sustainable transport—walk, cycle, or public transport instead of using cars. SDG 15 stands to protect, restore, and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, halt and reverse land degradation, and halt biodiversity loss. In addition, the goals and objectives of the UNCCD are in alignment with the complex challenges and interconnections between land, biodiversity, and climate change (UN 2012; Patz et al. 2012).

"Globally, greening trends (trends of increased photosynthetic activity in vegetation) have increased over the last 2–3 decades by 22–33%, particularly over China, India, many parts of Europe, central North America, southeast Brazil and southeast Australia" (source: IPCC 2019a, b, c). During the growing season, afforestation generally brings cooler days from increased evapotranspiration, and warmer nights. "During the dormant season, forests are warmer than any other land cover, especially in snow-covered areas where forest cover reduces albedo" (IPCC 2019a, b, c).

Biofuel programs need to be integrated within a broader context of investment in rural infrastructure and human capital formation (Tao et al. 2021). Thus, climate action including reforestation and afforestation, hydroelectric dams, wind or solar energy installations, and biofuel plantations pose risks to human rights including the right to housing and to a livelihood, the right to water and to food, and the right to take part in cultural life (Climate Changes and Human Rights (2015) (https://web.law.columbia.edu/sites/default/files/microsites/climate-change/climate_change_and_human_rights.pdf); Robinson and Shine 2018).

Climate action and protection of human rights and social justice have a role to play in determining future of mankind (Levy and Patz 2015). There are already examples from the UN Framework Convention on Climate Change's Clean Development Mechanism and Reduced Emissions from Deforestation and Forest Degradation (REDD+) where climate action has resulted in human rights violations (Wallbott and Schapper 2017; Robinson and Shine 2018). This means if climate adaptation and mitigation projects are designed without the participation of local people, they can lead to conflict or to the project being rejected by the community (Penz et al. 2011; Robinson and Shine 2018). These circumstances are shaped by the distribution of money, power, and resources at global, regional, national, and local levels, and are mostly responsible for health inequities (World Health Organization Commission on the Social Determinants of Health 2008; Roberts and Stott 2011). Through their effect on social determinants of health, some climate change strategies can *increase* the environmental, economic, and health burdens on communities already bearing the burden of cumulative environmental exposures, poor health, discrimination, and poverty (Shonkoff et al. 2011). However, the risks to human rights of climate inaction and of climate impacts far outweigh the risks to human rights posed by climate action consistent with meeting the 1.5 °C goal set in the Paris Agreement (Kumar et al. 2020).

Climate change has been described as the biggest global health threat of the twenty-first century (Costello et al. 2009; Cardwell and Elliott 2013). The 2015 Lancet Commission on Health and Climate Change has been formed to map out the impacts of climate change, and the necessary policy responses, in order to ensure the highest attainable standards of health for populations worldwide. The changes in the range and transmission of infectious disease, food insecurity, health impacts of air pollution and temperature stress, reduced access to safe water, and related psychosocial impacts are now central issue (Cardwell and Elliott 2013). According to Gould and Rudolph (2015), efforts to increase public health sector engagement should focus on education and communications, building leadership and funding, and increasing work on the shared root causes of climate change and health inequities. Malena-Chan (2019) reported that decades of widespread knowledge about climate change have not translated into adequate action to address impacts on population health and health equity. There is need to make impact of climate change from an environmental to a health issue (Gaba et al. 2015; Malena-Chan 2019).

3.11 Conclusion

The global changes that the humans are currently experiencing have never happened before. Climate and COVID-19 are two major challenges for the mankind for its survival. In order to contain the global warming, technologies that reduce greenhouse emissions and the consumption of water resources would be needed. This will need energy conservation in building design and urban planning, and reduced waste of energy for transport, building heating/cooling, and agriculture. Major effect of climate-sensitive diseases is the poorest populations. It would also involve shifts in agricultural production and food systems to reduce energy and water use particularly in meat production. Achieving green transformation of agriculture by implementation of crop diversification schemes can enhance targeted ecosystem services consisting of provision, regulation, and cultural services according to regional characteristics and social and economic demands.

In order to prevent the spread of infectious and vector-borne diseases, it would be necessary to establish an integrated notification network of veterinary, entomological, and human survey, with particular attention to avoid the introduction of new human and animal pathogens. Then, it is necessary to optimize existing and/or design novel cropping systems based upon farming practices and ecological principles, and to strengthen targeted ecosystem services to achieve the identified objectives.

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stress. In Global Land Outlook Working Paper, United Nations Convention to Combat Desertification (UNCCD), Bonn, Germany, 2019. Available online: https://knowledge.unccd.int/publication/landunder-pressure-health-under-stress (accessed on December 25, 2020). This is an open access article distributed under the Creative Commons Attribution License. Figure 3.6 Source: https://www.nbcnews.com/science/science-news/worlds-largest-iceberg-just-broke-antarctic-ice-shelf-rcna974; https://phys.org/news/2021-05-vast-antarctic-iceberg-drift-ocean.htm. Figure 3.7 Watts N, W Neil Adger, Paolo Agnolucci, Jason Blackstock, Peter Byass, Wenjia Cai, Sarah Chaytor, Tim Colbourn, Mat Collins, Adam Cooper, Peter M. Cox, Joanna Depledge, Paul Drummond, Paul Ekins, Victor Galaz, Delia Grace, Hilary Graham, Michael Grubb, Andy Haines et al. (2015). *The Lancet* 386: 1861–1914. Reproduced with permission from RightsLink license number 5060710880266, May 2, 2021. Figure 3.8 Perrotta F, Matera MG, Cazzola M, Bianco A. Severe respiratory SARS-CoV2 infection: Does ACE2 receptor matter? *Respir Med*. 2020 Jul; 168: 105996. doi: https://doi.org/10.1016/j.rmed.2020.105996. License number 5075330204643 dated 24th May 2021. Figure 3.9 Source: Triggle, C. R., Bansal, D., Ding, H., Islam, M. M., Farag, E., Hadi, H. A., & Sultan, A. A. (2021). A Comprehensive Review of Viral Characteristics, Transmission, Pathophysiology, Immune Response, and Management of SARS-CoV-2 and COVID-19 as a Basis for Controlling the Pandemic. *Frontiers in immunology*, *12*, 631139. https://doi.org/10.3389/fimmu.2021.631139. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). Figure 3.10 Source: Triggle, C. R., Bansal, D., Ding, H., Islam, M. M., Farag, E., Hadi, H. A., & Sultan, A. A. (2021). A Comprehensive Review of Viral Characteristics, Transmission, Pathophysiology, Immune Response, and Management of SARS-CoV-2 and COVID-19 as a Basis for Controlling the Pandemic. *Frontiers in immunology*, *12*, 631139. https://doi.org/10.3389/fimmu.2021.631139. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). Figure 3.11 Majumder J, Minko T. Recent Developments on Therapeutic and Diagnostic Approaches for COVID-19. AAPS J. 2021 Jan 5; 23(1): 14. doi: https://doi.org/10.1208/s12248-020-00532-2. PMID: 33400058; PMCID: PMC7784226. http://creativecommons.org/licenses/by/4.0/. Figure 3.12 Perrotta F, Matera MG, Cazzola M, Bianco A. Severe respiratory SARS-CoV2 infection: Does ACE2 receptor matter? Respir Med. 2020 Jul; 168: 105996. doi: https://doi.org/10.1016/j.rmed.2020.105996. Rahimi, H., Salehiabar, M., Barsbay, M., Ghaffarlou, M., Kavetskyy, T., Sharafi, A., Davaran, S., Chauhan, S. C., Danafar, H., Kaboli, S., Nosrati, H., Yallapu, M. M., & Conde, J. (2021). CRISPR Systems for COVID-19 Diagnosis. *ACS sensors*, *6*(4), 1430–1445. https://doi.org/10.1021/acssensors.0c02312. Permission granted ACS permission granted May 27, 2021. Figure 3.13 Source: Díaz, S., Demissew, S., Carabias, J., Joly, C., Lonsdale, M., Ash, N., Zlatanova, D. (2015b). The IPBES Conceptual Framework—connecting nature and people. *Current Opinion in Environmental Sustainability*, *14*, 1–16. https://doi.org/10.1016/j.cosust.2014.11.002. Reprinted with RightsLink license no 5067401150652 dated May 13, 2021. Figure 3.14 Gielen, D., Boshell, F., Saygin, D., Bazilian, M. D., Wagner, N., & Gorini, R. (2019). The role of renewable energy in the global energy transformation. *Energy Strategy Reviews*, *24*, 38–50. https://doi.org/10.1016/j.esr.2019.01.006. Figure 3.15 Gielen, D., Boshell, F., Saygin, D., Bazilian, M. D., Wagner, N., & Gorini, R. (2019). The role of renewable energy in the global energy transformation. *Energy Strategy Reviews*, *24*, 38–50. https://doi.org/10.1016/j.esr.2019.01.006. Reproduced with license number 5065640898563, dated May 10, 2021. Based on IRENA Global Energy Transformation. A Roadmap to 2050 IRENA, Abu Dhabi (2018). Figure 3.16 Vasić K, Knez Ž, Leitgeb M. Bioethanol Production by Enzymatic Hydrolysis from Different Lignocellulosic Sources. Molecules. 2021 Feb 1;26(3):753. doi: https://doi.org/10.3390/molecules26030753. Reproduced under articles published in an open access Creative Common CC BY license any part of the article may be reused without permission provided that the original article is clearly cited. Reuse of an article does not imply endorsement by the authors or MDPI. RightsLink licence.

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Environment and Green Technology

4

Moitri Let, Krishnendu Majhi, Ashutosh Kabiraj, and Rajib Bandopadhyay

Abstract

Due to the rapid increase in population, the process of industrialization and urbanization are also upsurge in a parallel manner. The nondegradable pollutants generated by anthropogenic activities show a detrimental effect on the biosphere. Various pollutants like heavy metals, oil spills, pesticides, fungicides, solvents, plentiful plastics, and industrial effluents cause several environmental health risks. For healing the environment, a sustainable solution, that is, green technology has come out. Green technology also known as clean technology reduces environmental pollution and conserves natural resources. Nowadays, the technology is very much acceptable for having advantages like zero or low emission of greenhouse gases, conservation of energy and natural resources, minimization of environmental degradation, and better use of renewable resources. There are some trending green technologies, like Bioremediation, Biomineralization, Phytoremediation, Bioaugmentation, Bioflocculation, Biosurfactant, Biocatalyst, Membrane Bioreactor (MBR), Wetlands, Nanogreen Technology (NGT), etc., which are used to rescue and secure the environment from the toxic pollutants. Various biological organisms like microbes (*Acinetobacter*, *Pseudomonas* etc.), algae (*Chlorella*, *Desmodesmus* etc.), plants (*Amaranthus*, *Camellia sinensis* etc.) or their byproducts (exopolysaccharides, enzymes, phenolic compounds, nanomaterials etc.) are involved in various mechanisms of green technology. In this chapter, various mechanisms of green technology and their widespread applicability have been compiled for environmental sustainable development.

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4.1 Introduction

Over the last few decades, various human activities like agriculture, urbanization, and industrialization release various toxic pollutants into the environment. Though various industries play an important role for developing the economy of every nation; meanwhile, these are also responsible for extensive environmental pollution. Toxic elements used in industrial processing are mainly discharged along with wastewaters, which shows severe adverse effects on the growth and metabolism of living organisms, soil fertility, and biogeochemical cycle. To overcome these problems some adequate treatments are needed for environmental protection and human health concern. A wide range of chemical and physical treatments like landfilling, excavation, soil washing, incineration, adsorption, precipitation, coagulation, filtration, photodegradation, chemical oxidation, etc. are available for mitigating inorganic and organic pollutants (Kumar et al. 2018). But these conventional approaches are very expensive, time-consuming, and environmentally destructive in nature. Due to the incompatible nature of these methods, some eco-friendly approaches are needed.

Searching for eco-friendly approaches, the world is moving toward the implementation of green technology to accomplish sustainable development. Green technology or clean technology generally defines the use of natural products or systems which protect the environmental natural resources also minimize the adverse effect of toxic pollutants (Bhardwaj and Neelam 2015). The main goals of green technologies is to conserve energy and natural resources, decreasing the emission of greenhouse gases, minimizing environmental degradation, and better use renewable resources. These days scientists use various green technologies like Bioremediation, Phytoremediation, Bioaugmentation, Bioflocculation, Biosurfactant, Biocatalyst, Membrane Bioreactor (MBR), Wetlands, Nanogreen Technology (NGT), etc. for protecting the environment from toxic pollutants.

Bioremediation is an emerging field of green technology which is a very effective approach to counter the ill effects of environmental pollution. In this system, microbial population like *Pseudomonas*, *Bacillus*, *Rhodococcus*, *Azotobacter*, *Mycobacterium*, etc. acts as bioremediating agents which absorb or adsorb the toxic pollutants as a source of nutrition (Verma and Kuila 2019). In phytoremediation, fast-growing plants are used to remove toxic pollutants from soil or water and reduce their bio-availability. Hyperaccumulator plant species like *Azolla pinnata*, *Ipomoea alpine*, *Eleocharis acicularis*, *Euphorbia cheiradenia*, *Pteris vittata*, *Pteris ryukyuensis*, *Phragmites australis*, etc. are applied for the phytoremediation process (Ashraf et al. 2019). Apart from these, a microbes-derived surface-active molecules (biosurfactant), extracellular polymers (glycolipids, exopolysaccharides, glycoproteins, proteins, and lipids) and microbial enzymes (biocatalyst) are significantly used as greener approaches.

The main motives of all the green technologies are the same, and social and economic development by sustaining nonrenewable sources (energy, fuels, and valuable metals), reducing environmental degradation, and also create sustainable green industrialization and economical market.

4.2 Nondegradable Toxic Pollutants

Pollutants are hazardous to environment and it also affects our physical and mental health. When pollutants become nondegradable and toxic, they become a nightmare to us. Heavy metals are one of the major nondegradable toxic pollutants. Among them, some are important for our basic metabolic activities; however, nonessential heavy metals are still dangerous at least concentrations (Ali et al. 2019). Severe effects on human beings and other organisms have been well established due to the rigorous toxicity of cadmium, copper, cobalt, chromium, mercury, molybdenum, etc. heavy metals followed by arsenic, a metalloid. These elements enter in cells vigorously and interact with different enzymes and metabolic products resulting retardation of cellular metabolisms (Engwa et al. 2019). Plastic has multi-dimensional adventurous effects on our environment (Kabir et al. 2020). We develop nondegradable plastic and plastic products for our daily needs, in exchange; persistent plastic steals our mental peace. Nondegradable plastic pollutes terrestrial and aquatic (mostly marine) environments, sometimes air at the time of burning.

4.3 Green Technology

The environment nourishes us in all aspects from birth to death, even after death also. In return, we drastically pollute it without thinking about the results. For the reduction of pollution and sustainable development, green technology is one of the best choices for scientists. In this section, we will discuss a very brief history and some mechanisms related to green technology.

4.3.1 History

It is a difficult work to find out the proper history of green technology. In the ancient ages or strictly before industrialization, we used different green products for our daily needs. Sudden change due to the industrial revolution, we acclimatized ourselves with synthetic products. To fulfill our needs, extensive use of fossil fuels by industries results in an energy crisis throughout the world. After the 1960s, people were interested to find out the solution to environmental degradation. For this purpose, eco-friendly, cost-effective, renewable green technologies such as the use of sunlight, airflow, water current speed to produce energy or other potent technologies gain much interest to scientists. Nowadays, green technology directly deals with living organisms and their potentiality in many aspects of environmental sustainability (Guo et al. 2018).

4.3.2 Trending Green Technologies

4.3.2.1 Bioremediation

Degradation or detoxification of environmental hazardous elements or organic materials by using biological organisms (mainly microbes like bacteria, fungi, protozoa, etc.) toward sustainable development is known as bioremediation (Leong and Chang 2020). This process has broad-spectrum implications on wastewater management, contaminated soil, sludge, synthetic dye degradation, etc. (Giovanella et al. 2020). The immobilization of harmful chemical compounds are carried out using partial degradation, transformation, or other mechanisms (Giovanella et al. 2020). Polycyclic aromatic hydrocarbons (PAHs), halogenated hydrocarbons are well-known environmental pollutants readily remediated using microbes. Heavy metals like cadmium, copper, mercury, lead, etc., or metalloids like arsenic are also consider using bioremediation techniques (Leong and Chang 2020). There are two broad-spectrum bioremediation classifications, that is, ex situ (excavating pollutants from polluted sites and removal) and less expansive in situ (without excavating materials, treating at the site of pollution) (Azubuike et al. 2016). However, the basic principle of bioremediation is situated in detoxification or partial degradation through enzymatic modification. Microorganisms evolved themselves by modifying their genetic makeup to tolerate multiple biotic and abiotic stresses. Heavy metal-reducing or oxidizing, hydrocarbon-degrading microbes have slightly bypassed in their metabolic network which leads to bioremediation. For example, textile synthetic dyes have a detrimental effect on the environment; some potent thermophilic bacterial strains are capable to degrade dyes using two well-studied enzymes—laccases and azoreductases (Giovanella et al. 2020). Metals and metalloids have an attraction to the thiol group of amino acids. This discovery opens a new window for researchers concerning metal bioremediation.

Metabolism-dependent remediation technologies show the best results only when biological organisms are grown in their optimum environmental conditions. The pH, temperature, relative humidity, the concentration of heavy metals, etc. highly affect the cellular metabolism of all organisms. Interestingly, these abiotic environmental conditions vary greatly from organism to organism; even, sometimes strains to strains. So, to get the highest outcome, one should optimize proper growth conditions for desirable organisms.

There are some basic mechanisms of bioremediation like biotransformation, bioaccumulation, biosorption, etc. Biotransformation means the enzymatic transformation of pollutants from one form to another. Oxidation and reduction reactions play a crucial role in biotransformation. Here, organisms take up pollutants, transform their chemical nature, ultimately release into the environment. Specific enzymes along with reductants or oxidants are crucial factors in biotransformation.

Two vital bacterial genes *crcB* and *eriCF* are involved for providing high fluoride concentration tolerance capability. These genes code the efflux pump for fluoride efflux (Katiyar et al. 2020). Cupric reductase is an enzyme of bacteria functions behind the reduction of copper.

Two other mechanisms are biosorption and bioaccumulation also been extensively studied. Both mechanisms are not involved in change the chemical nature of pollutants like biotransformation. In the case of bioaccumulation, microbes uptake actively heavy metals or other pollutants into their cells where biosorption deals with passive adsorption of metallic ions. Biosorption restricts metals in cell walls which could not be transported into cells. Gram-positive bacterial cell walls containing teichoic acids are a good accumulator of metal ions. Extracellular membranes of gram-negative bacteria, however, also show potentiality to stabilize ions (Hansda and Kumar 2016).

4.3.2.2 Phytoremediation

Phytoremediation, remediation of organic and inorganic materials by using green plants which is another promising, cost-effective, unconventional biotechnological approach to recover environmental health. Plants uptake enormous numbers of pollutants through their root system and detoxify pollutants through specific metabolic pathways (Ali et al. 2013). A few parameters such as plants with large roots, easily cultivable, ability to fast growing with large biomass are preferable for this noble eco-friendly remediation process (Irshad et al. 2015). Many members of Brassicaceae like *Brassica juncea* and *B. oleracea* as well as members of other families are responsible for phytoremediation of heavy metals (Salt et al. 1998). Like bioremediation, phytoremediation has a broad spectrum of applications, however, phytoremediation of heavy metal-contaminated soils and water are well studied (Irshad et al. 2015). Plants are ubiquitously distributed and grow under different environmental conditions, which may be hazardous or optimum. To survive themselves from biotic or abiotic stresses, plants have evolved into different morphological, physiological, or genetic changes. These evolved characters of plants are now a subject for discussion. Applications of these adapted, potent plants for the removal of environmental pollutions are under critical considerations of researchers.

Phytoremediation is taken place through a few strategies like phytoaccumulation, phytofiltration, rhizodegradation, etc. (Ali et al. 2013). Phytoaccumulation or phytoextraction is a procedure where plant roots absorb directly or indirectly pollutants and transport them through xylem, which finally accumulate in their stem and leaves. Accumulation greatly varies with plant species, their ecological parameters as well as their genetic, physiological, morphological, and metabolic behavior (Irshad et al. 2015).

Adsorption or absorption of metals by plant parts (root, stem, frond, etc.) from metal-contaminated water is a well-known procedure called phytofiltration (Yang et al. 2019). In this procedure, the nature of plant species and the availability of types of minerals in the water are considered as critical parameters.

Reduction for mobility or bioavailability of toxic compounds leads to a decrease in its abundance. Some plants, however, immobilize these metals through sorption by roots, metallic bond formation with metabolites. This well-known technique is named phytostabilization (Luo et al. 2019). The principle hidden in this technique is to restrict toxic metals (where the place is drastically polluted by single or more than one metal) at rhizospheric regions of plants, nearer to root surface (Shackira and Puthur 2019).

Phytovolatilization is a controversial method that remediates organic pollutants from soil to the atmosphere as volatile compounds. Here, plants uptake compounds and partially degrade into volatile forms distributed in the atmosphere (Ali et al. 2013). Phytovolatizing ability of plants was also studied for heavy metals or metalloids like arsenic, but not well documented so far (Guarino et al. 2020).

When plants exclusively degrade organic pollutants without the interference of microorganism is called phytodegradation. Enzymatic activities on organic pollutants produce simple forms of compounds that lead to phytodegradation (Newman and Reynolds 2004). Rhizodegradation, in some context, looks like phytodegradation, but in this procedure, rhizospheric microorganisms take part in the remediation of organic pollutants.

Interestingly, some plants can grow at high saline conditions and accumulate salts in their tissue leading to survival salt susceptible plants. The report says that halophilic plant varieties can tolerate heavy metals. This method of remediation is considered as phytodesalination (Ali et al. 2013).

4.3.2.3 Bioaugmentation

Bioaugmentation is the process where microbial strains were added to the specific contaminated environment for accelerate the rate of degradation process. Generally, bioaugmentation is categorized into two main approaches; first where indigenous microorganisms are used, and second where nonindigenous microorganisms are used. The microbes which are directly isolated from contaminated sites and can degrade toxic pollutants are known as indigenous microorganisms, whereas those microorganism which were not directly isolated from contaminated sites are exogenous microbes or nonindigenous microorganisms (Poi et al. 2017). The microbes which are used in the bioaugmentation process lead to reinforcing the biological pollutant-degrading colonies so that they can significantly reduce the toxic pollutants. Bioaugmentation is a very effective approach for mitigating stubborn pollutants like heavy metals, pesticides, dyes, hydrocarbons, etc. from the environment. For example, *Aeromonas hydrophila* LZ-MG14 was used in the bioaugmentation process for the removal of Cr(VI) and malachite green from the textile wastewater (Ji et al. 2020). The microbial strain increased the efficiency of malachite green degradation and Cr(VI) reduction in a membrane bioreactor (MBR). This system removes 96.8% of malachite green and 93.71% of Cr(VI) within 12 h (Ji et al. 2020).

4.3.2.4 Bioflocculation

Flocculation means floc formation by the aggregation of colloidal particles and suspended solids in presence of a stimulant (Ayangbenro and Babalola 2018). Flocculants are the compounds that are used in the separation of suspended solids.

Flocculants are classified into two types: chemically synthesized inorganic/organic flocculants and natural flocculants (Okaiyeto et al. 2016). Chemically synthesized inorganic flocculants (aluminum chloride, alum, aluminum sulfate, polyaluminum chloride, ferrous sulfate, and ferric chloride) and organic flocculants (polyethylene amine and polyacrylamide) are toxic, produce a huge amount of secondary waste, and are nondegradable, which causes environmental health risk factor (Farag et al. 2014). Whereas various natural products (cellulose, sodium alginate, tannin, chitosan, mucilage, gum, and microbial flocculants) are considered as natural flocculants. Generally, bioflocculants are extracellular polymers which mainly composed of glycolipids, exopolysaccharides, glycoproteins, proteins, and lipids (Subudhi et al. 2016). Bioflocculant potentially replaces synthetic flocculants due to their nontoxic, biodegradable, and eco-friendly nature (Bukhari et al. 2020).

Several mechanisms are involved in the bioflocculation process like adsorption bridging, charge neutralization, chemical reaction, and volume sweeping. In adsorption bridging, microbial flocculant adsorbed colloidal particles by intermolecular Van der Waals interaction and hydrogen bond, forming chain like 3D structure, which extended effectively to form bridges (Figdore et al. 2018). After adsorbing the negatively charged colloidal particles, neutralization has occurred because flocculant contains a positive charge on its surface area. The decline of surface charge leads to form zeta potential (reduction of repulsive force involving the particles) (Suopajärvi et al. 2014). It was reported that the performance of the flocculant is most excellent when the zeta potential is nearly zero. But when the charge has reversed, the attraction of the particles turns into repulsion and the particles may be discrete (Suopajärvi et al. 2014). Presently, bioflocculants are getting increasing attention in remediation processes for the treatment of wastewater, heavy metals and dye removal, nanoparticles synthesis, and recovery of biomass, potential emulsifier, etc. (Bukhari et al. 2020). The bioflocculants produced by two bacterial species namely *Pantoea* sp. and *P. koreensis* are potentially used for heavy metals remediation and they showed 71.3% and 51.7% of flocculation activity (Ayangbenro et al. 2019). The polysaccharide- and protein-based bioflocculants are consisting of hydroxyl, carboxyl, and amino groups which are responsible for heavy metals adsorption. The bioflocculant of *Pantoea* sp. showed 51.2% of Cd, 80.5% of Pb, and 52.5% of Cr removal while *P. koreensis* showed 48.5% of Cd, 73.7% of Pb, and 42.5% of Cr removal (Ayangbenro et al. 2019).

4.3.2.5 Biosurfactant

Biosurfactants mean surface-active molecules that are synthesized by microorganisms. They are also known as amphiphilic compounds because structurally it contains both hydrophilic and hydrophobic moieties. Hydrophilic segments may be ionic (negatively or positively charged), nonionic, or amphoteric, while hydrophobic segments contain hydrocarbon chains (Otzen 2017). These amphiphilic moieties are aggregated at the boundary of a liquid phase and forming a supramolecular configuration, which is called “micelle” (Campos et al. 2013). Various types of microbial biosurfactants have been reported which are categorized into lipopeptides (surfactin, cellobiolipids, etc.), glycolipids (rhamnolipids, trehalose lipids, sophorolipids, etc.), fatty acids, phospholipids (spiculisporic acid, phosphatidyl ethanolamine E, etc.), polymeric biosurfactant (liposan, emulsan, etc.), and particulate surfactants (vesicles, whole microbial cells, etc.) (Singh et al. 2020). Microbial biosurfactant shows great characteristics such as high surface movement activity and biodegradability, resistance to high temperature and pH, less toxicity, emulsifying or demulsifying activity, biofilm formation, etc. For having such superiorities, researchers have been motivated to explore more biosurfactant which replaces the chemical surfactant for sustainable development. These days biosurfactants are significantly applicable for heavy metals and hydrocarbon remediation.

Biosurfactants generally interact with these heavy metals via binding of ions, electrostatic interactions, exchange of ions, precipitation, etc. (Franzetti et al. 2014). The main mechanism for heavy metals mitigation by biosurfactant is chelation. In this process, metal ions are chelated with oppositely charged surfactant monomers and forming complex micelles configuration (Sarubbo et al. 2015). These micelles configurations help to recover heavy metals from soil.

Due to the amphipathic behavior of biosurfactants, it increases the solubility of both organic and inorganic compounds which leads to reduce surface tension (Patowary et al. 2017). Having such property, biosurfactants are used in hydrocarbon degradation from oil spillage. In the degradation process, biosurfactants increase the hydrophobicity of bacterial cell walls and entrapped the hydrophobic molecules which promote the bioavailability of hydrocarbons by bacterial cells (Xu et al. 2018). Rhamnolipid biosurfactant obtained from *Rhodococcus fascians* was able to remove 81% of anthracene a polycyclic aromatic hydrocarbon (PAH).

4.3.2.6 Biocatalyst

Biocatalyst is generally defined as natural substances from a biological origin that are used to speed up the various chemical reactions. Recently biocatalysts are used as alternatives to chemical catalysts because they originate from renewable resources, are nontoxic and biodegradable, potentially work at low pH and temperature, have selectivity for substrate and product, reduce cost and pollution, and increase sustainability (Jemli et al. 2016). Nowadays, microbial enzyme-based biocatalysts are increasing as green approaches for the degradation of various organic pollutants from wastewater. In this biodegradation process, organic pollutants act as a substrate for microorganisms and produce enzymes that help to convert the pollutants into nontoxic smaller particles (Tran et al. 2013). Among various enzymes, laccase and peroxidase (chloroperoxidase, horseradish peroxidase, soybean peroxidase, and manganese peroxidase) are widely used as biocatalysts for the bioremediation of wastewater (Zdarta et al. 2019). These biocatalysts catalyze the oxidation–reduction of various pollutants like phenols, cresols, chlorinated phenols, pesticides, herbicides, textile dyes, dioxins, pharmaceuticals products, etc. (Bilal et al. 2019).

4.3.2.7 Biocomposite

Composites are produced by the combination of two different constituents with distinct properties and forming matrix structure. Generally, composite materials, produced by the reinforcements of fiber and natural fibers, are gaining more attention for environmental concern. For these purposes, biocomposites have emerged for replacing synthetic composites materials. Biocomposites are considering composite materials derived from biological organisms. These are used in various natural fibers like hemp, flax, cotton, recycled products from wastepaper or wood, and byproducts of different crops for reinforcement (Fowler et al. 2006). Polyethylene, polystyrene, polyvinylchloride, and polypropylene are renewable thermoplastic which are used for biocomposites production. In biocomposites, natural fibers with thermoplastic resins like epoxy, polyester, phenol formaldehyde, and vinyl esters are used for reinforcement (Faruk et al. 2012). Biocomposites have several potentials for water purification like heavy metals and dye removal from wastewater. Clay cellulosic biocomposite is recently used for water purification. This biocomposite has been modified (with NaOH) for obtaining a more negative-charged surface area and shows maximum adsorption capacity for Pb(II) is 389.78 mg/g and for Cd(II) is 115.96 mg/g (Abu-Danso et al. 2020). Another study found that biocomposite which is made up of activated carbon and cellulose is efficiently used for dye remediation. Activated carbon and cellulose were synthesized from natural fiber, that is, sisal. The biocomposites have a maximum adsorption capacity of 103.66 mg/g for methylene blue (Somsesta et al. 2020).

4.3.2.8 Membrane Bioreactor (MBR)

A membrane bioreactor (MBR) is a system in which activated sludge suspended with solids particles is getting separated by a membrane filtration (microfiltration or ultrafiltration) process. In recent times, membrane bioreactor offers several advantages like a lower amount of sludge production due to the presence of a high concentration of biomass in the bioreactor, high water recovery, very high removal rate, short retention time (Wang et al. 2016). Having such advantages, membrane bioreactors have been extensively used for wastewater treatment. For example, an anaerobic dynamic membrane bioreactor is significantly used for the treatment of textile wastewater. It removes 98.5% of COD and more than 97.5% dye (Remazol Brilliant Blue R). In this MBR system, various phyla of the microbial communities like Spirochaetae, Proteobacteria, Euryarchaeota, Firmicutes, Bacteroidetes, Aminicenantes, Chloroflexi and Thermotogae are involved in the anaerobic degradation of wastewater (Berkessa et al. 2020).

However, some limiting factors prevent the further development of membrane bioreactors. The main bottleneck factor is membrane fouling. Membrane fouling in membrane bioreactors includes solutes, cell debris, colloids, biopolymer, and microorganism which lead to plugging pore of the membrane and resulting membrane flux decline (Wang et al. 2018). Recently, a membrane bioreactor modified with UV photocatalytic treatment has been developed for wastewater treatment, and it also mitigates membrane fouling. Under UV light treatment it shows good effluent and antifouling activity. The modified membrane bioreactors are effectively reduced by 83% of the cake layer, 88% of pore blockage substances, and also reduced foulants and microbial products which are deposited on the membrane surface during photocatalysis (Luo et al. 2020).

4.3.2.9 Constructed Wetlands (CWs)

Constructed wetlands (CWs) are artificial wetlands that are comprised of substrates, vegetation, soils, water, and microorganisms. CWs have been extensively used as a green technology for the treatment of a variety of wastewaters like industrial effluent, agricultural wastewater, domestic sewage, mine drainage, stormwater, polluted river water, landfill leachate, and urban runoff (Yalcuk and Ugurlu 2009). Constructed wetlands are classified into two groups such as free-water surface and subsurface flow constructed wetlands. Generally, free-water surface CWs are very much like natural wetlands in which shallow flow of wastewater has occurred. While in a subsurface flow system, wastewater flows vertically or horizontally over the substrate. The performance of CWs is significantly dependent on various optimal parameters like water depth, types of plant species, substrate and wastewater, hydraulic retention time, loading rate, operation design, and maintenance process (Wu et al. 2015). In CWs system, macrophytes plant species like *Phragmites*, *Hydrilla*, *Typha*, *Vallisneria natans*, *Nymphaea*, *Marsilea quadrifolia*, *Salvinia natans*, etc. are recurrently used.

Several substrates like artificial media, natural material, and industrial by-products like gravel, calcite, clay, sand, slag, vermiculite, dolomite, bentonite, limestone, zeolite, activated carbon, etc. are most commonly used in the CWs system (Yan and Xu 2014).

4.3.2.10 Nanogreen Technology (NGT)

Nanogreen technologies are significantly involved in the development of multifunctional nanoproducts which are used for renewable energy production, green packaging, nanosensors for detecting pollution, photocatalysis for pollutant remediation process, and forming a novel membrane for wastewater treatment (Ran et al. 2008). In this green synthesis mechanism, several biological organisms such as bacteria, fungi, yeast, and plants or their byproducts are involved for nanoparticle production. Nanoparticles that are produced via green synthesis methods include silver, gold, palladium, platinum, metal sulfide, metal oxide, nonmetal oxide, nanocomposites, alloy, and magnetic nanoparticles (Soundarrajan et al. 2012). Plants and microorganisms show have dissimilar mechanisms for nanoparticles synthesis. In the case of microbes, at first they entrapped the metal ions on the surface or inside the cells where these metal ions are reduced to nanoproducts by enzymes. While in plants, oxidation and reduction are the main reaction for nanoparticle synthesis. Plant-derived secondary metabolites like flavonoids, alkaloids, terpenoids, phenolic acid, etc. are responsible for the reduction of metal ions into nanoparticles (Makarov et al. 2014). Having a higher surface-volume ratio and surface reactivity, nanomaterials have prevalent applications in the environmental remediation field. Several approaches like nano-based adsorbents, membranes, and nanobiocomposites are involved for ecofriendly treatment. Orange peel waste which is chemically treated with silica nanospheres is used to develop a unique nanocomposite that enhances heavy metals adsorption (Saini et al. 2020).

4.4 Advantages of Green Technology

Green technology is an ecofriendly concept, and it is widely accepted for its importance. Green technology shows a remarkable impact on the environment and provides several advantages, such as the fact that green technology does not deal with anything harmful to the environment; and it generally involves the production of green products, waste recycling, energy saving, also it can prevent global warming by less CO_2 emissions. Apart from these, green technologies can give economic profit to companies by reducing sustainability costs and recover economic circumstances. For having some advantages, awareness of environmental protection is increased for consumers which lead to forming market opportunities for companies.

4.5 Disadvantages of Green Technology

Along with advantages, there are several disadvantages of green technology like very high implementation expenses, lack of knowledge, insecurity about performance, lack of skills, etc. Additionally, the installation cost is very high for new green technology. In the case of the hybrid vehicle, it shows good mileage, but the energy consumption efficiency is reduced and the cost of hybrid vehicles is more than thousands of dollars than other vehicles without a hybrid system. Another disadvantage is the marketing competition. In the marketing world, green technology is very attractive for consumers, but if the green improvements are not economically feasible it can create a competitive situation with other conventional technologies.

4.6 Marketing Prospects of Green Technologies

The market demand for green technology is improved because of environmental concerns and for the increased profit from the green patent discovery. Patent analysis of green technology is very effective for scientists, technologists, attorneys, business dealers, and policy planners to develop new technology, process, outcome, planning a research strategy, and future programming which provide environmental and economic benefits. However, patent analysis is the best measure of creative activities. Our present study deals with several aspects like to analyze the patent activity regarding green technologies (biosurfactant, bioflocculant, biocatalyst, biocomposite, membrane bioreactor (MBR), and bioremediation, and nanogreen technology), their composition, application, applicants, and publishing year and these are summarized in Table 4.1.

Table 4.1 List of available patents on trending green technologies in environmental applications

Patents	Composition	Application(s)	Applicant(s)	Year
Biosurfactant-based green technology				
CN107497364A	Several bacterial species like *Bacillus subtilis*, *Rhodococcus erythropolis*, and *Pseudomonas aeruginosa* are involved in producing biosurfactant which comprises of lipopeptide, phospholipid, and rhamnolipid	Applied the biosurfactant into the soil contaminated by organic matters and helps in soil remediation	Luoyang Tmaxtree Biotechnology Co Ltd	2017
CN107500407A	The biosurfactant specifically comprises of trehalose lipid	Effectively remove suspended solids and chemical oxygen demand (COD) from the water body and it can be used in landscape water treatment and domestic sewage treatment	Luoyang Tmaxtree Biotechnology Co Ltd	2017
CN106834189A	Comprise of phospholipids biosurfactant synthesized from *Serratia marcescens* O_2	Used for the treatment of petroleum hydrocarbons-rich waster and coking wastewater–contaminated water, and *Serratia marcescens* O_2 can degrade *n*-hexadecane	Univ Wuhan Science & Tech	2017
CN107988122A	It comprises of lipopeptide-type biosurfactant which are synthesized from *Bacillus subtilis* fermented culture	Help to degrade inorganic and organic matters in glass water formula and act as a substitute of chemical surfactant	Jiangsu Lopal Tech Co Ltd	2018
CN111205842A	Biosurfactants are produced from *Pseudomonas*	Act as a microbial oil displacement agent, and it is used in oil-recovery process	Shaanxi Wenling Microbiology Tech Co Ltd	2020
Bioflocculant-based green technology				
CN106830357A	Bioflocculants are produced from *Bacillus licheniformis*	The bioflocculant shows excellent decoloration effect, and it decolorized 94.5% of Congo red dye	Univ Xiamen	2017
CN110282823A	Algae used as bioflocculating agent	Use for the separation of wastewater and oil residues from wastewater, and it also degrades organic pollutants	Suzhou Feiniu Environmental Protection Tech Co Ltd	2019

CN105132302A	Bioflocculants are produced from *Bacillus cereus*	The bioflocculant is used for the treatment of tannery wastewater, and it shows 75.6% of COD removal	Univ Sichuan Sci & Eng	2015
CN106085923A	Bioflocculants are synthesized from *Bacillus amyloliquefaciens*	The bioflocculants are used for wastewater treatment, and it removes 76.7% of suspended solids	Univ Anhui Agricultural	2016
Biocatalyst-based green technology				
US6284012B1	Comprises of Kelp or seaweed extract	The biocatalyst is significantly used in wastewater treatment, and eliminates the grease from sewage systems and grease traps	Mundschenk David D.; Reid Paul	2001
CN103736241A	Comprises of laccase enzyme from fermented culture of *Armillariella tabescens*	Used for the degradation of chlorophenols in wastewater system	UnivLishui	2014
Biocomposite-based green technology				
WO2020150164A1	Biocomposites consist of biopolymers which are synthesized from fungal mycelium	Used as green approach	Nike Inc [US]; Nike Innovate Cv [US]	2020
SE1851589A1	Biocomposite materials comprises of cellulose, oat husk, and wheat bran	Used as green approach	OrganoclickAb [SE]	2020
CN104261550A	Biocomposites are comprised of soybean straws and wheat straws	The biocomposites are easily absorbed phosphorus, urea-suspended particles, and metallic elements like copper, zinc, and iron from the wastewater.	XuZhenlin	2015
CN103706333A	Biocomposites consist of eggshells which are loaded with zirconium oxide	The biocomposite are effectively used for water treatment and it can effectively remove phosphorous from wastewater body	UnivYanshan	2014
Membrane bioreactor (MBR)-based green technology				
CN102745804A	It comprises of cultivation stage, domestication stage, and running stage	This MBR treatment is used for nitrogen removal	Univ Southeast	2012
CN109437503A	Two technologies are combined in this MBR system, one is membrane bioreactor reduction	It used for the reduction of organic sludge produced from papermaking process		2019

(continued)

Table 4.1 (continued)

Patents	Composition	Application(s)	Applicant(s)	Year
	technology, and another is electrochemical reduction technology		Shandong Huatai Paper Co Ltd; UnivQilu Technology	
CN205803252U	The MBR system consisting of oxygen pond, aeration tank, coagulating and sedimentation tank, oxidation and filtration ponds	It shows good COD removal and decolorizing effect from sewage	Chongqing KaidaEnvProtEng Co Ltd	2016
WO2020015435A1	The MBR comprises of anaerobic and aerobic system which loaded with quinonyl containing fiber membrane	It effectively used for treating domestic wastewater	Univ Xiamen Technology [CN]	2020
Bioremediation-based green technology				
IN5050CH2013A	Involve *Escherichia fergusonii* strain	Used in bioremediation for textile effluents	Sastra University [IN]	2015
IN427KO2014A	Comprises of microbial consortium which includes *Aeromonas veronii*, *Bacillus barbaricus*, and *Stenotrophomonas maltophilia*	Used for heavy metals remediation	Gandhi Inst Of Engineering and Technology [IN]	2015
EP3626683A1	Comprises of two bacterial species one is *Pseudomonas*, and another is *Bacillus*	Used for hydrocarbon degradation in refinery wastewater	Indian Oil Corp Ltd [IN]	2020
WO2008104997A2	Comprises of biomass of *Aspergillus oryzae*	The fungal species are successfully reduced COD, BOD, and TDS from industrial effluent	AgnihotriSnehalNitin [IN]	2008
WO2017125943A1	Comprises of Bacterial consortium which includes *Serratia marcescens*, *Halomona* ssp., and *Bacillus* sp.	Effectively removed nitrate and perchlorate from contaminated water	Council ScientInd Res [IN]	2017
Nanogreen-based technology				
CN106966480A	Zero valent iron particles are formed by using perilla seed	This green nano-based system degrades crystal violet dye from wastewater	Univ Beijing Technology	2017
CN104174870A	Zero valent iron particles are prepared by using grape seed extract	This green nano based system is effectively degrading 98% of azo-dye and 95% of brilliant blue dye	Univ Beijing Technology	2014

Source: https://worldwide.espacenet.com/

4.7 Conclusion and Future Perspective

Green technology creates a balance between the environmental shield and economic development which significantly contributes to a sustainable environment. At first, we discussed non-degradable toxic pollutants such as heavy metals (copper, cobalt, chromium, mercury, molybdenum, etc.), plastic, and dye which showed multidimensional dangerous effects on the environment. For resolving this environmental condition, several green technologies (Bioremediation, Phytoremediation, Bioaugmentation, Bioflocculation, Biosurfactant, Biocatalyst, Membrane bioreactor (MBR), Wetlands, and Nanogreen Technology) are a move toward a sustainable solution. In the case of bioremediation biological organisms particularly bacteria and fungi are involved, and they adsorb or accumulate or enzymatically transform (along with reductants or oxidants) the heavy metals and hydrocarbon. While in phytoremediation (mediated by green plants), accumulation, filtration, and rhizodegradation are involved in mitigating toxic pollutants. Next, we studied microbial synthesized active molecules like biosurfactant (rhamnolipids, trehalose lipids, surfactin, etc.), bioflocculant (glycolipids, exopolysaccharides, glycoproteins, proteins, and lipids), and biocatalyst (laccase and peroxidase). Along with these, some natural fibers like hemp, flax, cotton, recycled products from wastepaper or wood, etc. (biocomposite), nano-based green technology, and an updated version of membrane filtration (membrane bioreactor) and wetlands (constructed wetlands) are also considered as green approaches. Having some advantages and disadvantages these green technologies are also studied by their patent analysis.

In the future perspective, we must focus on the field implementation of these green technologies. For this purpose, we need to set up effective systems, including apparatus and devices related to these green-based technologies. Not only execution we also focus on the recycling and regeneration part which contributes to a safe environment.

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Sustainable Technology: Foresight to Green Ecosystem

5

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Abstract

The global environmental problems such as pollution and degradation have enforced society to reconsider the ways of development and pursue sustainable technologies. Many eco-friendly initiatives have been taken to maintain and improve the quality of an ecosystem that might flourish on the green resources which are abundant in nature. The use of bioenergy in the form of biofuels and biogas as a potential source of renewable energy in recent times is a significant step toward sustainable future. The three primary domains of sustainable development are desegregated in this book chapter. Potential use of green technology in various sectors, their merits and demerits, along with the efficiency of such techniques are also mentioned. Green product innovation has reduced the impact on the environment as well as improved energy consumption efficiently. Advancements in the application of science and technology have given hope for the solution of the current environmental problems. The various ecological toxicants are assessed concerning the adverse effect on flora and fauna. The recent development in industrial, agricultural, and domestic sustainable technologies has hugely benefitted the environment, and has been a significant step toward weather change. Under sociological reform, various policies and international rules have been framed as precautionary measures to put a check on the usage of nonrenewable sources of energy and implement sustainable methods to reduce the degradation of the environment on a large scale. Furthermore, light is shed on the adaptability of newer technologies and their sustainability as compared to the conventional methods, and how these technologies will take the modern living toward a sustainable environment.

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Keywords

Bioenergy · Eco-friendly · Green innovation · Pollution · Sustainable technology

5.1 Introduction

5.1.1 Environment Sustainability

The term environment sustainability is concerned with the fact of whether the environmental resources and raw materials are used judiciously; thus, they stay protected/maintained for the future generation. Figure 5.1 shows the various concerns related to environmental sustainability.

The issues which get solved with environmental sustainability are as follows:

- Long-lasting health of ecosystem and the protection of long-term well-being of resources to meet the future socioeconomic needs such as protection of fishing stock and farmlands.
- Use of renewable resources such as solar and wind energy.
- Protection of ecological diversity and species structure, that is, the restriction of involvement of near-extinct flora and fauna as raw materials for production.
- Targeting social welfare and development over concerns such as gross domestic product (GDP).

5.1.2 Bioenergy

Bioenergy can be obtained from various biomass found in nature such as forest, livestock remains, and agricultural waste. They can also be produced from municipal waste and wastewater. These materials are directly or indirectly converted to produce electricity or heat. There is a vast range of bioenergy technologies, and its technological maturity varies sustainably. Some of the widely used bioenergy sources are the production of ethanol from carbohydrate and cellulose-based products, domestic heating systems, and varied scale boilers (Ottomar et al. 2011).

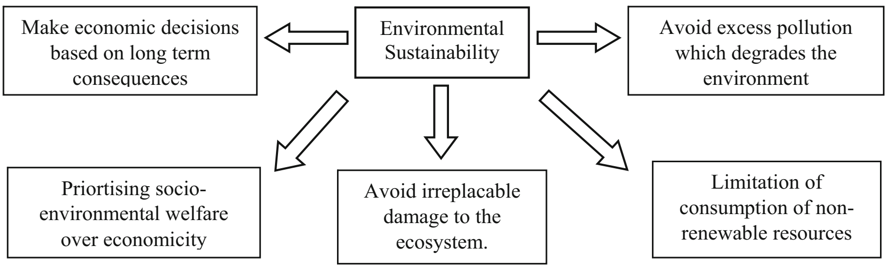

Fig. 5.1 Schematic diagram of the concerns of environmental sustainability

Advanced biomass-integrated combined power plants and cellulose-origin transport fuels are some examples of technology which are in their commercial stages of development. Bioenergy technologies with the traditional use of biomass in developing countries are one of the widely used sustainable technologies in recent years (Ottomar et al. 2011).

5.1.2.1 Biofuel

Biofuel is often referred to as substance which is produced from biomass and can be used to generate energy. It includes biomethanol, bioethanol, biodiesel, and biogas. Bioethanol is a petrol substitute made from wheat, corn, and sugar beet. Biodiesel is an eco-friendly alternative fuel, which can be used in place of conventional diesel in machines (Demirbas 2008).

5.1.2.2 Biogas

The organic formation of any biomass which includes waste materials from domestic outlets, sewers, and industrial pollutants can degenerate to a mixture of methane and carbon dioxide. This mixture is known as “Biogas.” They are very cheap, sustainable, and versatile form of biofuel (Kapdi et al. 2005). First methane digester plant was set up in 1859 in Bombay, India, by the leper colony. Methane digester plants are commonly called as anaerobic digester or AD (Ottomar et al. 2011).

5.2 Components of a Sustainable Technology

5.2.1 Domains

There are three major components/domains of sustainable technology, namely ecological domain, economic domain, and sociocultural domain. The first component, biological part, is present in connections with the physical and natural environment. The second component, the economic activity, is essential to fulfilling human’s requirements, resulting in the third component, sociocultural domain, which is induced by the internal human traits (Duran et al. 2015).

5.2.1.1 Ecological Domain

The environmental component of sustainable development is not only natural economic growth, but all ecological development in terms of resource capacity, access to waste, and direct use of resources. It is closely related to growth compliance with environmental laws and balance. This domain is designed for adequate operational requirements, and over time, promotes harmony and peace, without being independent of any branch or sector. The protection of environment considers the potential of the system physically and naturally, enhancing their ability to adapt to changes and the state of conservation that can be regarded as optimistic (Bran 1991). By adopting a complex structure, ecodevelopment term growth is categorized according to stage requirements and specific objectives. It requires care in environmental work and stimulates the development of system-based information, however planning opportunities are low. Various international organizations have been advocating ecological policy. As mentioned above, environmental action is a goal for developing countries in the long run (Duran et al. 2015). Resource management is a low-level approach based on economic data. Herman Daly suggested that there are three main benchmarks for environmental sustainability: Renewable resources should deliver a sustainable yield (yield rate should not exceed the renewal rate); with nonrenewable resources, there must be a consistent development of other nonrenewable resources; waste generation should not exceed the capacity of the ecosystem (Daly 1990). On a global scale and in a broader sense, environmental management includes oceans, clean water systems, land and air; however, the principle of sustainability can apply equally to any environment from tropical rainforests to home garden.

Atmosphere

At the Copenhagen Climate Council meeting in March 2009, 2500 climate experts from 80 countries made a significant statement that there is now "no reason" to fail to act on global warming, and that without strong carbon, emissions could change abruptly or permanently in climate. It can be challenging for existing communities to manage. Global space management now includes an examination of all aspects of the carbon cycle to identify opportunities to report human-made climate change, and this has become a milestone in scientific research due to the potential adverse effects on biodiversity and human societies (Adam 2009). Other impacts on the atmosphere include urban air pollution, pollution resulting from toxic chemical emission (i.e., SO_X, NO_X), photochemical smoke, and acid rain. Anthropogenic particles such as sulfate aerosols in the air reduce direct irradiance and earth reflector (albedo). Globalization can have a problem with the Earth's water cycle by causing evaporation and rain in some places. Reforestation is one of the ways to halt desertification supported by climate change and sustainable land use.

Water Usage

The Earth's surface contains about 71% water, of which only 97.5% is freshwater, and 2.5% are trapped in Antarctic ice. The remaining freshwater is found in glaciers, lakes, rivers, swamps, soil, water, and the atmosphere. Due to the water cycle, the supply of clean water is often supplemented by rainfall; however, a certain amount is still needed to manage this resource. Awareness of the global importance of conserving water in natural resources has recently emerged. By the twentieth century, more than half of the world's wetlands and their value-added services were lost. Urban sprawls pollute clean water, and most of the world does not yet have access to clean, safe water. Now more emphasis has been placed on blue (clean) and freshwater (groundwater available for plant use), which applies to all water management measurements (Hoekstra 2006).

Marine cycle patterns have a profound effect on climate and climate change, as well as food security and other ecosystems. Under the influence of climate change, scientists have warned that it may be a maneuver in some parts of the world. About 10% of the world's population—about 600 million people—lives in low-lying areas where sea levels are likely to rise (Kerr 2004). Water efficiency around the world has been enhanced through increased demand management, improved infrastructure, agricultural water production, reduced water (mixed water) supply of goods and services, addressing shortages in industrialized countries, focusing on more productive food production, and climate change planning—flexible planning. At the local level, people are now able to become self-sufficient by harvesting rainwater and reducing the use of ordinary water (Zhang and Babovic 2012; Hoekstra 2006).

Food Supply

"A sustainable diet is defined as the diet providing healthy food to meet current food needs while preserving healthy ecosystems that can provide food with a minimal negative impact on the environment for generations," as defined by the American Public Health Association (APHA) (Feenstra 2002; Harmon and Gerald 2007). A sustainable diet also encourages production and distribution of food locally and also ensuring nutritious food is easily accessible and available at a reasonable price for all. Moreover, it is a kind of action and protection of all the interdepended groups of people such as farmer, workers, consumers and communities. Industrial agriculture causes environmental effects that cause various health problems around the globe. It led to a strong movement toward a healthy, sustainable diet (Policy 2007). The environmental effects of different foods depending on several elements, including the animal and plant foods proportion and the food production model. A Global Strategy on nutrition, physical fitness, and health reporting, released by the World Health Organization (WHO) and approved by the World Health Assembly in May 2004 (Baroni et al. 2007) recommends a Mediterranean diet. It is low in meat; rich in fruits and vegetables; low in added sugar, salt, and also in saturated fatty acids; and associates itself with health and long life. The primary source of fat in this diet is olive oil, rich in mono-unsaturated fatty acids. Sustainable agriculture and organic farming address a global environmental impact on agribusiness. There are various movements toward local food production, including slow food, sustainable horticulture, and the use of domestic gardens in a more productive way (Organization 2004).

Land Usage

With the gradual conversion of natural capital into human-made capital, the loss of habitat and fragmented biodiversity results from the misuse of land for development and agriculture. A significant impact on biogeochemical cycles has been observed due to changes in the relative proportion of land dedicated to urbanization, agriculture, and forestry. At the grassroots level, large sustainability reimbursements grow from sustainable parks and gardens and green cities (Poh et al. 2008; Klintman and Boström 2004; Alibhai-Brown 2010). Since the Stone Age, the world's forests have been degraded by 50% due to human intervention (Gleick 1998). Forests today cover one-fourth the world's snow-free land, 50% of which covers the tropics. Deforestation is increasing gradually in temperate regions (except Siberia), but a significant concern in the tropics. If deforestation continues at the current rate for the next 20–40 years, it could cause complete extinction of humanity, according to a study published in the journal *Nature Scientific*.

Food is essential for life. To feed more than seven billion human bodies, it is severely affecting the Earth's resources. It starts with using about 38% of the Earth's land area and 20% of its net primary productivity. It includes everything from industrial agribusiness to resource-starvation activities—crop demand for irrigation water, synthetic fertilizers, and pesticides, to resource costs for food packaging, transport (now an essential part of global trade), and retail. Environmental issues related to industrial agriculture and agribusinesses are now solved through such movements pertaining to sustainable agriculture, organic farming, and more sustainable business practices (Imhoff et al. 2004).

5.2.1.2 Economical Domain

The economic component of sustainable development that seeks to generate maximum income flow based on rational use, resource performance, and incredibly rare resources. As an expression of macroeconomic dynamics, economic components require quantitative changes in the quality, structure of scientific research and production tools in organizational arrangements, and economic activity mechanisms (Becker et al. 2001). It can be seen that rapid economic growth, mainly for emerging countries, places enormous liability on the Earth's support by acquiring maximum benefits. As sustainable development states, economic growth must be in line with the control of adverse environmental impacts. The idea of sustainable development replicates a standard shift in this area—a discussion of sustainability is done not only in the context of environmental protection, but also concerning other regions, particularly those involving economic activities. Therefore, development must be considered in many dimensions, including significant changes in social structures, approaches of national institutions, accelerating economic growth, alleviation of poverty and inequality. Perhaps, the economic arena targets to maintain a manageable level of government and external debt, to avoid sector-to-sector severe imbalances affecting agriculture and manufacturing industries, and to make sure that a sustainably balanced economic environment is held by the continuous production of goods and services in an orderly manner. Training is required for the industry in competition, expanding industrial output and attracting investment.

On the contrary, the economic component of the scheme is being suppressed by other countries failing to progress from the financial crisis, making the survival of the project difficult. Furthermore, the common goals, which are required at a certain level in addition to this obstacle, may modify the above results (Lichtveld et al. 2016). Even if these issues are involved, they should be addressed in a way that encourages complete exhaust solutions by targeting the necessary technology and natural resources. Also, managing the economic purpose is possible by accessible training and methodical and logical spirit, thereby stimulating participation in economic activities, which leads to achieving the ultimate goal of the strategy (Duran et al. 2015).

The latest United Nations Economic Program (UNEP) report defines a green economy as "promoting human well-being and social equality, while at the same time significantly reducing environmental risks and environmental deficits." The information creates three significant findings (Duran et al. 2015): Greening not only increases wealth, but also creates a higher rate of GDP growth (over 6 years), particularly for the creation of environmental commodities or natural capital gains (Daly 1990); the presence of an inseparable connection among poverty alleviation, protection of common good and the enhanced care, which arises from the flow of benefits that the poor receive directly from natural capital (Adam 2009); creation of new jobs during the transition period to a green economy, which outweigh the jobs lost during the brown economy. However, there is a period of job losses in transition that require investment in reskills and reeducation of workers (UNEP 2011).

Environmental Economics

Traditionally, economic growth and environmental degradation are linked to each other as the growth of communities and declination of the environment are inversely proportional. This proportionality is verified in maps of economic growth as well as population index of social and environmental indicators (Diamond 2005; Diamond and Guns 1997). Sustainable economic development is entirely comparable to the growth of cancer as it rips ecological services of the Earth into pieces as it supports life. If resource utilization is not verified, there is a concern that present human society will be trapped into the footprints of distorted early human societies by overexploiting their resource base. Although the conventional economy is essentially linked to economic growth and effective allocation of resources, the ecological economy in that order has a clear objective of sustainable size (rather than sustainable development), efficient funding, and fair distribution (Daly 1990; Costanza et al. 2014).

The term decoupling is commonly used in the fields of economics and ecology concerning environmental quality and economic production. In this context, the term refers to the overall capability of an economy to develop without pressurizing the environment. Ecological economics refers to reviewing social metabolism and the performance of resources around the economic structure concerning environmental quality (Costanza et al. 2014; Cleveland 1984). The economy is said to be cut off, which could sustain GDP growth without harming the environment. Sustainability studies analyses ways to reduce the number of resources required for unit production, consumption, and disposal, whether it is derived by the use of advanced management of the economy, new technology, or design of products (Daly 1996). There are different opinions against the fact that improvements in the performance of technology and revolution can completely cut off economic growth from environmental degradation. Performance experts have repeatedly stated that the intensity of resource utilization can be decreased by at least five or six times in principle, thereby permitting the continuation of economic growth without increasing pollution and related resource exhaustion (Von et al. 2009). However, on analyzing the history of enhancements in the performance of technology, it is found that there is outperformance in the economic growth due to developments in the energy and material utilization, resulting in a net rise in resource (safety) utilization and associated pollution.

Moreover, all performance improvements have inherent thermal dynamics and practical limitations. It is nearly impossible to achieve sustainable and infinite economic growth without an association with resource depletion and environmental pollution which proves that the lack of resources and change in the economy can be controlled for a shorter period. Therefore, a changeover is required to a stable state economy, in which GDP remains more or less constant (Cleveland and Ruth 1998).

The usage of the services related to expression ecosystem highlights the economic and the market significance of nature, which is day by day becoming a little natural world that is neither unlimited nor free. Overall, as a product or service decreases in quantity, the price rises and this act as a check to encourage self-control, technical inventions, and alternative products. However, this only applies to the service which falls inside the market range. As these are usually considered as economic externalities, they don't have any stated price and thus overutilized and ruined (Hardin 2009).

5.2.1.3 Sociocultural/Human Domain

We assume that potential alternatives to maintain ecological balance have been identified, and the officials everywhere must meet the criteria of optimality and efficiency, as well as the professional and social quality of labor and life to adapt the raw materials purchased in the products. Human sustainability is related to interactions among people, relationships, behaviors, and human values (Dempsey et al. 2011). Our present generation is worried about the maintenance of cultural diversity—"World Village"—preventing contemporary "social ills": feeling of being alone or isolation, dissatisfaction with work ("Continue under the narrowing of expertise in terms of knowing" less and less. "Identifying the finished product for the worker and his work." "Difficult to understand the usefulness"), the relativity of values, the end of history, the skepticism about the future or the more distant "disease" of the age-specific postmodern world. It is crucial to realize the need to protect and improve the condition of the environment and social development, which represents the only opportunity to create and maintain the well-being of both the present and future generations. Equilibrium was a factor in ensuring growth throughout the company.

5.2.2 Principles of Sustainability

1. Interdependence, participation, information delivery, and advancing science
   (a) Encourage interdependence by being mutually responsible and sharing common principles with others.
   (b) Learn about the impact each of us has on a global scale.
   (c) We need to protect and manage natural resources for those who cannot do it themselves.
   (d) We need to encourage people to refine and access information.
   (e) We must work to improve scientific and technological knowledge through innovation.
   (f) We need to promote public awareness and engage in related issues.

   (g) The precautionary approach should be widely used to protect the environment.
2. Providing intermediate equity and life
   (a) Growth should be equal for current and future generations.
   (b) Individuals should be encouraged to reverse the effects of degeneration in areas beyond the individual.
   (c) Individuals need to minimize and eliminate lasting patterns of production and consumption.
   (d) Notice of natural disasters and emergencies is required.
   (e) Buffers between development and natural systems should be provided.
   (f) Encourage efforts to increase the availability of locally available and drought-tolerant water.
3. Supporting and improving the rules of governance
   (a) Informed democracy should be encouraged to avoid making inadequate or nonexistent decisions.
   (b) The government should be held accountable for its actions.
   (c) Acceptable environmental standards, policies, and management objectives are paramount.
   (d) Must accept cooperation to create good environmental legislation.
4. Maintaining the quality of the community and improvement if possible
   (a) Organizations should be the primary focus of responsibility for creating a sustainable society.
   (b) A sustainable community must respect cultural diversity.
   (c) A community must have an adequate distribution of open space.
   (d) Individuals should have the opportunity to receive education and training to develop work and life skills.
   (e) The integration of transport systems should be encouraged and encouraged.
   (f) Materials and construction methods should be specific to climate and region.
   (g) Measures to reduce the impact of vehicles and traffic should be encouraged and encouraged.
5. Maintain and, if possible, improve the quality of human life
   (a) Humans have the right to live meaningfully and to act responsibly.
   (b) The participation of tribals should be encouraged.
   (c) Efforts should be made to reduce and eradicate poverty.
   (d) The bare necessities of life should be available to all.
   (e) Safety requirements include protection from harm and the absence of anxiety and encouragement.
6. If possible, improve the economic power
   (a) Landowners have the right to use resources responsibly.
   (b) Sustainable economic growth is essential.
   (c) For the creation of a pulsating local economy, the government, the community, education, and the business sector need to work together.

(d) To encourage more job opportunities and expansion of small businesses around the local community, significant changes should be made in favor of the supporting companies and local entrepreneurs.
(e) Communities need to promote decisions that lead to a unique identity.

5.3 Assessing Potential Green Technology

Technology means the application of innovative ideas for practical purposes. Green technology originated in the early 1970s, where much attention was directed to a sustainable approach (Billatos 1997). Green technology is a system-level approach to the production process with the primary objective of an environmental attribute (Billatos 1997). It can change the production pattern, which won't harm the environment and the planet. The purpose of Green Technology is to minimize waste and energy usages. This technology eliminates all toxic material from the waste. It mainly focuses on utilizing energy like solar thermal energy, wind energy, solar electric energy, geothermal energy, etc., which are non-fossil fuel sources (Anastas and Zimmerman 2012).

5.3.1 Principles of Green Technology

The primary goal of green technology is to make products recyclable and reusable. It introduces sustainable living by developing a renewable source of energy and decrease waste production. It also conserves our natural resources (Iravani et al. 2017). The main objectives of green technology are reduce, recycle, refuse, renew and responsibility.

5.3.2 The Various Types of Green Technology

Green technologies are divided into two parts—Green Chemistry and Green Energy.

5.3.2.1 Green Chemistry

Paul Anastas introduced the term "Green Chemistry" in 1991. It also referred to as sustainable chemistry. It encourages chemical research and engineering to minimize hazard production. Green chemistry is a combination of physical chemistry, organic chemistry, and inorganic chemistry (Iravani et al. 2017). The 12 principles of green chemistry are prevention of waste, atom economy, minimize hazardous chemical synthesis, designing unharmed chemicals, use of safer solvent, energy-efficient production designing, use of renewable raw material, derivatives reduction, use of catalysis reagent, degradation designing, pollution-preventive measure for analyzing real time, and choose unharmed chemical for chemical accident prevention.

5.3.2.2 Green Energy

Green energy is also called renewable energy. It comes from natural resources like rain, sunlight, wind, tide, etc. In this era, the most critical objective of green technology is to develop alternative energy. All of these energy resources are renewable. India aims to meet 20% of country's total requirement of energy from renewable sources by 2020 (Iravani et al. 2017). Green energy can be harnessed from various sources such as the follows:

Solar Energy

Solar energy is converted into electricity by using photovoltaic cell. This electricity can be used in the water pump, refrigerator, and charging batteries. The solar road is made of concrete modules in which solar panels are embedded.

Wind Energy

The kinetic energy of wind is converted into mechanical energy by using a wind turbine to run a generator. After running the generator by using that mechanical energy, it produces electricity. A total of 1% of global electricity generation is contributed by wind energy. In 1920 it stated by Betz that a wind machine might be able to capture a maximum of 59.3% energy of wind. Size of the turbine and the quality of wind resource is responsible for the amount of electricity produced. If appropriate wind resource is available, a 2-megawatt (MW) turbine can produce enough electricity to power almost 400 homes for a full year.

Water Energy

The energy of running water is used to make electricity, which is called hydroelectricity, where the running water is stored and hydroelectric stations are built. It is the most eco-friendly electricity-generation source. The first Hydroelectric Power Station was the Rance tidal power plant situated at La Rance, France. It took 6 years to build this station (1960–1966). The Pelamis has converted the wave energy into electricity. The machine operates in 50 m deep water.

5.4 Development of Newer Technology from Conventional Methods

Air pollution occurs when the different components of air get changed by various gases and particles that cause serious health issues and environmental degradation. Now the solutions for global sustainability is to invent and implement newer technologies. For every industry, the critical factor is the power source, that is, electricity. Now the industries are using renewable sources of energy instead of using conventional sources. Some of them are solar energy, wind energy, water energy, and biofuel (Sabban 2020).

5.4.1 Solar Energy

It is the most commonly used renewable energy source, where solar cell converts solar energy into electric energy via the process of photovoltaics. It has grown from 5 GW in 2005 to 510 GW in 2018 (Sönnichsen 2020). The main advantages of using solar energy are as follows:

- It's a renewable energy resource.
- It reduces the cost of production of electricity.
- It does not create any pollution.

The maintenance cost is significantly less as compared to any other nonrenewable energy sources.

Some of the disadvantages of using solar energy are the solar panels take a lot of spaces; it is weather dependent so the production rate changes with it; and apart from the production, energy storage is also a big concern and the production rate is prolonged as compared to other energy sources (Sönnichsen 2020).

5.4.2 Wind Energy

The wind's kinetic energy can be used to rotate the blades of the windmill that is connected to the turbine, which converts wind energy into electric energy and creates no pollution. However, there are some problems with this windmills, that is, these cannot be operated in the residential area and thus require a lot of open space and also depend on the wind's turbulence. Currently, India is the second-highest producer of electricity from wind in Asia and fourth in the world. India produces about 35 GW from wind energy.

5.4.2.1 Advantages of Wind Energy

1. Wind energy is a green form of energy with less carbon emission rate.
2. It is nondegradable for nature.
3. It is an economical source of energy.

5.4.2.2 Disadvantages of Wind Energy

1. It is dependent on natural conditions.
2. It cannot be operated in urban and congested areas.
3. A vast area of land is needed for setting up of wind energy generators such as windmills.
4. Dangerous for airborne birds (Sabban 2020).

5.4.3 Water Energy

Hydropower electricity provides about 16% of the world's total electricity production and it constitutes 70% of the total renewable energy. The entire global installed capacity of hydropower energy is around 1150 GW (by 2019). China, Brazil, Canada, the United States, and Russia are the five largest producers of hydropower. The world's largest hydroelectric plant in terms of installed capacity is Three Gorges (Sanxia) on China's Yangtze River (Evwind 2020).

5.4.3.1 Advantages of Water Energy (Santamarta 2020)

1. Greener source of energy with less carbon emission.
2. Economical source of energy with abundant availability.

5.4.3.2 Disadvantages of Water Energy (Santamarta 2020)

1. Dependent on the water stream.
2. Cannot be operated from urban areas.
3. Dams and waterfalls are the only sources of energy for such kind of setups.

5.4.4 Recycling

Recycling is the process of collecting and processing waste materials that would otherwise be thrown away as trash and turning them into new products. These create an opportunity for the industries to turn unique technology and methods to implement in sectors. The most common industrial recycling techniques are bulking facilities, materials recovery facilities, household reuse and recycling centers, in-vessel and windrow composting, anaerobic digestion, etc. Figure 5.2 shows the various steps involved in the recycling of a product from a preexisting work.

The advantages of recycling process include conservation of natural resources such as timber, water, and minerals; reduction of the amount of waste sent to landfills and incinerators; increase in economic security by tapping a domestic source of materials; prevention of pollution by reducing the need to collect new raw materials; savings in energy and support of manufacturing; and conservation of valuable resources (Sabban 2020).

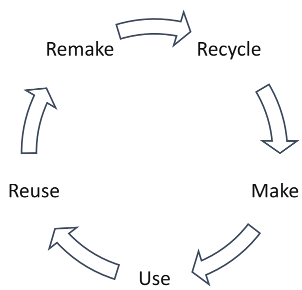

Fig. 5.2 The five steps involving the recycling of a product

5.5 Application of Modern Technology in Different Sectors

5.5.1 Agricultural Sector

With an increase in population, the amount of food requirement is tremendously high. It leads to the implementation of newer technology into agricultural sectors (Matthew and Schrimpf 2017). From machine learning to artificial intelligence (AI), modern technology has been successfully incorporated to fulfil the need of ordinary people. The following are some examples to enhance the Green Revolution.

5.5.1.1 Machine Learning

The implementation of machine learning is the ability of a computer to accumulate knowledge about an action or a thing and begin to “make decisions” on its own. This technology is having a significant impact on agricultural sectors as it can make patters on weather conditions, soil, use of fertilizer, pesticides, and other things and implement this information in real time. Relatively tricky tasks, the implementation of computational technology, and machine learning makes it very simple. It increases the production in agricultural sectors rapidly.

5.5.1.2 Irrigation Control

An irrigation controller is a tool to perform computerized irrigation structures which includes garden sprinklers and drip irrigation structures. Systems that serve up popularity reviews on pivot performance, soil moisture sensing, weather, and different area records to cellular telephones and computer systems are commonplace, and supplying end customers with on-the-cross equipment to make and put into effect irrigation control decisions (Matthew and Schrimpf 2017). Some benefits of using the same are saving a ton of money by reducing water wastage, enhanced landscape health and beauty, helping you prepare for the future of water, smart irrigation controllers help you reduce hardscape loss, avoiding fines with your smart irrigation controller (Landscaping 2019).

5.5.1.3 Liquid Nanoclay (LNC) Technology

LNC is a revolutionary technology which converts sandy deserts into fertile lands. It is a natural component which does not make any environmental pollutions. This nanoclay is obtained by a mixing method utilizing subsequent changing of flow regime between laminar and turbulent. The mixing is done on-site, and the LNC is spread onto sandy soil using traditional irrigation systems like sprinklers or water wagons. The ground is rinsed until the mix has saturated down to root depth, a process that usually takes 7 h. The LNC treatment gives sand particles a nanostructured clay coating. The treatment completely changes the physical qualities of the sand particles, allowing them to bind water. As a result of using LNC, it reduces the use of water by 65% and increases the yield by five to six times (Tabarsa et al. 2018).

5.5.1.4 Biopesticides

These all are essentially made from natural resources which do not create any environmental pollutions and maintain the balance of the ecosystem (Agency 2017). Biochemical pesticides are naturally occurring substances that control pests by nontoxic mechanisms. Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus, or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest. Plant-incorporated protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant. Some advantages of using biopesticides are that they are usually inherently less toxic than a conventional one and generally affect only the target pest and closely related organisms (Agency 2016).

5.5.1.5 Vertical Farming

Vertical farming is one of the modern strategies to reap crops, vegetables, herbs, etc. in vertically stacked layers. It pursuits to optimize plant growth, and soilless farming strategies together with hydroponics, aquaponics, and aeroponics. The primary blessings of hydroponics are the capacity to growth yield consistent with the location and decrease water utilization for cultivation as evaluate to standard methods.

5.5.2 Domestic Sector

5.5.2.1 Solar Heaters

Conventional ways of making hot water for domestic and household uses consumes a lot of power from electricity, diesel, coal, timber, natural gases, or any kind of fossil fuel. On the contrary, the solar water heater uses solar energy to make hot water without causing any pollution and save 50–80% on water-heating bills by saving energy. The heated water stays in an insulated tank, much like with a conventional water heater. It also can act as a backup.

5.5.2.2 Solar Panel

The term “solar panel” is used colloquially for a photovoltaic (PV) module. Photovoltaic cells use sunlight as a source of energy and generate direct current electricity. Since solar energy is a renewable resource, by installing solar panels, its renewable electricity can be generated. Some advantages of using the panel are that it is an environment-friendly source of energy, it reduces electricity bills, it has low repair cost, etc. Some significant disadvantages were also seen, which include high installation charges, season dependency, required power storing devices to store solar energy, slow production rate, etc.

5.5.2.3 Geothermal Heat Pump

This type of pump additionally makes use of the Earth thermal strength to supply power without causing any pollution. Ground supply heat pumps (GSHPs) are most recent of the leading strength green technologies for providing heating, ventilation, air conditioning (HVAC), and water heating. Traditional combustion furnaces and electric powered warmers can by no means exceed 100% efficiency. In short, the advantages of geothermal heat pump are that it lasts more than the conventional heat pump, it has a shallow environmental impact as compared to a traditional heat pump, it can be used for heating as well as for cooling purpose, and it does not make any noise (Hepbasli and Akdemir 2004).

5.5.3 Social Architecture Sector

The Concept of Green Architecture, in any other case called "affordable engineering" or "inexperienced structure," is the hypothesis, technological know-how, and fashion of systems deliberate and advanced as in line with ecologically cordial standards. Green layout endeavors to restrict the amount of property wolfed withinside the structure's development, use, and activity, as merely lowering the harm executed to the weather via the discharge, infection, and misuse of its parts (Ragheb et al. 2016). To limit those influences and configuration ecologically, vital and asset-efficient structures are required. Also "inexperienced shape frameworks" have to be presented, explained, comprehended, and rehearsed. It may have many of these characteristics:

- Ventilation frameworks intended for significant warming and cooling.
- Energy-effective lighting and machines.
- Water-sparing pipe apparatuses.
- Landscapes wanted to boost uninvolved sunlight-based energy
- Minimal damage to the characteristic living space
- Alternate power sources, for example, sun-oriented force or wind power
- Nonmanufactured, nonpoisonous materials
- Locally acquired woods and stone
- Responsibly gathered wood
- Adaptive reuse of more seasoned structures (Ragheb et al. 2016)

5.5.3.1 Passive Solar Design

Inert sun-oriented arrangement for the utilization of the sun's vitality for the warming and cooling of living spaces. The structure itself or a few components of it abuses ordinary vitality qualities in its materials to absorb and transmit the warmth made by presentation to the sun.

5.5.3.2 Green Building Material

Green structured substances are generally comprised of countless non-sustainable assets. They are biologically cautious because their effects are taken into consideration over the lifestyles. Green shape substances may be selected with the aid of surveying traits, for a case reused, and unexpectedly maintainable accumulated substances, tall recyclability, sturdiness, lifestyles span, and adjoining creation (Spiegel and Meadows 2010).

5.5.3.3 Green Walls

Otherwise referred to as vertical greenery, it is truly supplying flora onto the shape façade. Some of the usual green walls are as follows:

1. Wall-climbing green wall: These are an ordinary and inexperienced divider. Despite the truth that it miles a tedious cycle, mountain-climbing flowers can cowl the partitions.
2. Hanging-down green wall: These are a mainstream technique for simple dividers. They can indeed form a complete vertical inexperienced belt on a multi-tale operating via planting at every tale assessment with the divider mountaineering type.
3. Module green wall: This is the latest concept contrasted with the beyond kinds. It calls for a more generous confounded plan and arranging contemplations earlier than a vertical framework. It is also probably the most expensive inexperienced dividers technique (Ragheb et al. 2016).

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Green Technology for Bioplastics Towards Sustainable Environment

6

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Abstract

This chapter addresses recent developments in eco-friendly and economically suitable bioprocesses developed for the production of polyhydroxyalkanoates (PHAs). Present-day polyethylene plastic, which is nonbiodegradable, creates permanent harm to the environment. Thus, biodegradable plastics offer a great range of applicability. This green innovation of selecting renewable resources as raw material proves beneficial for circular economy. The two utmost key conditions for industrial-scale production of polyhydroxyalkanoate (PHA) are supply and cost. Bio-based materials are catching the eye of the researchers due to the faster rate of fossil depletion. In addition, advanced methodologies like co-production of PHAs with other value-added products for economically feasible and energy-efficient production. The operational cost of PHAs production route can be considerably reduced by using high-yielding strains. To satisfy the global demand for PHAs, the downstream processing techniques and economics must be studied. PHA extraction techniques which require hazardous solvents are now being replaced with suitable and efficient green solvents. Among different extraction techniques involving high-cost and hazardous solvents, green solvent extraction, aqueous two-phase extraction proves to be advantageous in terms of

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nontoxicity. Nowadays, detailed study on bio-extraction techniques for highly pure PHA with desirable properties is needed for adoption of green methodology.

Keywords

Fermentation · Media · Polyhydroxyalkanoate · Recovery

6.1 Introduction

Environmental threats posed in the surroundings by synthetic plastics and their trashes continues to be a chief problem nowadays. Likewise, efforts are associated for plastic waste collection, its recycling, and reuse. The widespread use of such synthetic plastics in packaging for medical areas is commonly known. Several risk factors are associated with plastic production and its aggregation in the environment along with its residual monomers. In particular, a very huge damage to human health is caused due to its degradation during recycling with the release of toxic gases in the surrounding environment, which also affects terrestrial and sea animals. Bearing all these points, a serious step to stop the exploitation of such fossils-derived plastics must be followed. A need arises for their replacement with green biodegradable plastics. Thus, bioplastics are accepted as potential replacement to synthetic plastics industry, thus leading to cleaner environment. Monomers like hydroxyalkanoic acids, lactic acid, bio-ethylene, 1,3-propanediol, bio-propylene, and bio-ethylene glycol are exploited for the production of bio-based plastics. Enormous use of synthetic plastics is leading to severe health and environmental risks, adding a lot of CO_2 in the environment. These synthetic plastics are consumed by several sea and terrestrial animals, and also directly harms their habitat. Conclusively, petrochemical-derived plastics are not sustainable for environment leading to the requirement of biodegradable plastics to avoid surrounding damage. Monitoring proper disposal of such plastics is required as it leads to release of greenhouse gases, toxic compounds, along with nondegradable materials to the soil. Thus, an alternative with biodegradable plastics like polyhydroxyalkanoates (PHAs) with adequate waste management system must be adopted. PHAs are stored as carbon reserve material inside the bacterial cells accumulated under excess of carbon present in the medium. Also, the type of bacterium and the nutrient source affect the quality of obtained PHA for various applications.

6.2 History

"Plastics" term was coined by Leo Baekeland, who invented world's first synthetic plastic called Bakelite in 1907. These synthetic plastics were composed of very large molecules which when discarded as trash led to serious environmental issues, and efforts toward recycling of these materials were then undertaken. Polyesters, polyethylene terephthalate, polyethylene, high- and low-density polyethylene, polyvinyl chloride, polyvinylidene chloride, polypropylene, polystyrene, polyamides, polycarbonate, and polyurethanes are commonly used plastics in various areas. Synthetic plastics have been extensively exploited in our everyday life owing to their strength and durability. Polyethylene, polyvinyl chloride and polystyrene (molecular weight: 50,000–1,000,000 Da) are generally used in the fabrication of thin films and fibers. Several microorganisms including cyanobacteria are known to accumulate PHAs intracellularly (Bhati et al. 2010). Due to their high resistance toward various chemicals, plastics have been explored for their durability as packaging materials. But severe environmental damage is caused due to its direct disposal in large numbers in land and oceans (Reddy et al. 2003). Molecular size of the material shows a significant part in its persistent nature in soil. Xenobiotic nature of petroleum-derived plastics increases the chances of unusual changes in the natural biota. An expensive alternative is incineration of such plastics releasing deleterious hydrogen chloride and hydrogen cyanide. These bear negative effects on landfills due to excessive waste accumulation including recalcitrants (Reddy et al. 2003). Another approach is recycling with three different methods—thermal recycling, material recycling, and chemical recycling (Goto 2009). The challenge for new eco-friendly plastics is to sustain their thermal and mechanical properties along with their biodegradability. These biodegradable plastics can benefit the environment by overcoming the pollutants from synthetic plastics. This has encouraged researchers in exploiting biodegradable plastics. The polymer industry is trying to resolve these problems by developing biodegradable polymers.

Various polymers like starch, cellulose, and PHAs were checked for their efficient commercial usages. PHAs gained specific concerns due to their biodegradable and biocompatible nature. Structural properties of the polymer could be achieved with the selection of type of bacteria determining stereospecificity with effective biodegradability and biocompatibility. It has also enormous prospective for medical applications, thus increasing its usage in wide areas. The kind of bacterium and growth environments governs the composition and molecular weight of PHAs ranging from 2×10^5 to 3×10^6 Da. The production costs of 1 kg of polyhydroxybutyrate was about US$15–10, which is much higher compared to synthetic plastics. Many investigators are emphasizing on economic aspects of PHA production utilizing waste substrates. Two groups of PHAs are mainly found on the basis of type of microorganism selected, that is, short-chain-length thermoplastic and crystalline PHA (SCL-PHA) with C_3–C_5 carbon, and the other group consists of medium-chain-length elastomeric PHA (MCL-PHA) with C_6–C_{14} carbon. It has been reported by researchers that depending upon the type of carbon source, pseudomonads can synthesize MCL or SCL PHA (Thakor et al. 2005).

Structural properties of PHA could be explored for its wide range of applicability. Polyhydroxybutyrate, a homopolymer of polyhydroxyalkanoate, is less tough compared to its copolymer counterparts. This can be overcome by blending the homopolymer with other polymer materials depending upon the target application area.

6.3 Structural Properties of PHA

The structural property of PHA that has fascinated several researchers as a substitute polymer extracted from bacterial source utilizing waste substrates. The performance properties of these PHAs could be improved by PHA–PHA blend properties adding advantage over fossil-derived plastics. Advanced modeling on chain growth kinetics for PHA accumulation could be monitored for its end applications. The properties of PHA basically depend upon the carbon feedstock fed in the process, the producer organisms, the metabolic pathway involved, and enzymes involved. The homopolymer poly-3-hydroxybutyrate is stiff due to its highly crystalline nature while copolymer hydroxyvalerate is highly ductile. SCL-PHA comprises of 3–5 carbon atoms while MCL-PHA comprises of 6–14 carbon atoms. The MCL-PHA in the PHA polymer chain affects the thermal and crystalline behavior of PHAs. The most commonly observed crystal structure of P(3HB) is its α-form. Both P(3HB) and P (3HV) are orthorhombic with left-handed symmetry. Results from infrared spectra indicates C–H···O=C hydrogen bond along the a-axis between C=O group of one axis with CH_3 group of another axis (Sato et al. 2009). While the β-form paracrystalline with zig-zag conformation. Yokouchi et al. (1973) first observed the β-form of P(3HB) film. The below mentioned table indicates the examples of PHA polymers with both homopolymers and copolymers. Below is the general structure of PHA (Fig. 6.1, Table 6.1).

6.4 Production

Production media, conditions, and time taken for maximum polymer productivity are very crucial factors for setting up large-scale production units. Several reports on the media composition have been put forward by researchers. Depending upon the

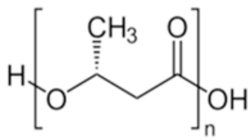

Fig. 6.1 General structure of PHA

Table 6.1 Examples of PHA polymers

Homopolymers	Copolymers
Poly(3-hydroxybutyrate)	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
Poly(4-hydroxybutyrate)	Poly(3-hydroxybutyrate-co-4-hydroxybutyrate)
Poly(3-hydroxyvalerate)	Poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate)
Poly(3-hydroxyhexanoate)	Poly(3-hydroxybutyrate-co-3-hydroxyoctanoate)
Poly(3-hydroxyheptanoate)	Poly(3-hydroxybutyrate-co-3-hydroxydecanoate)
Poly(3-hydroxyoctanoate)	
Poly(3-hydroxynonanoate)	

Source: Li and Loh (2015)

carbon feed and composition different types of homopolymers and copolymers could be obtained. Nutrient sources consisting of fructose, 1,4-butanediol and ammonium sulfate was used to obtain copolymer with varied ratio of 3HB:4HB (Chanprateep et al. 2010). Major content of lignocellulosic feedstock that is xylose was explored as nutrient source screening the PHA positive isolates (Lopes et al. 2009). Similar studies were conducted with glycerol for P (3HB) production (Ibrahim and Steinbüchel 2010). Crude glycerol generated during biodiesel production could be the suitable raw material as feed for the cultivation of PHA-producing bacteria. It not only solves the problem of huge amount of waste generated in the biodiesel production unit but adds advantage by creating value-added products like PHAs. Cycle length of the whole production process is a crucial factor in lowering the overall production cost of the product. Various feast and famine strategies to improve the PHA productivity was applied and similar study under various cycle length concluded to produce polyglucose and PHA under 6 and 24 h interval (Moralejo-Gárate et al. 2013). Submerged and solid state fermentation strategy was followed to check its effect on PHA productivity by Castilho et al. (2009). Various batch and fed batch studies are continuously applied by researchers. Fed-batch culture studies with phosphorous limitation were studied by Cavaillé et al. (2013). Dairy waste and seawater was used as nutrient medium in fed-batch study carried out by Pandian et al. (2010).

6.4.1 Coproduction of PHAs

Engineering microbial cells for several products are gaining interest in the eyes of the researchers. Exploiting suitable strains utilizing economical substrates for efficacious product and coproducts production at a commercially available scale are carried out nowadays. PHA coproduction can balance cellular metabolism with positive effect on various other coproducts. Also, in various studies researchers have utilized the anaerobic condition strategy to cut down the aeration process utilizing engineered microorganisms.

6.5 Microorganisms

PHA production by bacterial enrichment has gained momentum nowadays. PHA-producing microorganisms originate from freshwater to areas affected by anthropogenic activities. Both gram-negative and gram-positive bacteria are reported to accumulate PHAs intracellularly. PHAs not only act as carbon reserve material for these microorganisms but also support to overcome various external stress factors (Obruca et al. 2015). Reports on its role in triggering sporulation in *Bacilli* have been studied. *Bacillus cereus* has been reported to accumulate different monomers and copolymers utilizing various carbon sources. *Bacillus thuringiensis* was capable of producing copolymer with 13.4% HV when supplemented with high nitrogen with propionic acid (Kumar et al. 2015). Many halophilic bacterial strains are reported to accumulate PHAs. The advantage of exploring such microbes is ease in developing open system cultivation without any sterilization cost. *Halomonas profundus*, a deep-sea hydrothermal vent isolate was stated to accumulate P(3HB) and P(3HB-co-3HV) with different carbon substrates (Simon-Colin et al. 2008). Halophilic bacteria *Halomonas elongate* accumulates PHA and ectoine under unoptimized conditions (Mothes et al. 2008). Moderately haloalkaliphilic *Halomonas campisallis* with 51.56% PHA yield was obtained in an experiment carried out in 120 L fermenter (Kshirsagar et al. 2013). Various thermophilic bacteria like *Caldimona staiwanesis*, *Caldimonas manganoxidans*, *Chelatococcus thermostellatus*, *Thermus thermophiles* HB8, *Chelatococcus daeguensis* TAD1 have been reported to accumulate PHAs. *Pseudomonas extremaustralis* isolated from polar region has also been reported to accumulate PHB.

6.6 Media

Van-Thuoc reported that *Halomonas boliviensis* accumulates PHA utilizing wheat bran hydrolysate as carbon source. Additionally, reduction in 50% production cost could be possible with the exploitation of such waste substrates. The selection of media should be economically feasible at an industrial level but also result in high volumetric productivity of the product competing with the traditional plastics. Thus, efforts by several scientists are focused on the exploitation of inexpensive media/ waste resources meeting the requirements for industrial-scale process. Extensive studies to select waste substrates as media containing food waste (Reddy and Mohan 2012), by-products from biodiesel industry (Ghosh et al. 2014; Shrivastav et al. 2010; Bhattacharya et al. 2016), sea salt (Dhangdhariya et al. 2015; Dubey and Mishra 2021a), waste glycerol (Cavalheiro et al. 2012), animal waste (Muhr et al. 2013), paper mill wastewater (Jiang et al. 2012), municipal solid waste (Korkakaki et al. 2016), municipal wastewater (Valentino et al. 2015), olive mill wastewaters (Martinez et al. 2015), algal biodiesel waste (Dubey and Mishra 2021b). The selectivity of media also depends upon the selection of wild or recombinant strain along with the requirement of homopolymer or copolymer form of PHA as the final product (Gumel et al. 2012). Also, the results obtained by Gumel et al. (2012) indicates 49.7–68.9% (w/w) PHA accumulation of 3HB to 3HTD when fed with $C_{8:0}$ to $C_{18:1}$ fatty acids. In the same study, 3HHp (7 carbon atom length) polymer was obtained with substrate as oleic acid. Due to the increasing bioethanol production rise in glucose as carbon substrate, utilization of glycerol waste generated during biodiesel production could be the possible substitute. Pure glycerol has broad range of applicability from food to pharmaceutical industries requiring refined glycerol. This glycerol with high purity requires expensive refining techniques which is difficult for medium and small biodiesel producers. Posada et al. (2011) reported the production costs of total PHB production to be in between US$2.11/kg and US $2.44/kg with 88 wt% glycerol while the cost was between US$1.94/kg and US $2.38/kg with 98 wt% glycerol. Bera et al. (2015) reported the copolymer accumulation when crude levulinic acid was added as a co-substrate along with crude

Table 6.2 PHA production by bacteria-utilizing glycerol as nutrient source

Microorganism	PHA (g/L)	Reference
Escherichia coli CT1061	51	Nikel et al. (2008)
Burkholderia cepacia ATCC 17759	7.4	Zhu et al. (2010)
Ralstonia eutropha	55	Gözke et al. (2012)
Bacillus thuringiensis EGU45	1.5–3.5	Kumar et al. (2015)
Bacillus megaterium	62	Naranjo et al. (2013)
Halomonas hydrothermalis	75%	Shrivastav et al. (2010)

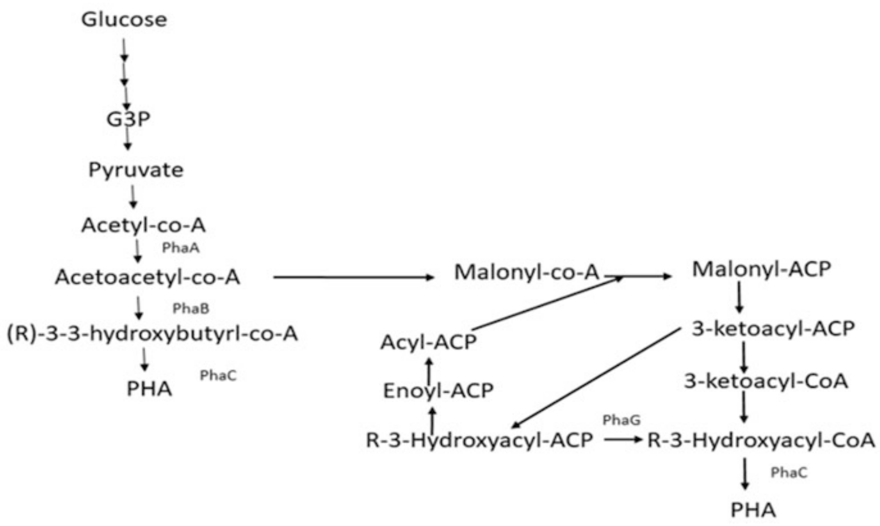

Fig. 6.2 The biosynthetic pathway of PHA synthesis

glycerol derived during *Jatropha* biodiesel production. Table 6.2 indicates few studies carried out using glycerol for PHA production.

6.7 Biosynthesis of PHA (Fig. 6.2)

Microorganisms convert various substrates into PHAs through metabolic pathways with acetyl-CoA as an intermediate followed by PHA synthases for monomer polymerization. The substrate specificity of PHA synthases of different microorganisms to synthesize PHAs can be divided into four classes: Class I that utilize CoA thioesters of 3-HAs, 4-HAs, and 5-HAs comprising 3–5 carbon atoms; Class II polymerases with specificity to CoA thioesters of 3-HAs with 6–14 carbon atoms, 4-HAs and 5-HAs. These two classes are encoded by PhaC gene. Class III synthases are composed of two genes (PhaC and PhaE) with similar substrate specificities with class I. Also, class IV synthases consist of two genes (PhaC and PhaR) that utilize 3-HA monomers with 3–5 carbon atoms. Basically, three enzymatic reaction steps are involved in PHB synthesis. Initially condensation of two acetyl coenzyme A molecules into acetoacetyl-CoA by β-ketoacyl-CoA-thiolase takes place followed by reduction of acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA by an NADPH-dependent acetoacetyl-CoA dehydrogenase. The P(3HB) polymerase polymerizes (R)-3-hydroxybutyryl-CoA monomers to PHB. Propionate, the precursor of propionyl-CoA, combines with one acetyl-CoA to produce hydroxyvaleryl-CoA.

6.8 Fermentation

There are various crucial factors affecting PHA accumulation in bacteria. Both wild-type and recombinant strains have been explored for its potential to accumulate PHAs intracellularly. Various strategies have been looked upon till date for maximum polymer productivity. Albuquerque et al. (2011) reported that continuous-feeding strategy compared to pulse-feeding strategy could alleviate volumetric productivities with simultaneously broadening polymer structures with higher hydroxyvalerate content. While Reddy and Mohan (2012) stated higher PHA productivity under anoxic microenvironments, higher substrate degradation under aerobic microenvironment was reported using aerobic mixed culture as biocatalyst. *Halomonas* TD01 was checked for PHA production under unsterile and continuous fermentation process with 80 g/L dry cell weight and 80% PHB on glucose salt medium decreasing the cost in PHA production (Tan et al. 2011). To date, all PHA production process exploits microbial discontinuous fed-batch fermentations which leads to varying product quality. To overcome such problem, Atlić et al. (2011) chose a multistage system comprising of five continuous stirred-tank reactors in series for PHA production process. In this process, the first stage consists of inoculum development; thereafter, the fermentation broth was fed continuously into the subsequent reactors resulting in 77% w/w PHA productivity. A constant nutrient-feeding approach was implemented based on the estimated biomass content in the first phase with PHA accumulation rate inducing PHA (125 g/L) under nitrogen limitation (Mozumder et al. 2014). Another approach using mixed microbial culture under feast/famine conditions with maximum PHA content of 9.2% g/g dry cell weight was obtained in a sequencing batch reactor with bio-oil obtained from fast pyrolysis of chicken beds (Moita and Lemos 2012).

6.8.1 Outlook for Industrial-Scale Production of PHA

1. *Selection of potential strain*: Rapidly growing bacterial strain; high PHA accumulation; acclimatized to grow in crude substrates.
2. *Study at shake flask level*: Optimizing various conditions such as temperature, pH for maximal growth, and PHA productivity; downstream processing.
3. *Fermenter study*: Process development and optimization at lab-scale fermenters; downstream processing.

4. *Scaling up the process*: Robust growth with high product development along with the exploitation of crude substrates.

For scaling up the process, cost is an important factor. Therefore, process requiring minimum sterility must be adapted without the need of sophisticated sterility equipment, thus cutting down the energy consumption cost. Secondly, the waste materials should be explored for the growth of such bacteria overcoming the commercial substrate cost. Also, the time required for maximum product development should be paid attention to that is competent to grow in low aeration accumulating PHA within a reasonable period of time.

6.9 Recovery

Extraction of PHA from bacterial biomass generally requires harsh treatments involving environmentally harmful solvents. Thus, detergents such as linear alkylbenzene sulfonic acid which is biodegradable was tested for extracting PHA from *Ralstonia eutropha* and *Escherichia coli* cells producing high-purity PHA (Yang et al. 2011). A procedure for copolymer poly (hydroxybutyrate-*co*-hydroxyhexanoate) containing high levels of HHx (>15 mol%) recovery using ethyl acetate from *Ralstonia eutropha* biomass with high recovery purities (99%) in case of dry bacterial biomass. While in wet biomass, methyl isobutyl ketone resulted in 99% purity and total 84% recovery yield (Riedel et al. 2013). We in our previous studies have reported about the use of ionic liquids as potential extractant to recover PHAs from bacterial biomass (Dubey et al. 2018).Dissolution of PHAs were studied in different green solvent systems by Sequeira et al. 2020. Figure 6.3 indicates the general recovery process from bacteria.

6.10 Applications of PHA

PHAs are excellent biomaterial for its widespread applications. They have been explored from preparing household articles, packing, coatings on paper, fabrics, adhesives, films, and additives. Among biopolymers, these biogenic polyesters correspond to a potential sustainable replacement for synthetic thermoplastics. These PHAs extracted from the bacteria cell can be further formulated and processed by extrusion modifying its properties depending on the area of application. Improving and regulating industrial microorganisms for higher yield of fermentation products like PHAs are helpful in product improvement. Various companies are supplying PHAs in the market, for example, Imperial Chemical Industries under the trade name "BIOPOL"; Kaneka in Japan under the trade name "Kaneka"; Mitsubishi in Japan with "Biogreen"; PHB Industrial Brasil under the trade name "Biocycle"; Telles PHB copolymers under the trade name "Mirel"; Tinan in China produces PHBV from corn sugar under the trade name "Enmat."

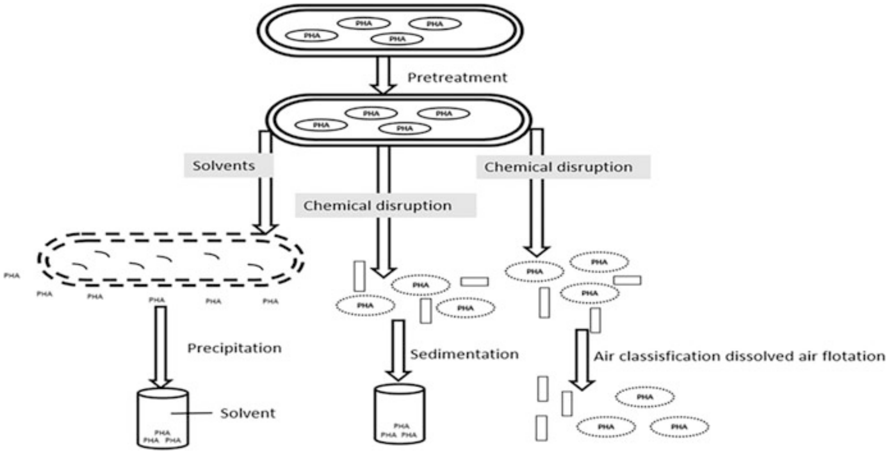

Fig. 6.3 General diagram representing PHA recovery from bacterial cells. (Ref: Madkour et al. 2013)

Applications of PHAs in various sectors are gaining momentum due to its durable and tunable behavior for manufacturing commodity plastics (Reddy et al. 2003). PHAs have attracted interest of various researchers in controlled drug delivery systems due to its nontoxicity and biocompatibility (Nigmatullin et al. 2015). Purity level of PHA is of utmost importance in medical applications. Modification in PHAs could improve thermal, mechanical, and hydrophilic properties of PHA increasing its application in cardiovascular patches, wound dressings, tissue engineering, drug carrier, cancer treatment, etc. (Ali and Jamil 2016). Bourbonnais and Marchessault (2010) showed the ability of PHB and P(3HB-co-3HV) as sizing mediator measuring resistance to penetration by aqueous fluids. Additionally, the study indicated that the sizing efficacy of the copolymer was observed to be poor comparatively to the PHB homopolymer. These biodegradable PHAs could be used as polymer carrier with regulated release of pesticide embedded in the PHA matrix to the soil. Thus, release of pesticide in the soil could be controlled by changing PHA: pesticide content (Voinova et al. 2009). On the other hand, blending Distillers' Dried Grains with Solubles (DDGS) with PHA at three load ratios (90/10, 80/20, and 70/30) were mixed with a twin screw extruder. These composites could be suitable material for horticulture-oriented biobased crop containers (Lu et al. 2014).

6.11 Conclusion

PHAs are accumulated under stress conditions with both biodegradability and biocompatible properties. Studies on the industrial-scale production, extraction, and application of PHA are extensively carried out with interdisciplinary approach.

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Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Statement This study does not involve any human or animal testing.

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Green Vaccination: Smart Plant Health Care for Human Welfare

7

Prashant Singh

Abstract

One of the twenty-first century's greatest food security issues is increasing crop yield stability through the production of disease-resistant crops. Plants are constantly exposed to potentially pathogenic microbes present around them. Managing plant health is a major challenge to modern food production, and exacerbated by the lack of common ground among the various disciplines involved in disease control. Food production is highly dependent on the use of chemical to combat pathogens, in the absence of genetic resistance in crops. Given their efficacy, plant protection dependent on chemical substances has negative environmental effects and creates risks for the wider ecosystem. Reducing the food production's reliance on chemical control is a key goal of plant pathology science. One of the main objectives of plant research in the twenty-first century is to improve our knowledge of the plant protection mechanism and uncover how pathogens exploit it to create crops with both long-lasting pathogen resistance and enhanced yields. Plants also developed a complex immune system designed to withstand pests and diseases. In addition to their innate immune system that regulates preprogrammed defensive reactions, plants may also increase their immune system's sensitivity in response to selected environmental signals. This phenomenon is known as "defense priming." Although defense priming seldom offers maximum security, its wide-spectrum efficacy, long-lasting longevity, and inherited from future generations make it attractive to integrated disease control. This article highlights the role of the plant defense priming in sustainable agriculture.

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Beta aminobutyric acid (BABA) · Priming · Transgenerational immune priming (TGIP) · Vitamins

7.1 Introduction

With the increasing volume of world, there is relatively increase in food demand among the population. Globally, bursting population is a major problem and by 2050 we need to feed two billion extra people. Conventional breeding strategies to solve this condition on longer remain viable. From the very start of agriculture, plant diseases have been a concern for humanity. Throughout the intervening decades we have learned a lot of plant diseases and how to manage them, but diseases still takes its toll on our crops every year. The issue is the result, to a large extent, of the genetic adaptability of the pathogens responsible for causing plant diseases: They establish resistance to our crop protection chemicals, and they easily overcome the resistance produced in our new varieties. Therefore, it is important that we keep one (or ideally several) steps ahead of the pathogens in the fight against plant disease (Dong and Ronald 2019). Weeds, pests, and pathogens report up to 40% of our main crop yields, given current control measures. Without control, crop losses could be as high as 80% (Dong and Ronald 2019).

In addition to the selection of resistant crop varieties and crop rotation, the use of pesticides represents an effective strategy against these threats. However, pesticide use is increasingly regarded as problematical for two key reasons: the potential impacts of pesticide application on health and the environment (Stanley et al. 2015), and the ongoing evolution of pesticide resistance. Furthermore, strict European regulation is leading to a reduction in the number and diversity of pesticides available to farmers (Hillocks 2012). Thus, the situation arises where production must increase without relying heavily on the use of pesticides. Therefore, the sustainable maintenance of agricultural productivity requires new strategies for crop protection.

Plants possess several inducible, systemic defense responses to pests and pathogens (Hammerschmidt 2009). In particular, two signaling pathways are known to regulate plant defense against biotic stress: one regulated by salicylic acid (SA), which provides systemically acquired resistance (SAR) to microbial pathogens; and the other regulated by jasmonic acid (JA), which provides resistance to herbivorous insects and necrotrophic pathogens. Activators of natural plant defenses have been proposed as useful tools within integrated pest management (IPM) strategies that aim to minimize the use of toxic products (Bruce et al. 2017). Key advantages of induced resistance responses are that they are compatible with, or may even enhance, biocontrol techniques by promoting plant attractiveness to natural enemies of plant pests (Peterson et al. 2016), and that they depend on multigenic outputs, so it is less likely that resistance can be acquired by target pests and pathogens. Many chemical activators of induced defenses against biotic attackers are known, and some of these, such as acibenzolar-*S*-methyl, an activator of SAR, have been commercialized for crop protection (Tripathi et al. 2010). However, sustained activation of defense throughout the plant is costly in terms of resources, and long-term activation of induced defenses can result in yield penalties (Douma et al. 2017).

7.2 Priming: An Alternative to Direct Activation of Defense

To prime means to prepare or make ready in everyday language. In plant, defense priming is a physiological mechanism by which a plant prepares to react to potential biotic or abiotic stress faster or more actively (Fig. 7.1). The level of readiness reached by priming was called the "primed state" (Conrath et al. 2006).

A range of plant taxa, including wild species and cultivated varieties, and from herbaceous to long-lived woody plants have been reported for defense priming (Hilker et al. 2016). Defense priming is postulated as an adaptive, low-cost defensive mechanism, because defense responses are not triggered by a given priming stimulus, or only slightly and transiently. Alternatively, defensive responses are applied in a quicker, better, and/or more sustained manner, flowing with the perception of a later demanding signal (triggering stimulus); that is, in times of stress (Conrath et al. 2015).

Because priming initiates a state of readiness that does not impart resistance in itself but instead allows for accelerated induced resistance once a triggering stimulus

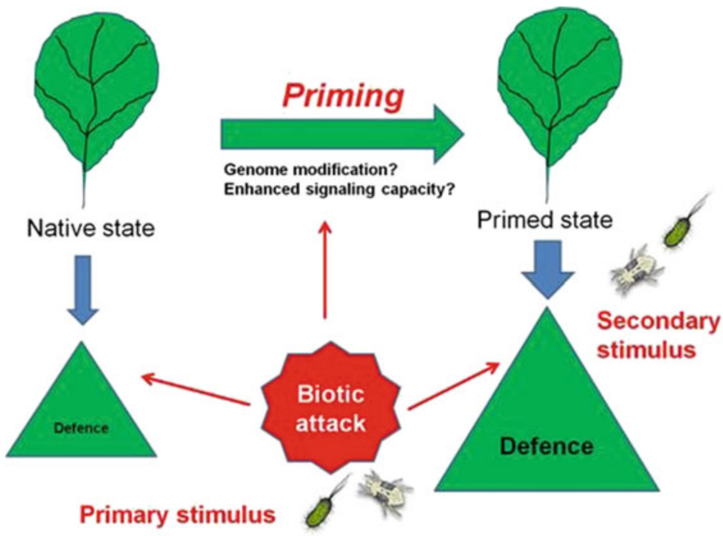

Fig. 7.1 Diagram illustrating the principle of priming for defense. Leaves previously exposed to a priming signal are somehow able to responds more effectively to biotic attack, and consequently generate higher levels of resistance

occurs. A major advantage of priming is that it does not enforce the costs associated with completely enforcing an induced response to defense (van Hulten et al. 2006). Priming of protection is sometimes defined as plant vaccination compared to the animal immune system. Priming provides efficient enhanced basal resistance, mediated by a multitude of genes; therefore, basal resistance priming is successful against a wide range of biological threats (Ahmad et al. 2010; Conrath et al. 2015).

7.2.1 Priming Stimuli

Priming stimuli include a wide range of physical, biological, or chemical inputs from the environment to which a plant reacts by developing a memory. These inputs induce plant changes that involve the accumulation of various metabolites at low cost. Many of the priming agents used to activate protection mechanisms that are not harmful to plants and mammals at low concentrations; consequently, they can be used to reduce stress and to increase crop yield and quality without any adverse effects on the crop and the environment (Kercheva et al. 2020). Many of these natural molecules can act as priming stimuli when applied exogenously, generating a plant memory that boosts defenses and improves the performance of the plant when challenged (Mauch-Mani et al. 2017).

7.2.1.1 Chemical Compounds and Plant Hormones

Plant defense priming can be induced by chemical compounds (BABA, salicylic acid, jasmonic acid, hexanoic acid, pipecolic acid, or volatile organic compounds), plant pathogens, insect herbivores (Conrath et al. 2015; Llorens et al. 2016). BABA is usually present at levels so low that it was only recently proven to be synthesized in highly diverse plant species in response to several kinds of pathogens (Thevenet et al. 2017). BABA-priming of *Arabidopsis* plants has provided strong protection against bacterial diseases and, to a lesser degree, fungal diseases. Wilkinson et al. (2019) also detected strong long-lasting effects of BABA against *Botrytis cinerea* postharvest infections in tomatoes with no yield penalty.

Oligogalacturonides (OGs), unlike BABA, tend to have low priming efficiency against fungal diseases (Westman et al. 2019). OGs comprise a diverse group of defense signaling molecules in the cell walls of plants that are degradation products of pectin. They break into fragments when disrupted, or so-called Damage Associated Molecular Patterns (DAMPs), which induce immune responses from plants via an MPK-dependent pathway. OGs have not scored highly as priming may be due to their diversity, and the specificity of OGs may warrant further attention for their potential role and utility as priming agents in future studies.

It is highly effective to prime plants with jasmonate or jasmonic acid, and also deters fairly large insects such as pine weevils from debarking coniferous plants (Berglund et al. 2016). Martinez-Medina et al. (2016) showed that priming may come at an initial cost, which is outweighed by later benefits, but methyl-jasmonate (MeJA) tends to come at relatively high costs as it slows growth and causes plants to reallocate resources to advanced metabolism, for example, as stated in spruce

(Sampedro et al. 2011). Li et al. (2018) found that MeJA applications in different species (e.g., tomato, sunflower, and soybean) have increased densities of trichomes and stomata, as well as reductions in height and biomass. Therefore, although MeJA's application effectively enhances herbivorous resistance its utility as a priming agent is questionable due to its unwanted phenotypic effects.

Elevated ozone (O_3) modulates phytohormone signals which eventually alter the interplay between plants and herbivorous insects. It has recently been shown that callose deposition acts as an as effective O_3-induced priming defence response of tomato plants against *B. tabaci* infestation (Guo et al. 2020).

7.2.1.2 Herbivore

Herbivores appear to be generally weak priming agents although they induce immediate activation of defensive pathways and increased regulation of specialized defense products pools (Ameye et al. 2015). Internal herbivore feeders (e.g., miners and gallers) have more intimate relations with their hosts, but it is challenging to maintain them in culture for experimental purposes, which may explain the lack of mechanistic and thorough literature studies of their effect on defense priming (Westman et al. 2019). Long-lasting transgenerational resistance formed by herbivory in *Arabidopsis* and tomato has been reported (Rasmann et al. 2012). Several studies have found that seed priming in different crop systems effectively prevented herbivore (Rasmann et al. 2012). Consequently, we cannot rule out the possibility that the effectiveness of priming treatments against herbivore attacks can depend on the stage of development at which they are applied to plants. In addition, recent results indicate a trade-off between herbivore-induced priming of volatile plants (HIPVs) and constitutive defence in tomato plants (Zhang et al. 2020).

7.2.1.3 Reactive Oxygen Species (ROS)

Many of the biotic stresses lead to increase in oxidative stress, that is, an increase in the cellular levels of reactive oxygen species (ROS). Transient or/and more prominent increase in endogenous ROS tiers can function as a signal that switches on diverse defense-associated genes, in turn, resulting in the accumulation of stress protective enzymes, proteins, and metabolites (Petrov and Van Breusegem 2012), a mechanism that can be mimicked by exogenous H_2O_2. H_2O_2-induced protection has been documented against pathogens, and other stresses in a number of model and crop species such as mustard, maize, pea, potato, rice, tobacco, and wheat), suggesting at a general phenomenon that could be used as a strategy for sustainable crop improvement (Gondim et al. 2012; Hafez et al. 2012). Although the precise molecular mechanisms of ROS priming are a long way far from understood, antioxidants, which include glutathione and stronger antioxidant enzyme activities, are possibly involved in the organization of a strong defense (Bhattacharjee 2012; Hafez et al. 2012).

7.2.1.4 Vitamins

Recent studies have shown that vitamins are effective chemical priming agents and are important, organic natural substances, and many have additional defensive functions in planta (Westman et al. 2019). For priming purposes, they also have commercially desirable properties, including non-phytotoxicity and potentially growth-stimulating properties, and B vitamins in particular are commonly used as priming agents (Boubakri et al. 2016).

Vitamin-B2 (riboflavin) is also naturally produced in plants, but its effects as a priming agent have not always been studied. Nevertheless, Azami-Sardooei et al. (2010) reported that priming with B2 improved pathogen resistance to *B. cinerea* on bean plants but not on tomato plants. The authors cited arguing that the latter could have been resistant to B2 priming because the tomato plants already had ample endogenous levels of B2, lacked or could not sufficiently absorb riboflavin receptors.

Vitamin B3 is another natural plant metabolite, niacin, which has been used extensively for prime plants. Plants primed with B3, either in forms of nicotinic acid or nicotinamide (Sasamoto and Ashihara 2014), have reportedly improved tolerance to both subsequent biotic stressors (Berglund et al. 2016) and abiotic stressors (Berglund et al. 2016). For a variety of crops (including barley, wheat, and pea), vitamins B1 and B3 were also used as priming agents without causing observable phenotypic changes. Hamada et al. (2018) and Hamada and Jonsson (2013) found that vitamin B1 protected a variety of crops from aphid attacks, while Berglund et al. (2016) found that B3 protected spruce from pine weevils. The lack of observed phenotypic responses to such agents is very positive as the ideal safety priming agent should have no or minimal performance penalties for the host as opposed to unprimed controls (Martinez-Medina et al. 2016). These studies confirm that vitamins may be attractive agents for priming plants of various species, applied either in vegetative stages (*Arabidopsis* studies) or seeds (assorted non-model crop species).

Enhanced defense responses to *Pseudomonas syringae pv. tomato* bacteria have been observed in various plants following thiamine foliar applications, confirming that it has diverse, species-specific, and genotype priming effects. In addition, thiamine also increases the resistance of many crops (including pea, barley, oat, wheat, and millet) to aphid pests (Hamada et al. 2018; Hamada and Jonsson 2013) and fungal infections (Pushpalatha et al. 2011) when used in seeds.

7.2.1.5 Biostimulants

In current years, eco-friendly strategies are increasingly taken into consideration to support agricultural sustainability. Biostimulants are natural molecules or plants extracts regarded to stimulate plant growth (Chiaiese et al. 2018). Recently, several biostimulants had been proven to enhance broad-spectrum defense systems in plants (Shukla et al. 2019). Stress tolerance is perhaps the most crucial and consistent benefit achieved by treatment of plants with biostimulants (du Jardin 2015). Pretreatment of crops with the *Trichoderma* fungus has been demonstrated to combat disease and pests (Brotman et al. 2013). Root colonization by *Trichoderma* can bring about elicitation of plant defense responses and induction of antimicrobial compounds through the phenyl propanoid pathway, a process that can restrict pathogen ingress. Tomato plants pretreated with *Trichoderma harzianum* T22 were modified at the epigenetic level (Kercheva et al. 2020). The belowground interactions between beneficial bacterial strains and plants have the potential to prime and spark off protection responses (Mhlongo et al. 2018). Another sort of priming agents, chitin and chitosan, are known as elicitors that can prime and induce plant self-defense against plant pathogens (De Vega et al. 2020). Beneficial microorganisms such as rhizobacteria and rhizofungi may also provide plant defenses (Pieterse et al. 2014).

Biostimulants have the benefit to be naturally derived compounds, to be nontoxic at low concentrations, and not only to mitigate stress, but additionally promote plant growth. The molecular mechanism and in-depth understanding is still required to develop these biostimulants as priming agents to counteract biotic stresses. Such in-depth molecular research will useful resource enhances the efficacy of these naturally derived extracts and will help optimize their applications in sustainable agriculture.

Priming can involve diverse mechanisms depending on the nature of the priming stimulus and the stressor (Singh et al. 2014; Balmer et al. 2015). Primed plants can exhibit a wide range of responses, such as stomata closure control (Jakab et al. 2005), reactive oxygen generation (Pastor et al. 2014), hormonal homeostasis changes (Aranega Bou et al. 2014), and plant volatile emissions (Winter et al. 2012).

In comparison to humans, plants have a nonadaptive immune system, based on biochemical changes. Nonetheless, the priming of induced resistance affects responses after an initial stimulus, and thus it represents a form of immunological memory that enables plants to remember stressful situations. Epigenetic modifications are one of the mechanisms by which plants can acquire memory and induce long-term gene sensitivity alterations (Singh et al. 2014; Balmer et al. 2015; Mauch-Mani et al. 2017). Recent advancements have been made in understanding the molecular mechanism behind priming. For example, chemically induced priming is associated with the accumulation of inactive mitogen-activated protein kinases in *Arabidopsis* (Beckers et al. 2009). Priming was also linked to di- or tri-methylation of histone H3 lysine 4 (H3K4me2 and H3K4me3, respectively) and histone H3 lysine acetylation of lysine 9 (H3K9) or histone H4 lysine 5, 8, or 12 (H4K5, H4K8, and H4K12, respectively) in the promoter regions of defense-related genes (Jaskiewicz et al. 2011; Singh et al. 2014).

7.2.1.6 Tree Priming

In the sense of long-lived plants, such as trees, a primed condition may persist over several growing seasons, a phenomenon widely referred to in the ecological literature as “delayed induced resistance” (Kuc 2006). Defense priming has been widely studied for short-lived model plants such as *Arabidopsis*, but little is known about this phenomenon in long-lived plants. Much of our knowledge on plant protection priming comes from model plant lab studies such as *Arabidopsis* (Conrath et al. 2015; Mauch-Mani et al. 2017).

Regulated experiments using genetically specified model plants were used to elucidate the molecular mechanism underlying the priming of defense. These mechanisms include changes in histone alteration, DNA methylation, changes in gene expression, accumulation of dormant signaling kinases, primed deposition of callose, and glycosylated hormone accumulation (Annacondia et al. 2018; Conrath 2011; Hilker and Schmülling 2019; Wilkinson et al. 2019).

While experiments in model plants are central to our understanding of plant protection priming, they do not provide much knowledge about the costs and benefits of priming in an environmentally friendly setting. Model plant experiments, for example, frequently show a faster and stronger control of inducible defenses in primed plants but rarely test plant efficiency and health in natural environments (Martinez-Medina et al. 2016). Numerous research papers have been recently written on priming for defense. Resistance in fungus-inoculated trees has been shown to be due to direct defense induction, while resistance in MeJA-treated trees is due to defense priming (Mageroy et al. 2020). This research extends our knowledge of model plant protection priming to an ecologically important tree species.

Increased tolerance to Phytophthora cinnamomi has been observed in small-sized offspring of ink-diseased chestnut trees, suggesting a virulent pathogen may cause a defense response to trees in subsequent generation (Camisón et al. 2019). Plant responses to sex pheromones emitted by a herbivorous insect will enhance plant defensive reactions to insect egg deposition, thus highlighting a plant's ability to activate its defenses very early against an initial phase of insect assault, egg deposition (Norbert et al. 2019).

7.3 Proteomics and Priming

As it is a cost-effective technique that improves the plant's ability to cope with stress, the agricultural importance of priming in plants has inspired scientists to unravel the underlying cellular mechanisms. Last year, studies using transcriptome and metabolome techniques were conducted identifying transcriptional regulators and metabolite switchers and providing fundamental clues about how various networks are influenced and communicate during the priming process (Luo et al. 2009). However, shortcomings in estimating rates of gene expression; mRNA degradation or inefficiently translation; gene alternative splicing; as well as protein PTMs, processing, and protein turnover make the use of proteomics an important method to cover the gap between the transcriptome and the metabolome. In addition, proteomics studies provide an opportunity to track subcellular proteomas and protein complexes (e.g., plasma membrane proteins, chloroplasts, mitochondria, and nuclei) and primarily priming-related PTMs (Tanou et al. 2012).

The relevance of proteomics to characterize seed imbibition and priming mechanisms is best illustrated by studies showing that early stages of seed germination do not require de novo transcription while protein synthesis from the mRNAs stored in mature seeds is absolutely required, thus revealing the function of proteins stored in dry mature seed or translated from stored mRNAs. SA reinduced the late maturation program in the early stages of germination, impacted protein translation efficiency, primed seed metabolism, triggered antioxidant enzyme synthesis, and mobilized seed storage proteins, as evidenced by a proteome-wide study (Rajjou et al. 2006). Ironically, SA strikingly influenced the oxyproteome of *Arabidopsis* seeds (Rajjou et al. 2006). A reverse genetics approach in *Arabidopsis* revealed that priming is dependent on increased accumulation of mitogen-activated protein kinase 3 (MPK3) after exposure to biotic or abiotic stress (Beckers et al. 2009), indicating that MPK3 is a possible candidate for priming signaling.

The use of proteomic approaches in the priming agent-induced acclimation of plants to environmental challenges is one of the most promising fields of basic and applied research for many years to come, while future proteomic studies on seed priming will also help reveal novel seed quality markers that are useful in ensuring the best crop yields (Catusse et al. 2011).

7.4 Transgenerational Immune Priming (TGIP)

Stress exposure may also create long-term immunological "memory" which helps the person to develop faster and stronger responses to future exposures. Evidence has accumulated in recent years that an individual's exposure to stress can also influence future stress responses in his offspring. These effects are referred to as "transgenerational" and are thought to have developed in future generations to improve the longevity of an individual's gene pool which is likely to face similar stresses. Although defense priming rarely offers maximum protection, its wide-ranging efficacy, long-lasting durability and hereditary to future generations make it attractive tool for integrated disease management.

Recent studies have shown that defense priming can be inherited, a phenomenon called transgenerational immune priming (TGIP), or transgenerational plant protection memories. Progeny of parental plants primed by BABA treatment or infection with avirulent *P. syringae* bacteria has been shown to have increased expression of SA-dependent defense genes and increased resistance to both virulent *P. syringae* and downy mildew pathogen *Hyaloperonospora arabidopsidis* (Slaughter et al. 2012). Remarkably, these transgenerationally primed plants were often "primed to be primed," as their offspring, when treated with BABA themselves, displayed higher priming rates than first-generation plants (Slaughter et al. 2012). Likewise, chemical barley elicitation (*Hordeum vulgare*) with acibenzolar-*S*-methyl or saccharin also primed the subsequent generation for leaf blotch resistance caused by the fungal pathogen, *Rhynchosporium commune* (Walters and Paterson 2012). At repeated inoculations of parental *Arabidopsis* plants with virulent *P. syringae*, Luna et al. (2012) also demonstrated priming of the progeny stage. In this analysis, not only was the primed state transferred to the immediate offspring generation, but it was possible to detect increased disease resistance in the grandchildren of the original infected plants, and thus was inherited over one stress-free generation. The β-aminobutyric acid priming agent induced resistance to *Pseudomonas syringae pv. phaseolicola*. This has been correlated with gene expression patterns of the PvPR1 gene (*Phaseolus vulgaris* PR1), a highly sensitive gene for priming, and shows that a transgenerational priming response to pathogen attack will last for at least two generations. This suggests that an easily identifiable, generational, and transgenerational defense-resistant phenotype and "primed patterns" of gene expression are excellent indicators of the priming response in crop plants (Ramírez-Carrasco et al. 2017).

These finding suggests that the increased resistance does not compensate for the maternal effects, which implies that the phenomenon is genuinely epigenetically controlled. Transgenerational priming at the promoters of the SA-regulated genes, PATHOGENESIS-RELATED1, WRKY6, and WRKY53, was shown to be correlated with chromatin modifications. In addition, evidence was given for the role of DNA methylation because the DNA methylation-related drm1drm2cmt3 triple mutant, which is mutated in DOMAINS REARRANGED METHYLASE1 AND 2 AND CHROMOMETHYLASE3 and exhibits genomic DNA hypomethylation, exhibits a constitutively elevated phenotype of acquired resistance but is not responsive to further parental stress priming (Luna et al. 2012). Transgenerational priming can therefore be inherited through the hypomethylation of defense genes which confer resistance to the SA-dependent pathogen. In transgenerational acquired resistance (TAR), there is a connection between DNA methylation patterns expressing plants at different generations after initial exposure to disease (Stassen et al. 2018).

Recently, Singh et al. (2017) made the significant discovery that the offspring from diseased plants are more resistant than the offspring from healthy plants which are genetically identical. This increased resistance continued, indicating epigenetic inheritance, for at least two stress-free generations. In the absence of pathogens, the resistant progeny did not display increased defense activity, but rather demonstrated an increased response of defense genes to infection. This is a classic example of transgenerational immune priming, since progeny plants are "primed" to respond to infection more quickly.

Transgenerational immune responses have significant implications for populations of natural plants and offer an opportunity for sustainable agriculture to be exploited. The ability to enhance pest and disease resistance through epigenetic manipulation provides a new mechanism through which reliance on chemicals can be reduced without changing the genetic make-up of our elite crop varieties.

7.5 Priming: Green Vaccination

Priming is an important technique for mitigating biotic and abiotic stress, and thus represents a possible solution for enhancing plant safety in agricultural systems (Walters et al. 2013). Given the urgent need for new strategies that do not rely on pesticides or single resistance genes, the exploitation of plant immune system ability in combination with other strategies that hold the potential for better crop defense. The attractiveness of priming for agricultural defense is also related to the fact that, unlike direct activation of defenses, this mechanism does not incur significant developmental costs (Douma et al. 2017). A substantial transfer of expertise from the laboratory into the field has already taken place (Walters et al. 2013).

Resistance caused by defensive priming is robust. When induced, priming can be sustained during a plant's life, and epigenetically inherited to future generations.

Transgenerational plant defenses priming in agriculture has the potential to lead to a sustainable intensification. Plant-induced resistance offers wide spectrum protection against pests and pathogens, and is long lasting. When triggered, priming can be sustained during a plant's life, and thus primed crops would need fewer applications of pesticides in order to achieve comparable rates of safety. By reducing the inputs of pesticides, the introduction of transgenerational priming into existing crop protection schemes could offer several benefits for both farmers and the environment (Singh and Roberts 2015). A successful introduction of TGIP would allow poor farmers to collect more resistant crop varieties from their own seed stocks, thereby making their food production less vulnerable to pest and disease outbreaks.

Priming will help grow new varieties of crops better suited to modern farming. I suggest that the efforts to develop crops must include the use of elicitors for priming or triggering induced resistance in the field and, above all, the selection of induced heritable epigenetic states in progeny prepared for defence. Putting all of these into account, some challenges need to be addressed in order to manipulate the plant's immune system (priming) to provide IPM strategies. Importantly, it is important to consider such side effects before the priming can be completely incorporated into an agricultural environment. For example, an excessively high stimulation level may lead to direct induction of resistance, thereby sacrificing health. In addition, plants might be more prone to react to false alarm signals that do not pose a danger, leading them to reallocate energy resources unnecessarily. The most prominent challenge lies in the fact that both biotic and abiotic stresses occur concurrently in the region. Nothing is known about how plants then set their defensive goals, making it hard to predict their reaction and obtain a robust and wide-spectrum response to resistance. The correct formulation therefore requires the implementation of a battery of measures to achieve an efficient, less complex, nondetrimental, and easy-to-apply priming response in order to overcome these potential obstacles.

Many studies have tried to provide IPM strategies that involve defensive priming as one of the components and potentially partially resolve the above-mentioned obstacles. For example, the combination of favorable genetic traits and fungicides has been shown to have a synergistic effect in protecting Arabidopsis from *Hyaloperonospora arabidopsidis* (Friedrich et al. 2001). A new product called TrichoPlusTM (BASF) has recently been produced in crops using a fertile substratum *Trichoderma asperelloides* strain JM41R that increases resistance to soil-borne pathogenic fungi while also growing plant growth (Diez 2016). Moya-Elizondo and Jacobsen (2016) have incorporated more control measures and demonstrated a combination of fungicide, resistant varieties and induction of SAR-reduced crown rot disease (caused by *Fusarium pseudograminearum*) in a dryland wheat field trial. These integrative studies show that leveraging the plant's immune capacity to reduce disease represents a benefit, which in combination with a battery of measures to provide effective disease control would greatly increase (Diez 2016) (Fig. 7.2).

We propose here that modern methods of plant breeding and efforts to improve crops should include the use of elicitors for the prime induced resistance in the field and, above all, the selection in progeny that is prepared for defense for induced heritable states.

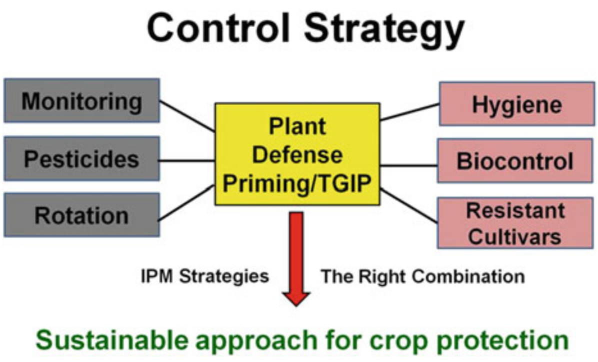

Fig. 7.2 Schematic representation of the integration of priming into IPM strategies to obtain the best formulation for sustainable crop protection. Priming could be integrated pet of IPM–non-pesticide alternative with broad effectiveness

7.6 Future Prospect: Plant Defence Priming and Nanotechnology

Nanoparticles (NPs) are particles with unique optical, magnetic, electric, and thermal properties in a nano-range structure. Nanobiotechnology has the ability to revolutionize various sectors including medicine, livestock, clothing, textiles, and pharmaceuticals. Numerous research papers and patents filed in this area indicate developments in crop safety and disease control nanotechnology (Elmer and White 2018). A detailed review on the role of NPs as a biocontrol agent in plant defense and is disease management has been published (Elmer and White 2018). Less is known about the capacity for prime resistance of nanoparticles to biotic stresses such as insect attacks or pathogens. Looking into the science underpinning NPs for inducing resistance to pests and diseases is important in the future. This may have two main aims: First, to establish the molecular and biochemical basis for increased resistance to biotic attack in plants treated with nanoparticles; and second, to test the ability of NPs to function individually and in combination, to enhance various aspects of plant defense. Investigating the role of NPs in defense priming and their effect on the resistance of broad spectrum diseases would be important.

The concept of treating seed with NPs for plant resistance against pests and diseases is novel as such approaches have previously been limited to enhancing plant resistance against abiotic stresses such as salinity or drought. Much remains to be learned about priming phenomena in plants and in particular the mechanism by which it works. Furthermore, this will develop the underpinning science for new agricultural demands. On a global scale, there is burgeoning human population growth and only a finite land area suitable for agricultural production which is expected to be reduced by climate change. Increasing efficiency of production by reducing yield losses caused by pests and diseases is one way by which future needs could be met. There is consumer concern about pesticide applications to arable and horticultural crops and the development of pesticide resistance poses a serious challenge to effective crop protection. There is also increasing regulatory pressure for reduced chemical inputs of all kinds, but especially pesticides. This concept of

NPs and defense priming will provide an alternative approach by developing sustainable resistance in plants in which natural plant defense mechanisms against insect and pathogen attack are enhanced. This will provide an opportunity for exploitation of NPs in sustainable agriculture.

7.7 Future Challenges

Despite advances in priming awareness, many problems need to be addressed before priming can become commercially viable. For example, reliable priming methods with detailed knowledge of priming strength for relevant crop species and detailed information on priming stability and reliability should be established as a matter of urgency. Effective priming improves the overall performance of plants under stress (Hilker et al. 2016; Martinez-Medina et al. 2016) and it is necessary to consider and include both physiological costs and performance benefits in any experimental test to assess the priming strength. Benefits under pressure outweigh disadvantages, so priming in a hostile atmosphere may be a beneficial tactic. This isn't so straightforward though. Positive effects on antagonist's yield and negative effects are compelling indicators of effective priming, but bioassays are costly and elaborate to conduct and require standardization. A mechanistic understanding of priming may imply simple, cost-effective ways of evaluating priming. Promising possibilities may be epigenetic responses (histone modifications and DNA methylation and acetylation) (Westman et al. 2019). They cannot stand alone, however, and must be calibrated according to the performance of the plant and the antagonist.

A potential disadvantage of priming for crop improvement is that the epigenetic states may not be transmitted to the next generation under specific circumstances, or they may show partial heritage within a population (Ramírez-Carrasco et al. 2017). However, this provides the potential to explore novel natural priming activators, examine new epialleles, or explore new combinations of priming treatments and turn natural plant defenses into practice.

A priming agent can be applied in a variety of ways, for example, spraying or submerging seeds, foliage, or roots and drilling soil. Seeds have obvious advantages, as they can be treated with little or no environmental impact or nontarget ramifications evenly and precisely. In contrast to plant priming, seed priming will also protect plants from earliest germination stages and during their development. Seed treatment thus promises to be less labor-intensive and more cost-effective than plant treatment (Sharma et al. 2015). However, the production of robust seed priming procedures will require several phases of optimization including plant system and priming agent choices, test conditions, stresses, and plant and antagonist responses. Ugena et al. (2018) recently developed a multi-trait high-throughput biostimulant screening method on *Arabidopsis* seeds to determine growth and germination effects in response to subsequent salt stress. This work provides a rare resource toward plant health care for characterizing biostimulants.

7.8 Conclusion

Sustainable crop protection strategies are desperately needed, and the priming of seed defence seems to be an appealing method. Resistance caused by defensive priming is robust. When induced, priming can be sustained during a plant's life, and epigenetically inherited to future generations. In addition, the activation of priming and the selection of transgenerational defence priming cultivars have many advantages for breeding programs to establish beneficial new crop traits. The ability through this mechanism to enhance resistance to pests and diseases offers a new mechanism by which dependence on chemicals can be minimized without having to alter the genetic makeup of our elite crop varieties. This could also provide a valuable tool for reducing residues of chemical pesticides in fruit and generate valuable knowledge for aid programs in India, where poor infrastructure and limited financial capacity require a small-scale, self-sustaining farming mode. In these conditions, the farmers themselves usually retain crop seed stocks. A successful introduction of TGIP would allow poor farmers to collect more resistant crop varieties from their own seed stocks, thereby making their food production less vulnerable to pest and disease outbreaks.

Priming may be useful in developing alternative methods for battling plant pathogens. Additionally, induced resistance does not provide the "standard" degree of protection we typically experience after pesticide application; however, priming may be used in conjunction with pesticides, biological control, resistance breeding, or any other integrated pest management strategy. Green vaccination/priming could therefore be a sustainable crop protection approach and could be integrated with broad effectiveness as part of the IPM–non-pesticide alternatives. We are at the start of an exciting new research path, where the mechanisms, ecological significance, and potential applications of transgenerational plant protection are only beginning to be revealed. We are beginning to unravel the molecular mechanisms prompted by the priming agents with the advances of the "-omics" technologies. This technology has vast potential in the future with its efficiency against multiple stresses, applicability to many crop species, affordable/less price, and environmentally safe mode of action.

However, many aspects, beginning with the priming mechanism(s), must be elucidated before it can be implemented commercially. Also required are evaluations of the efficacy of priming treatments (duration of the priming effects) and the range of biotic and abiotic stresses they may protect against are also warranted. Mechanism elucidation behind these various effects can provide highly interesting insights and opportunities in sustainable agriculture. I do not think it would be exaggerating to say that plant defence priming/green vaccination is an intelligent plant health treatment for human well-being.

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8 Role of Emerging Green Technology in Remediation of Toxic Pollutants

Priya Rai and Anjana Pandey

Abstract

Bioremediation—a process of cleaning toxic pollutants from contaminated soil and water—has gained enormous attention in the last few years. The chemical route of remediation has put the environment at high risk. So it has directed researchers toward a greener approach for more study in remediation. A route of greener approach for pollutant clearance from contaminated sites is greatly accepted owing to be no side effects of the current approach on the natural environment. Novel technologies such as bacterial quorum sensing, genetically modified microorganism (genetic engineering technology), transgenic plants (transgenic technology), electrochemical technology, nonmaterial, and biofilm-assisted remediation have been included in the class of green technology domain. These emerging technologies, when applied in the remediation process, exhibit tremendous results in the cleaning of hazardous pollutants from contaminated sites. Thus, the application of these technologies has widened the area for keen research in the remediation sector. Detoxification of pollutants (PAHs, isoproturon, acenaphthalene, naphthalene, Cr(VI), acenaphthylene, and fluorine, phenanthrene, etc.) through this eco-friendly route is highly effective than the regular chemical route. Thus, the use of advanced biotechnological interventions in bioremediation would surely enhance the efficiency and productivity of the process, as well as the quality of the environment.

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Keywords

Bioremediation · Electrochemical technology · Genetic engineering technology · Nanotechnology · Quorum sensing · Toxic pollutant

8.1 Introduction

Rapid progress in industrialization, urbanization, and population density has put our environment and ecosystem in danger due to water, soil, and air pollution (Wang et al. 2013). Besides these sources, some other sources, such as advanced agricultural processes, food processing sector, have enhanced the number of pollutants released in the environment, with subsequent negative results on human health. Excessive release of these toxic pollutants from the industry and households is progressively escalating majorly due to anthropogenic activities. It has polluted our environment on a large scale. These hazardous pollutants have deteriorated the natural cycle of the environment and endangered the earth in alarming levels. These pollutants are toxic gases (NO_x, SO_x, CO, NH_3), heavy metals, organics, inorganic, and bio-toxics. Removal of these pollutants from the environment is a burgeoning task for the current research, and it requires multidisciplinary approaches for attaining the relevant solutions. Detoxification of these hazardous pollutants through chemical and physical methods causes the processing cost high and indirectly adds many other pollutants in the environment. Some of the remediation techniques, such as surface capping, encapsulation, chemical immobilization, landfilling, vitrification, are acceptable but impact of these existing methods is not as successful as the remediation process (Liu et al. 2018). So, focusing on biological approaches is not only beneficial for environmental protection but also profitable for human health (Trapani et al. 2010). The involvement of various technologies has been depicted in Fig. 8.1 in

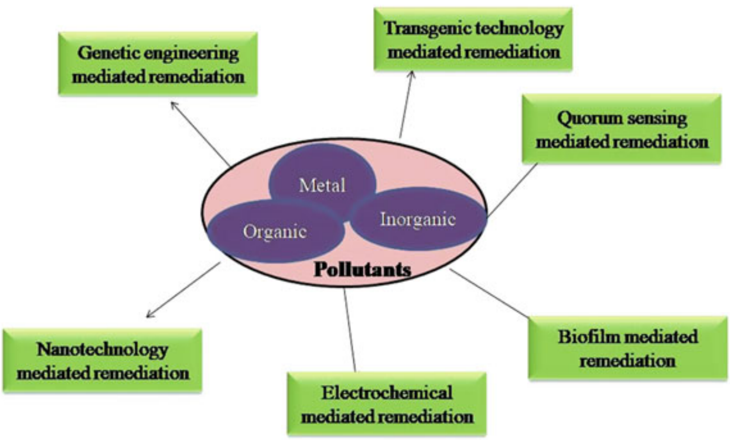

Fig. 8.1 Remediation of pollutants using green technologies

the remediation of pollutants. Various biological approaches for remediation of pollutants are in the current research, such as remediation through plants (phytoremediation), genetically modified organism, microbes, biofilm, and transgenic plants. While the incorporation of novel technologies like genetic engineering technology, transgenic technology, electrochemical technology, nonmaterial, and bacterial quorum sensing has brought a new revolution in the field of remediation, and thus has been discussed in this chapter.

8.2 Role of Nanotechnology in Remediation of Toxic Pollutants

Nanotechnology is an emerging and novel field in the area of research. The inclusion of nonmaterial is positively proved due to its effective role in the enhancement of attaining value-added products in various fields, such as remediation, medical, food, cosmetics, etc. Noria Taniguchi was the first who used the term “Nanotechnology” (Rajendran and Gunasekaran 2007). Nanomaterial mainly encompasses the size range from 1 to 100 nm and its reactivity depends on size, shape, as well as on surface area. The unique size of nanoparticle and its corresponding bulk form has different properties. The role of nanoparticles in remediation of soil and water pollutants has been explored through a diverse group of technology, such as surface plasmon resonance, optical detection systems, Raman scattering, and fluorescence spectroscopy. Nanomaterials, or nanoparticles (NPs), have a unique property in terms of activity, high surface energy, and it is considered as potential adsorbents for detoxification of pollutants from contaminated sites.

8.2.1 Titanium Dioxide Mediated Photodegradation of Pollutant

Titanium dioxide (TiO_2) is used as a catalyst in photocatalytic remediation of pollutants. It has the capability of removing traces of organic hazardous pollutants from water. It is operational as an industrial photocatalyst owing to its cheap cost, nontoxic quality, and potential activity. However, the high band gap energy (3.2 eV for anatase phase and 3.0 eV for rutile phase) of TiO_2 makes it ineffective in the remediation of contaminants from the polluted water (Gunti et al. 2016). So the application of dopants in TiO_2 photocatalyst plays a key role in enhancing the photocatalytic activity of the titanium oxide in visible light by lowering the band gap or band gap energy (Ihara et al. 2003; Gunti et al. 2016). Hence, the investigation is directed toward modifying the TiO_2 nanoparticle by doping with a transition metal, nonmetal, iodate, carbon, and nitrogen to make its photocatalytic properties effective.

8.2.2 Effect of Green Synthesized Carbon-Doped Titanium Dioxide

A green synthetic method used glucose and economical $Ti(SO_4)_2$ as a basic precursor for carbon-doped TiO_2 (anatase phase) nanoparticle synthesis. This greener approach avoids costly routes (expensive precursors, high temperature, and pressure) for the synthesis of a doped nanoparticle. This approach of carbon-doped TiO_2 synthesis is environment-friendly, and no other toxic by-products are formed during the synthesis process. The affectivity of carbon-doped TiO_2 in photocatalytic activity is increased due to the substitution of oxygen with carbon (O–Ti–C) atoms that help in narrowing the band gap. This carbon-doped TiO_2 can efficiently degrade toluene in comparison to TiO_2 (without doping) owing to enhanced photocatalytic activity by lowering the band gap energy (Dong et al. 2009). Carbon-doped titanium oxide (NPs) displayed an enhanced photocatalytic activity in the visible light and is successful in the degradation of a lower concentration of methylene blue solution (Liu et al. 2011). One study explored the TiO_2 NPs@C synthesis by using peach juice through hydrothermal method. Here peach juice has been used as a natural source for carbon that contains nitrogen and hydroxyl functional groups which have enhanced the photocatalytic activity of TiO_2 NPs@C. This composite was successful in the degradation of organic dye (MB) within 40 min under UV irradiation (Atchudan et al. 2018).

8.2.3 Effect of Green Synthesized Gold and Palladium-Doped Titanium Dioxide

The synthesis of gold and palladium (Au and Pd) doped titanium dioxide (TiO_2) use a greener approach via hydrothermal route by using aloe vera gel as a reducing and capping agent instead of a synthetic chemical agent. The performance of photocatalytic activity of gold and titanium oxide was comparatively tested in the removal of picric acid. Both the dopant with TiO_2 showed effective results but Pd-doped TiO_2 (35 min for 0.015 M) displayed an excellent performance in comparison to gold-doped TiO_2 (55 min for 0.015 M) (Hariharan et al. 2019a, b). Similarly, palladium-doped titanium oxide was synthesized through a green approach using aloe vera gel as a reducing agent. The comparative study between palladium-doped titanium dioxide and pure titanium dioxide exhibited that doped titanium oxide presented remarkable results with enhanced photocatalytic activity for the degradation of picric acid within 70 min. The reason for increased photocatalytic activity is due to aloe vera supported synthesized palladium-doped titanium oxide that produced an enormous amount of electrons within the picric acid suspension. These electrons mediate the synthesis of large free radicals in the suspension that helps in the mineralization of pollutant (picric acid). Palladium-doped titanium oxide solution under visible light irradiation generates electrons and hole pairs. The electrons react with dissolved oxygen in the conduction band and thus this process will lead to the synthesis of superoxides. The holes pairs follow either picric acid absorbance from the solution or synthesis of hydroxyl radicals when reacted with water. Thus, both the superoxide and hydroxyl radicals will help in the breaking of pollutant (picric acid molecule). So the overall procedure is leading to a large number of radicals' formation from green synthesized palladium-doped titanium oxide in the picric acid solution under visible light, and these radicals help in the degradation of picric acid from the suspension (Hariharan et al. 2019a, b).

8.3 Role of Quorum Sensing and Biofilm Technology in Remediation

Quorum sensing (QS) is defined as a type of response, stimuli, and an intercellular signal that can be analyzed in microbes in the form of various phenotypic appearances. QS is a cell-to-cell communication process that is regulated through autoinducers (AIs) which is released by the microorganisms. Microbes regulate the concentration of AIs to observe the changes in cell number or density and also for altering the expression of genes. QS regulation is different in gram-positive (oligopeptide/two-component-type sensor histidine kinases) as well as in gram-negative bacteria (LuxI/LuxR type). Furthermore, AHL (acylated homoserine lactone) is a type of AI that is organized in gram-negative bacteria and AIP (autoinducer peptide) is conserved in gram-positive bacteria. AHL-mediated quorum sensing is highly crucial in the field of bioremediation and is highly applicable in bioremediation technology.

Biofilm development is a type of adaption cycle by the microbial community, regulated by quorum sensing (QS). QS serves as a checkpoint for the propagation of bacteria and is also involved in the development of biofilms (Mangwani et al. 2012).

8.3.1 Application of Quorum-Sensing Technology in Remediation

Remediation of toxic pollutants using quorum sensing is an emerging technology without any harmful by-products formation (environment friendly). The influence of a wide variety of microorganisms in the remediation of specific pollutants through QS has improved the sustainability and capability of the technology. *P. Aeruginosa* is a microbe that is widely used in QS-mediated degradation of pollutants associated with food, pharmaceutical, oil, and cosmetic industry. QS-based degradation of different pollutants is specifically associated with unique inducer molecules like biodegradation of anthranilate and is mediated through autoinducer (*N*-decanoyl-L-HSL).

Water and soil pollution by hydrocarbon has completely altered the real characteristics of water and soil. The release of effluents that contain hydrocarbons (benzene or its derivatives, nitrogen alkanes, naphthalene, phenolic compounds anthranilate, hexadecane) has made the soil and water polluted with decreasing nutrients, salt, and nitrogen concentration in soil as well as decreasing dissolved oxygen level in water (Maddela et al. 2019). So the degradation of these pollutants by microbes following QS technology is reported in various studies. Some of the microbial species (*Enterobacter* sp., *Pantoea* sp., and *Klebsiella* sp.) have been explored for biodegradation of hydrocarbons. The role of AHL (acylated homoserine lactone) in QS is highly acceptable for the biodegradation of various pollutants either organic or inorganic. One study stated that the *P. aeruginosa* CGMCC 1.860 is capable of AHL production along with effective degradation ability for aromatic pollutants while anthranilate degradation by the same microbe is indirectly under the control of AHL. Furthermore, one other microbe (*Acinetobacter* sp. DR1) has proved the importance of QS in hydrocarbon degradation. The mutated microbe did not exhibit effective degradation capability for hexadecane due to inhibition in AHL release owing to changes in their genetic material while the wild strain showed significant degradation of hexadecane along with its growth when supplemented with AHL. So, this study has proved the keen role of QS technology in bioremediation (Sarkar et al. 2020). The degradation route in microbe has a direct relation with QS based on the type of inducer molecules. *Burkholderia* sp. DW2-1 has been reported for the degradation of pesticides available in water as well as in soil owing to its effective QS influence in their remediation route. CepI and CepR are two keen molecules (QS) that regulate the microbe for their growth along with biosurfactant production in glucose-nitrate-supported media and indirectly mediate the degradation of nitrate and glucose from the waste medium through enhancing their degradation capability (Sarkar et al. 2020).

Application of the QS system containing microbes in the densely polluted area would surely minimize or degrade the pollutants from the affected areas with effective and enhanced bioremediation along with the minimum cost.

8.3.2 Application of Biofilm Technology in Remediation

The increasing concentration of pollutants in water has degraded the purity of drinking. A variety of microorganisms is found in our ecosystem as natural filters for the purification of the environment. Focusing on the remediation of toxic pollutants through these natural filters, we will apply new bourgeoning technology, i.e. remediation through biofilm. Various studies have been published regarding pollutant remediation by bacteria and research is also under process for remediation using bacteria. Currently, a new technology is emerging, i.e. biofilm technology for pollutants (organic, inorganic, chemical, and heavy metals) remediation. Biofilm-mediated remediation is cost-effective, eco-friendly, and also applicable in the natural system without any negative impact on the environment.

Biofilm forming microbes naturally persist in the environment, and they survive in the environment and showed tolerance for the pollutants in the bioremediation process. These natural microbes manage their existence against a wide variety of factors, such as antimicrobial molecules (O'Toole 2011) temperature, pH, salinity, high concentration of nutrients, and UV radiation (Espeland and Wetzel 2001) during the remediation process of pollutants. Biofilm can be a group of single or multiple species (microbes, fungi, algae, and archaea), and communication among the single or multiple species of microbes in biofilm is well organized and cooperative. Various studies supported that the biofilm organization helps in the degradation or removal of pollutants from the contaminated sites. An assumption regarding biofilm is stated that as contaminated water passes through this biofilm nexus, the microbial species present in the biofilm start to engulf or consume harmful organic pollutants from the contaminated sources. It is stated above that biofilm is a well-organized group of microbes that are immobilized in self-synthesized support matrix such as EPS (extracellular polymeric substances). EPS protects the living cells from the harsh environment stresses, and it consists of nucleic acids, proteins, lipids, and polysaccharides. Protein and polysaccharides are the two major constituents of the biofilm. Hydration in biofilm is mainly due to water that is found almost ~97% in the film and availability of water in the biofilm helps in the movement of the nutrients from one cell to another. The total thickness of biofilm varies from 10 to 30 mm depending on the species while the total thickness of EPS varies from 0.2 to 1 mm (Maurya and Raj 2020).

8.3.3 Remediation of Heavy Metals Using Biofilm

Economically, industrialization has certainly participated in the development of the whole world but, contrarily, an effluent discharge from industry has completely degraded the biological and chemical characteristics of the water and soil. The introduction of heavy metals in water and soil is highly hazardous for the natural environment and human health due to possession of various toxic groups.

Two international agencies, i.e. World Health Organisation (WHO) and United States Environmental Protection Agency (US EPA), monitor and analyze the concentration of heavy metals to keep the environment healthy. Rapid depletion in non-renewable energy resources has directed the use of nuclear energy for electricity fulfillment. So, uranium is used as fuel for nuclear reactors, and the demand for this fuel is continuously increasing for one decade. The impact of two different forms of uranium, i.e. uranyl nitrate ($UO_2(NO_3)_2 \cdot 6H_2O$) and uranyl ion (UO_2^{2+}), has a severe impact on the health of living beings. Hence, remediation of heavy metal as uranium by a biological method, despite the chemical method, is highly encouraging. Biofilm technology is being used efficiently for the degradation of pollutants and the robustness of biofilm makes it highly effective for the degradation purpose (Shukla et al. 2020a, b). As concern related to uranium degradation, one study displayed that three microbes *Exiguobacterium profundum* MS-8, *Pseudomonas putida* MS-11, and *Bacillus marisflavi* MS-13 were isolated from the soil. Among them, the biofilm of *Pseudomonas putida* MS-11 showed the highest results in terms of uranium degradation up to 65% with 100 mg/L of initial uranium concentration. Whereas sequestration of uranium was up to 88% when the initial concentration of uranium was tested from 1 to 50 mg/L. The study concluded by stating that the biofilm of *Pseudomonas putida* MS-11 was successful in uranium degradation at both low and high initial concentrations (Manobala et al. 2019). Similarly, another study showed that *S. aureus* V329 biofilm has proved 47% sequestration of 10 ppm of U(VI) when exposed for 1 h. An enzyme (acid phosphatase) is speculated for its role in bio-precipitation of U(VI). So considering this, an acid phosphatase activity in the biofilm of *S. aureus* V329 has been detected and found high (26.9 ± 2.32 μi.u./g of biomass) in comparison to its planktonic form (Shukla et al. 2020a, b). The tolerance of *Stenotrophomonas acidaminiphila* NCW-702 has been studied and analyzed against heavy metal Hg (5 ppm), Cd (60 ppm), and Pb (250 ppm), suggesting that it's potential for heavy metal bioremediation is significant, and it can be processed for bioremediation purpose at a large scale. EPS (extra polysaccharide substance) is associated with a biofilm of *Stenotrophomonas acidaminiphila* NCW-702 and has shown the presence of nucleic acid, lipid, polysaccharides, and protein. Hence, the ionic nature of EPS makes it suitable for affinity with metal ions, and their assistance results in the remediation of heavy metals (Mangwani et al. 2014).

8.3.4 Remediation of Aromatic Hydrocarbons Using Biofilm

A new challenge is being faced for polycyclic aromatic hydrocarbons (PAHs) degradation due to its complex ring structures. The more number of rings in PAHs makes it less soluble in water and highly soluble in organic solutions (Krell et al. 2013). PAHs can be reached in aquatic, soil, and marine environment by an effluent that is released from industries, oil spills, and urban areas. US-EPA reported 16 PAHs as a dangerous pollutant and reviewed that PAHs are carcinogenic and their severe impact can be seen on human health (Perelo 2010; Pan et al. 2010).

A study regarding different phases of biofilm formation including cell attachment, cell differentiation, adhesion, EPS secretion, colony organization, and maturation was analyzed in *Stenotrophomonas acidaminihila* NCW-702. The surface property of EPS (*Stenotrophomonas acidaminihila* NCW-702) assists in increasing the solubility of hydrocarbon (PAHs) with the help of hydrophobic interactions. The microbe has shown 85.6 ± 2.9% degradation for phenanthrene using its biofilm, while its planktonic cultures showed 73.3 ± 1.5% degradation (Mangwani et al. 2014). Similarly, PAH persistence in the soil is found for a long time and the degradation of PAH in soil involves some biofilm-forming marine microbes (*Pseudomonas pseudoalcaligenes* NP103, *Alcaligenes faecalis* NCW402, *Pseudomonas aeruginosa* N6P6, *Stenotrophomonas acidaminiphila* NCW702, and *Pseudomonas mendocina* NR802). Phenanthrene and pyrene are the two hydrocarbons that were studied for their degradation using marine consortium in soil microcosm. The high hydrophobicity and poor solubility of PAH restrict it for their degradation. It is stated in various studies that the role of EPS is significant in the degradation of pollutants (Mangwani et al. 2017) due to its various active compounds. Hence, the biofilm–EPS combination facilitates in improving the solubility of pollutants (PAHs). This study reported that the degradation of pollutants increases as the concentration of EPS increased (Mangwani et al. 2017). A recent study reported that *P. putida* as well as *B. cereus displayed positive results in toluene remediation. These bacterial cells showed a hydrophilic pattern on their surface*, *and waste wood was selected for the* *bacterial cell attachment and further for biofilm formation. It was concluded from the study that the* cold low-pressure nitrogen plasma (LPN-plasma) pre-treated waste wood displayed the highest hydrophilicity in comparison to nonpretreated waste wood. So, the hydrophilic patterns of both the bacterial surface make it viable for attachment to the hydrophilic wood waste (pretreated). Furthermore, this attachment significantly mediates the biofilm formation and its potential viability in toluene remediation with 91% (*P. putida*) and 89% (*B. cereus*). The attachment (bacterial cell surface between solid surfaces) is followed by various forces such as hydrophilic, electrostatic, dipole, ionic, hydrophobic (Farber et al. 2019).

8.4 Role of Genetic or Transgenic Technology in Remediation of Pollutants

Genetic manipulation has been in the use by humans since 1970s, and information regarding transgenic plants was first time observed in 1984. After 2 years, success in the form of the first genetically engineered plant (tobacco) which showed resistance against herbicides was achieved, and transgenic tobacco was trailed first time in the fields in United States and France in 1986. Further exploration of gene(s) and their associated metabolic routes can be targeted in comparison to physicochemical techniques for the remediation of pollutants (Liu et al. 2017). The natural route for remediation by plants, microbes, and their interaction is quite a slow process. As the latest techniques (recombinant DNA methods, gene transfer, transgenic technology) along with engineered microbes and plants is increasing in the field of biotechnology with enhancement in the expression of specific enzymes or gene(s) to promote degradation or remediation of toxic pollutants from soil and water (Pandotra et al. 2018).

8.4.1 Application of Plant in Remediation

Massive exploration of pesticides, organic chemicals, non-renewable resources, industries, constructions, and transportation is continuously deteriorating the environment quality either directly or indirectly. The persistence of toxic pollutants (phenols, toluene, hydrocarbons, polyaromatic, biphenyls, and benzene, etc.) that is released from these sources was observed in the environment for a longer period. They can enter into the living body through the food chain and can cause cancer in living beings. Inorganic pollutants such as Co, Zn, Fe, Cu, Mo, Zn, and Mn act are also entered into living beings but concentration above the threshold value becomes toxic for them. Heavy metals like chromium, mercury, cadmium, uranium, lead, arsenic, and cobalt are such metals that are deadly even at lower concentrations (Clemens and Ma 2016). Removal of these toxic pollutants through the physical and chemical process is quite costly, laborious, and inefficient. An application of the chemical process in remediation indirectly releases another pollutant into the environment. Hence, the route is being diverted from chemical route to biological route

(Phytoremediation) for the decontamination of pollutants. Thus, phytoremediation is a process in which plants accumulate this deadly metal in its tissues or cells and mediate remediation or stabilization.

8.4.2 Application of Transgenic Plants in Remediation

The plants should have distinct characteristics (such as fast-growing along with high biomass production, hyperaccumulation of heavy metals, high efficiency in the uptake of pollutants, its translocation, and high tolerance capacity against odd environmental conditions) for a successful remediation process (Bell et al. 2014). Hyperaccumulators are the plants that exhibit the potential ability to accumulate a considerable amount of pollutants in their cell. Sometimes, hyperaccumulators are not considered suitable for phytoremediation, despite having a good accumulation of heavy metals due to slow growth with low biomass production. So, transgenic technology or genetic engineering has played as novel technology in the field of plant-mediated remediation. Now transgenic technology has improved the efficiency of hyperaccumulation in terms of metal absorption along with good biomass production due to gene transfer.

Genetic engineering or transgenic technology is one of the competent approaches to improve the remediation efficiency in plants by modifying the mechanism at genetic level (gene-based modification) and molecular level modification by using omics technologies (transcriptomics, proteomics, metabolomics). Genetic and molecular-based studies in phytoremediation have assisted biotechnologists to engineer non-hyperaccumulators to hyperaccumulators based on overexpression of genes that are involved in chelation, transportation, uptake, sequestration, and tolerance against xenobiotics (Mosa et al. 2016; Rai 2019). The insertion of these potential genes from animals, microbes, and plants is mediated either through traditional method (*Agrobacterium tumefaciens* mediated transformation) or technical method (direct DNA methods of gene transfer) to develop an engineered or transgenic plants (Seth 2012). The gene-based manipulation research has been studied in some of the model plants like *Oryza sativa*, *Populus*, and *Arabidopsis thaliana*, while transgenic technology has been applied in *Liliodendron tulipifera*, *Brassica juncea*, *Salix*, *Helianthus annuus*, and *Populus* for improving the remediation process (Luo et al. 2016). *Nicotiana tabaccum* (overexpression of yeast metallothionein) and *Arabidopsis thaliana* (overexpression of mercuric ion reductase) were the transgenic plants that show an efficient tolerance against Hg and Cd, respectively (Rugh et al. 1998). Now various transgenic plants are being developed after successful sequestration of heavy metal by transgenic *Nicotiana tabaccum* and *Arabidopsis thaliana*. Thus, genetic and transgenic technologies can play a successful role in developing transgenic plants at a large scale, and application of these transgenic plants at contaminated sites would surely contribute to remediating pollutants.

8.4.3 Role of Target Genes Involved in Remediation

Improved phytoremediation can be enhanced with some modification or manipulation in the target gene(s) that results in transgenic plants with effective tolerance and remediation quality. The modification can be possible for effective remediation (1) manipulation in transport proteins that monitor uptake, translocation, and storage of metals, (2) to enhance tolerance of plants and mobilization of metals by overproduction of some metal detoxifying ligands (metallothioneins, organic acids, phytochelatins, amino acids, and glutathione), (3) changes in metabolic route for conversion of more toxic form to less or non-toxic form (Luo et al. 2016).

Furthermore, the introduction of novel genes from bacteria and plants to model plants can result in better remediation process by the overexpression of target genes in model plants (Kaur et al. 2019). As concerns related to the degradation of organic pollutants in plants, there is no real transporter for their degradation. However, some enzymes and their modified form can assist in the degradation and translocation of the pollutants in plants. The nature of organic pollutants (hydrophilic and hydrophobic) decides the route for their degradation in plants. Hemicellulose and lipid bilayers can play an initial role in binding with organic contaminants (hydrophobic) and pollutants transported to the aerial parts of the plants (Rai et al. 2020). Execution of some potential enzymes (peroxidase, dehalogenases, nitroreductase, and laccases) can result in the conversion of high toxic pollutants to less toxic substances (Cherian and Oliveira 2005).

8.4.4 Target Gene(s) in Transgenic for Remediation

Transgenic organisms (model organisms) have an immense capability in the field of remediation. The potential of these model organisms is mainly due to the gene(s) that is transferred from the potential one to non-potential through transgenic technology. The expression of these gene(s) in the model one can enhance its efficiency in many folds in comparison to the non-model one. The transfer of gene(s) from microbes to non-model ones makes it transgenic with improved capability in remediation. Interestingly, microbes have pollutant degrading multiple potential gene(s) and transfer of these gene(s) to produce transgenic with effective remediation is highly acceptable. Transgenic-mediated remediation with a supported study that naphthalene dioxygenase complex was observed in *Pseudomonas* and transfer of this complex to *Arabidopsis* and rice has developed the remediation capacity of the transgenic plants and improved the tolerance capacity of the transgenic against phenanthrene. Roots and shoots of the transgenic plants have a lower concentration of phenanthrene in comparison to a wild one. Transgenic plants with naphthalene dioxygenase have efficiently displayed the role of eco-friendly technology in the remediation of phenanthrene (Peng et al. 2014). Similarly, to enhance the remediation of polychlorinated biphenyls (PCBs) and 2,4-dichlorophenol (2,4-DCP), transgenic BB11 (alfalfa) was produced due to the expression of 2,3-dihydroxybiphenyl-1,2-dioxygenase (BphC.B) with the help of promoter

CaMV 35S using Agrobacterium-mediated transformation. The expression of BphC. B in transgenic line BB11 (alfalfa) has enhanced the remediation of mixed pollutants in a short time in comparison to wild type (Wang et al. 2015). As we concern about xenobiotics degradation, wild plants have shown the degradation of many pollutants but the efficiency is poor owing to low tolerance capacity toward the pollutant. Hence, transformed *Oryza sativa* (rice) with enhanced tolerance capacity has been developed due to the expression of cytochrome P450 monooxygenases CYP1A1, CYP2C19, and CYP2B6. Furthermore, it has shown the potential results in the degradation of atrazine and metolachlor from the soil than that of non-transgenic *Oryza sativa* (Kawahigashi et al. 2008). The cytochrome P450 monooxygenases (CYPs) are reported for the degradation of various pollutants such as 9-methylanthracene, acenaphthalene, naphthalene, acenaphthylene, and fluorine. Alterations in the active site can increase the enzyme activity in many folds such as manipulation at the Y96 and F87 domain in CYP101 enzyme has enhanced the activity (oxidation potential) of a concerned enzyme in *Pseudomonas putida* (Harford-Cross et al. 2000). Similarly, mutation at three sites (A74G/F87V/L188Q) in CYP enhanced 30-folds degradation for polyaromatic hydrocarbons (Hussain et al. 2018), and mutation at Y51F and R47L active sites in CYP102 displayed 40-fold and tenfold enhancement in oxidation potential of the enzyme for the degradation of phenanthrene and fluoranthene in *Bacillus megaterium* (Carmichael and Wong 2001). Interestingly, the plant and microbe interaction can also cause efficient degradation instead of using individual one. Here the transgenic unit (plant-microbe) is reported for an efficient remediation of phenylurea herbicides from the polluted sites. A transgenic plant (*Arabidopsis thaliana*) was developed for the synthesis of bacterial *N*-demethylase PdmAB in its chloroplast and the transgenic has shown potential tolerance against isoproturon (IPU). This transgenic receive isoproturon from the roots, and it is carried to the leaves where isoproturon is majorly converted to 3-(4-isopropylphenyl)-1-methylurea (MDIPU). The MDIPU is released to outside from the roots. Further MDIPU-degrading *Sphingobium* sp. strain 1017-1 potentially breaks it in the rhizosphere. The whole process degrades the pollutants (IPU + MDIPU) effectively by the mutual interaction of transgenic unit (*Arabidopsis thaliana* + *Sphingobium* 1017-1) (Yan et al. 2018).

8.5 Application of Electrochemical Technique in Remediation of Toxic Pollutants

As we concern about pollutants generation, one emerging technique (Electrochemical) is highly appreciated due to many reasons, such as no requirement of costly reagents, no other waste product generation during the process of pollutants degradation. Various techniques are available for pollutant degradation, but the application of the electrochemical technique in the degradation of contaminants is highly advantageous over other methods due to cost-effectiveness, amenability to automation, energy efficiency, versatility, environment compatibility. Electrochemical techniques were started in the early 1960s for wastewater treatment. This technique has vast adaptability in various fields due to possessing various types of process reactions, i.e. concentration, reduction, dilution, oxidation, phase separation, along with directly or indirectly involvement in biological reactions (Yasri and Gunasekaran 2017).

8.5.1 Basic Approach of Electrochemical Study for Process Achievement

The basic concept of the electrochemical study relies on the conduction of electric charges from one stage or phase to another stage or phase (one stage or phase is metal electrode while the other stage or phase is an electrolyte solution that contains ions). When these two stages or phases are in contact with each other, the transfer of ions from one stage to another is started. This process leads to electric charge generation that creates a potential difference across the area between the two stages. This area is referred to as an interface or electrode interface and the potential is referred to as interface potential or electrode potential. In an electrochemical unit, at one interface (electrode-electrolyte), as electrons start to leave the cathode (electrode, an electron source), the cations or particles of the solution start to become reduced. While on the other interface, an anode (electrode, an electron sink) receives electrons and the process of oxidation is completed. Hence, the anode is considered as a site of oxidation, while the cathode is considered as a site of reduction.

The type of electrochemical cell decides to measure the potential and currents at two locations (cathode or anode). As consequence, in an electrolysis cell where current and potential are enforced to accelerate the chemical reaction (process), an anode is attributed for positive. Generally, the electrolysis process is used for wastewater treatment, and in this process, both the reactions (oxidation and reduction) take place at the electrodes. Oxidation takes place at the anode by the action of diffused anions toward the surface that generates anodic currents and, on the other hand, cations diffuse toward the cathode for reduction to take place at the surface (e.g. deposition of metal ions on cathode) that generates cathodic currents. However, in galvanic cell, i.e. batteries, where chemical reactions are casual, the cathode is attributed for positive (electron shrink act as reduction place) (Yasri and Gunasekaran 2017).

8.5.2 Electrochemical Remediation of Cr(VI)

The electrochemical technique is being extensively used on the large platform for the treatment of Cr(VI) (Dubrawski et al. 2014). To run this process, electrode selection and its participation in mutual oxidation/reduction for remediation are highly effective and imported. Carbon-based electrodes have proved its conductivity and anticorrosion characteristics in many earlier studies (Duan et al. 2017; He et al. 2020). Graphene–polyaniline electrodes are highly operative for Cr(VI) and exhibit effective electrocatalytic performance in Cr(VI) removal due to various active sites and potential efficiency of electron migration (Yang et al. 2014). It has been shown in a previous study that electrocoagulation is 100 times appreciated in terms of removal efficiency in comparison to pre-precipitated methods (Rodrigo et al. 2010). Cr (VI) follows two pathways for its remediation in the electrochemical system. One pathway follows the receiving of electrons from the anode electrode by the Cr (VI) and it is reduced or converted to Cr(III) that associates with hydroxide to synthesize precipitate, and the first pathway follows the electrochemical reduction process. While in the second pathway, Cr(VI) is adsorbed by Al(III) or Fe(III) hydroxide precipitate, and the second pathway is considered as an adsorption process (He et al. 2020).

8.5.3 Process of Electrochemical Remediation of Organic Pollutants

A simple biological process is effective and appreciated for the degradation of the organic compound due to its simple degradability nature. Sometimes high toxicity of the organic pollutants can damage the natural cleaner (microbes) in the treatment plants. Hence, in such cases, biological treatment for organic pollutant remediation is not successful. As a consequence, natural inhibitors (aldehyde compounds and aromatic phenolic compounds) are produced during the hydrolysis of polysaccharides (a portion of the plant). These compounds become toxic for the microbes when the concentrations are above the threshold value, and they persist in the environment for a long time and their aggregation causes severe problems for humans (Suanon et al. 2016). Hence, electrochemical technique selection for remediation of pollutants is preferred due to its ability to regulate the electron transfer in both oxidation/reduction processes. The chemical reaction that participates in the process is completely considered as a treatment step for the remediation and is highly favorable over the biological treatment due to its effective results and durability of the process. Remediation of some organic inhibitors can be achieved using a biological process following either oxidation or reduction in the electrochemical cell. In the process of oxidation, the electrode interface receives an electron from the organic waste by using the electric energy that will lead to the dissociation of organic bonds. While in the reduction process, organic inhibitors (furfural or hydroxymethyl furfural) receive electrons from the electrode surface and are reduced to alcohol and other reduced compounds. Such electrochemical process directly or indirectly depends on the concentration, type of organic pollutant, and other physiochemical conditions of the pollutants (Yasri and Gunasekaran 2017; Cha and Choi 2015).

8.6 Conclusions

Remediation of pollutants through green technology has exhibited its potential and non-toxic impacts on the environment. Various new, simple, and effective technologies have been introduced in this chapter for the conversion of a highly toxic component to a less toxic component, complete degradation of organic

Table 8.1 Green source in remediation of pollutants

S. No	Remediation technology	Pollutants	References
1	Iodine-doped Titanium Dioxide (Nanoscience)	Phenol	Hong et al. (2005)
2	Phytoremediation	Heavy metal	Luo et al. (2016)
3	Plant microbe	Phenylurea herbicides	Yan et al. (2018)
4	Microalgae–bacteria biofilms	Metal	Abinandan et al. (2018)
5	Microbe-mediated remediation	Arsenic	Shukla et al. (2020a, b)
6	Transgenic plants	Radionuclides	Reddy et al. (2019)
7	Microbe-mediated	Cadmium toxicity	Shahid et al. (2019)
8	Electrochemical	Printing ink	Ramos et al. (2019)
9	Biofilm-mediated	Organophosphorus pesticides	Dash and Osborne (2020)

compounds, and degradation of pollutants in two phases (i.e. complete degradation of intermediate pollutant along with main toxic pollutant). Different pollutants, such as Cr(VI), PAHs, isoproturon, acenaphthalene, naphthalene, acenaphthylene, and fluorine, phenanthrene, have been detoxified by applying these new technologies instead of other physical and chemical methods. Furthermore, exploration and more advancement in these new technologies will make our environment cleaner, sustainable, pollution-free, and rich in oxygen that is the need for a healthy life.

Participation of remediation technology in pollutant degradation has been shown in Table 8.1.

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Biofuel as a Sustainable Option to Control Environmental Changes

9

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Abstract

Biofuels are getting attention as an alternative to fossil fuel for a lot of reasons, and one of them is their capability to neutralize carbon. Biofuels actively reduce the toxic levels of greenhouse gases from the environment, and the combustion of biofuels result in the net-zero emission of carbon. The carbon used by plants during growth is recycled, so it does not harm the environment as fossil fuel does by releasing GHG. Biofuel also controls the carbon emissions from biomass facilities which would have been released back into the atmosphere through natural decay or disposal through open burning. Biofuels from degraded land and non-food crops are promising and will help in climate change mitigation. Proper planning in land use and identifying the most appropriate policies for promoting will help in combating the global issue. The technology utilizing

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carbon isolation in various sources, for ethanol, biodiesel, and other biofuels production, is a sustainable solution to climate change rather than biofuels from food crops.

Keywords

Biobutanol · Biodiesel · Biofuel significance · Climate change · GHG emission control · Carbon isolation

9.1 Introduction

In the current epoch, reduction in the usage of fossil fuels has become critical for the regulation of alarming CO_2 levels in the atmosphere (Kothari and Gujral 2013), as the usage of fossil fuels introduces 70% of CO and 20% of CO_2 into the atmosphere (Dechambre et al. 2017). It has now become impossible to live in the modern world by reducing the consumption of energy resources or in other words limit the use of resources (Kothari and Gujral 2013). The generation of the fuel from the plants and animal waste is termed as biofuel. Biofuel provides hydrogen, clean up oil for vehicles, cooking oil, and also works as a carbon-neutralizing agent by reducing the emission of carbon into the environment. Plants fix CO_2 by converting it into useful products, such as polysaccharides (cellulose and hemicellulose). The production rate of biofuel increased up to 105 billion L in 2010 (Shalaby 2013), and a 17% increase until last year, while today the demand for biofuel is increasing at 3.6% per annum. In 2017, biofuel production will be 143.3 billion L all around the world (Prasad et al. 2014). In 2018, the demand for biofuel remained at 115 million metric tons. The history of biofuel is ancient as civilization, Rudolf Diesel was the first person who made biodiesel from vegetable oil in 1890. Environmental protection agency (EPA) in 1970 and 1980 stated that fuel should be free of poisonous gases like CO (carbon monoxide), SO_2 (Sulphur dioxide), and NO (Nitrogenous Oxide). Biofuel was commercialized in 1998 after approval by EPA as an alternative source for fossil fuel, as the usage and production of biofuel products increased in European countries up to 53%. The International Energy Agency (IEA) has set an aim to reduce the fossil fuel usage (petroleum and coal) and shift all energy plants to biofuel by 2050.

9.1.1 Classification of Biofuel

Organic materials are used for the production of biofuels, the conventional method of obtaining biofuel is from the biomass of sugar, starch, and oil-producing crops, whereas the unconventional biomass source is algae and lignocellulosic material. Based on crops types and cultivation, biofuel production is widely divided into four categories:

9.1.1.1 First-Generation Biofuel

A conventional approach is used for the production of first-generation biofuels as the biodiesel comes from the harvested components like canola, soya bean, and *Jatropha*, while corn, wheat, sugarcane, and sweet sorghum resulted in the production of ethanol. The whole process is very naive and cost-effective and requires only a portable source of water for cultivation. For the proper growth of the crops, there should be optimum temperature and humidity and also fertilizers, pesticides for growth promotion (Table 9.1).

9.1.1.2 Second-Generation Biofuel

The second-generation biofuels are also called advanced biofuels as they result from the waste of different biomasses like wheat straw, waste of plastics, shells, stalks branches, straw dust, thinning, and wood (Dahman et al. 2019). Another term “lignocellulosic waste”, or the byproduct of nonedible food crops, is also used for this generation. Due to less emission of carbon in the atmosphere and a negative impact on CO_2, it is also taken as a carbon-neutral technique. The additional benefit of using this waste is that it does not require more land, spray, and water for cultivation so these raw materials can be harvested at a cheaper rate as compared to the first generation. Poplar, eucalyptus, and perennial vegetative grasses are the crops that can be used for this purpose. All the second-generation lignocellulosic wastes consist mainly of cellulose and hemicellulose and lignin (Yuan et al. 2019), then further converted into bioethanol after passing through a series of thermochemical and biological steps (Fig. 9.1).

It is suggested by scientists that a total of 476 million tons of biomass of lignocellulosic waste is required for the production of second-generation biofuels to accomplish the demand of bio-products by 2030 (Author links open overlay panel) (Hassan et al. 2019).

9.1.1.3 Lignocellulosic Feedstock

Agriculture Residues

Crops used in this feedstock are sugarcane, barley, and rice as they produce waste as a byproduct which is further used in biofuel production. In this way, producers save the land from the extra workload and other essential factors like water, nutrition, and fertilizers which make second-generation biofuel cost-effective; it also saves the environment from the greenhouse gases like carbon. It also helps in maintaining the

Table 9.1 Comparison between first- and second-generation biofuels (Camia et al. 2018)

First-generation biofuel		Second-generation biofuel	
Sunflower	0.06%	Palm	1.21%
Sugar	0.06%	Rapeseed	4.07%
Soybean	0.68%	Pellets	6.3%
Wheat	0.77%	Wastes	84.9%
Corn	0.77%		
Other biomass	1.21%		

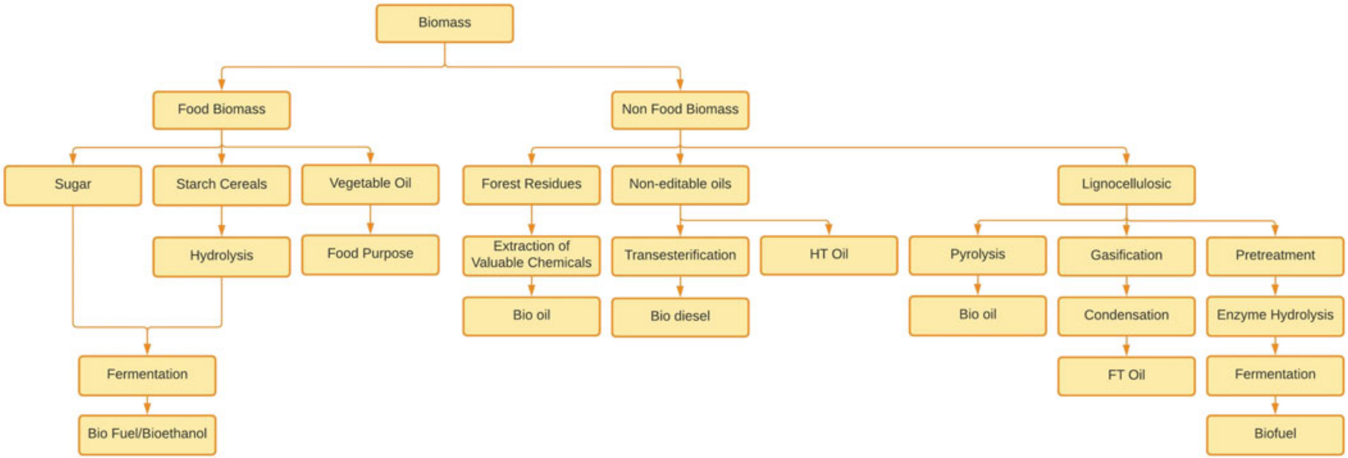

Fig. 9.1 Development of second-generation biofuel from biomass

soil temperature which is suitable for some crops in spring by removing residues and also helpful in maintaining the soil fertility by controlling disease-causing pests. It should be in complete balance; otherwise, it may harm the soil fertility, humidity, water balance, and also the environment.

Forest Residues

The wood obtained from the forest after the harvesting and cultivation of plants and trees is used in the second-generation of biofuel. Some other secondary forestry products are obtained from grapes juice, olives, and spent coffee ground. In Europe, the residues collection at the end of the year is approximately 43.5 Mt/year (million tons per year) especially from Ukraine as well as from Belarus. For example, 205 million residues including stumps, treetops, needles and branches, are produced worldwide (Ho et al. 2014).

9.1.1.4 Production of Second-Generation Biofuel

Thermochemical Conversion

Second-generation biofuel is produced either by the thermochemical or biological process. The thermochemical procedure further consists of gasification, pyrolysis, and liquefaction. Figure 9.2 explains these steps clearly. When the residue is burnt in the absence of oxygen, it produces oxygen or syngas (CNG) which comprises carbon monoxide (CO) and hydrogen, respectively. So, the conversion of this biomass is the same as that of coal by chemical or thermochemical.

Pyrolysis

It is the process of conversion of biomass into energy in the presence of heat, halogen, and in the absence of oxygen; fuel gaseous and bio-oil are the final products resulting from it. There is minimum usage of calorific power for the recovery of biofuel. Pyrolysis further divides into following subclasses.

Conventional Pyrolysis

This process is known as slow pyrolysis or may be called carbonization. It works under a slow heating rate of 0.1–1 K/s, a lot of wood, and a staying period of 45–450 s. In the first step, the heating required is 550–950 K for the decomposition of biomass, and it is called pyrolysis. In the meanwhile, bond breakage or ion formation has occurred, as well as the formation of hydroxide and carboxyl groups

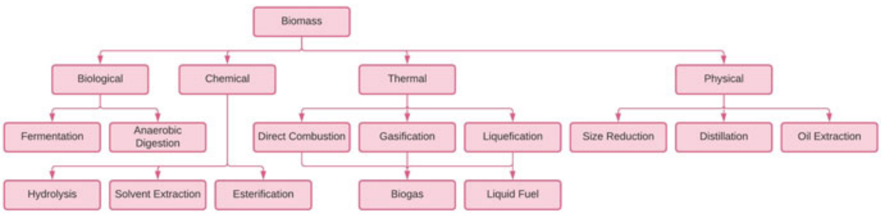

Fig. 9.2 A flow diagram for the conversion of biomass into biofuel

also happened. The second step involves the main procedure of pyrolysis which consists of the decomposition of solid biomasses and required a higher heating rate. In the third step, there is decomposition of char at a slow rate and as a result it produces carbon-rich byproducts (Al Arni 2018).

Fast Pyrolysis

In fast pyrolysis, the procedure of conversion occurs at the high temperature of 850–1250 K, fast rate of heating 10–200 K/s, the residence time is 0.5–10 s and the final product is fine particles of size less than 1 mm. It converts biomass into vapors, char, and aerosol. After this, vapor is converted into a condensed liquid which is brown. It produces end products bio-oil, solid char, and non-condensed gas with the ranges of 60–75%, 15–25%, and 10–20%, respectively (Bridgwater and Peacocke 2000).

Biochemical Conversion

Numerous steps are involved in the conversion of biomass into biofuel after passing through the chemical and biological procedures. Genetically modified micro-organisms like bacteria are also used as biomass for biofuel production and the fermentation procedure is also a most popular procedure. Landfill gas as well as municipal waste is used for the second-generation biofuel (Nogueira et al. 2020) (Table 9.2).

Table 9.2 Comparison between first-, second-generation and petroleum refinery (Naik et al. 2010)

	Petroleum refinery	First-generation fuels	Second-generation fuels
Feedstocks	Crude petroleum	Vegetable oil and corn sugar, etc.	Nonfood, cheap, and abundant plant biomass (agricultural and forest residue, grass, aquatic biomass, and water hyacinth, etc.)
Products	CNG, LPG, Diesel, Petrol, Kerosene, Jet fuel	FAME or Biodiesel, corn ethanol, sugar alcohol	Bio-oil, hydrotreating oil, FT oil, lignocellulosic ethanol, butanol, mixed alcohols
Problems	• Declining petroleum reserves	• Limited food stock	
	• Environmental pollution	• Blended partly with conventional fuel	
	• Economic and ecological problems		
Advantages		• Environment-friendly	• Noncompeting with food
		• Economic and social security	• Environment-friendly

9.1.2 Third-Generation Biofuel

The bioenergy is derived from photosynthetic micro-organisms like microalgae and macroalgae (Piwowar and Harasym 2020). Algae is present in a wide range from unicellular to multicellular such as *chlorella* and kelp, which is grown under the feet of 50 m. It can be autotrophic and requires only CO_2, salts, and other inorganic nutrients, as well as they require light energy for their growth. While, on the other hand, they can be heterotrophic and need organic compounds as nutrition from outside and require light energy to grow fully in a smaller surface area and also contains a higher amount of lipids. It can grow on saline conditioned soils, brackish, and coastal sea-water, respectively; the energy required for its growth is just 20–30% of the total cost. Flocculation, sedimentation, and flotation are the techniques used for the cultivation of biomass on a large scale (Abdullah et al. 2019). The total amount of biofuel energy produced by the algae is just up to 57,000 gallons per acre and it is ten times more than that of other generations (Deshmukh et al. 2019). The most frequently used algae for the production of oil and their properties in detail are given in Table 9.3 (Fig. 9.3).

9.1.2.1 Cultivation of Algal Biomass

Algae can grow everywhere and it has a diverse way of cultivation due to high temperature. There are three places in which it grows mostly or more efficiently.

Open Ponds

The most simple and easiest way to grow algae is in open ponds. In this condition, availability of natural light provides the required nutrition for its growth, it does not need any other enhancement. It is less effective and fruitful than the other methods (UK Biofuel 2020a). But there is another condition that can create a problem, that is, the presence of other species which could kill algae or may damage its growth (Zheng et al. 2012).

Table 9.3 Different species of algae and their properties (Sajjadi et al. 2018; Shuba and Kifle 2018)

Class of Algae	Strain	Lipids	Proteins	Carbohydrates
Eustigmatophyceae	*Chlorella vulgaris*	41–58%	51–58%	12–17%
	Chlorella sorokiniana	22–24%	40.5%	26.8%
	Chlorella pyrenoidosa	2%	57%	26%
Chlorophyceae	*Scenedesmusobliquus*	30–50%	10–45%	20–40%
	Dunaliellatertiolecta	11–16%	20–29%	12.2–14%
	Dunaliellasalina	6–25%	57%	32%
	Scenedesmusdimorphus	16–40%	8–18%	21–52%
	Scenedesmusquadricauda	1.9%	40–47%	12%
Bacillariophyceae	*Phaeodactylumtricornutum*	18–57%	30%	8.4%
	Thalassiosirapseudonana	20%	–	–
Cyanophyceae	*Spirulinaplatensis*	4–9%	46–63%	8–14%

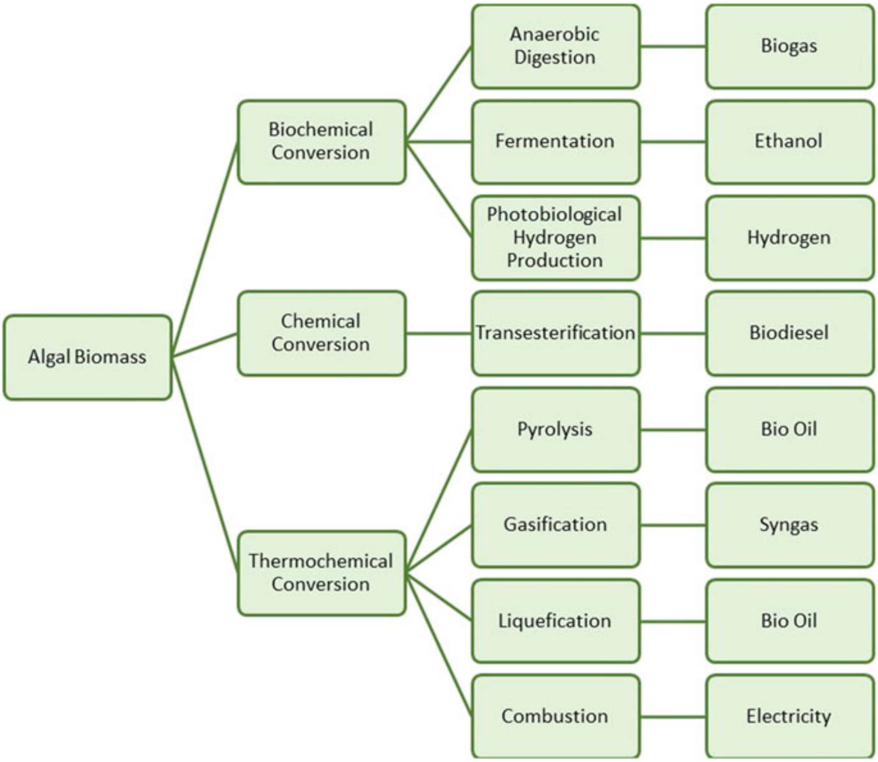

Fig. 9.3 Conversion of algal biomass into biofuel

Closed-Loops System

This system is more efficient than that of the open ponds and it grows in a sterile environment (Cohen and Arad 1989). It connects to the carbon dioxide directly and uses this gas before going to the environment (UK Biofuel 2020a). It is cost-effective as compared to photo-bioreactors but expensive than that of open ponds (Ullah et al. 2014).

Photo-Bioreactors

It is the most effective method than that of the above-mentioned methods. It is a closed and most advanced system for the growth of algal biomass. Algae can also grow in waste material and requires optimum light with the wavelength of 400–700 nm; nutrition required are carbon, hydrogen, and phosphorus, etc. (Acién et al. 2017).

9.1.2.2 Harvesting of Algal Biomass

There is a benefit of unicellular microalgae as they have a cell wall that contains lipids and fatty acids in a higher amount; this makes them very unique and different from other higher organisms. Harvesting is an important step for the recovery of oil from algal biomass. The two types of algae are micro-algae and macro-algae; in microalgae, there is a need for higher energy and it is collected by conventional methods like centrifugation, flocculation, and fractionation (Sydney et al. 2019) while the macro-algae needs less energy as it can be collected with the help of a simple net.

9.1.2.3 Conversion of Algal Biomass into Energy

There are numerous methods to extract oil from the algal biomass. These methods can be mechanical or chemical-based, but the extraction of oil from unicellular is very difficult and consumes high energy for completion.

Mechanical Extraction

Extraction of oil from algae can be done with the help of expellers and presses similar to oil extracted from the nuts and oils. First, algae should be dried and then a further extraction procedure is done to extract oil. The cell wall is broken down with the help of an expeller, then the oil comes out from it: no special training is required and a maximum of approximately 75% oil is extracted by this method. There is another method which is used for the extraction of oil, which is the osmotic shock method; this method also gives approximately 75% of oil.

9.1.3 Fourth-Generation Biofuel

This type of biofuel is obtained from genetically modified crops, and these crops can grow on any harsh medium or water bodies. These are specially designed crops have less barrier to the breakdown of the cellulosic cell wall. It is an ecofriendly generation (Shokravi et al. 2019).

9.1.3.1 Growth of Genetically Modified Algae

Contained System

In contained system, there is less exposure of algae to the environment; thus, the chances of contamination are very less and work is being done in slightly controlled conditions. Although it provides a secure environment for algae to grow, in return this system is expensive.

Uncontained System

In this system, algae are grown in a shallow artificial pond. It is not as expensive as the contained system but the rate of contamination is much higher because it is fully exposed to other species like animals, aerosols, and other kinds of sprays. For the proper experimentation of algae, there is a high demand of proper environment and management. GM helps in assimilation, bioremediation, and also in reduction or removal of CO_2 from the atmosphere (Abdullah et al. 2019) (Fig. 9.4).

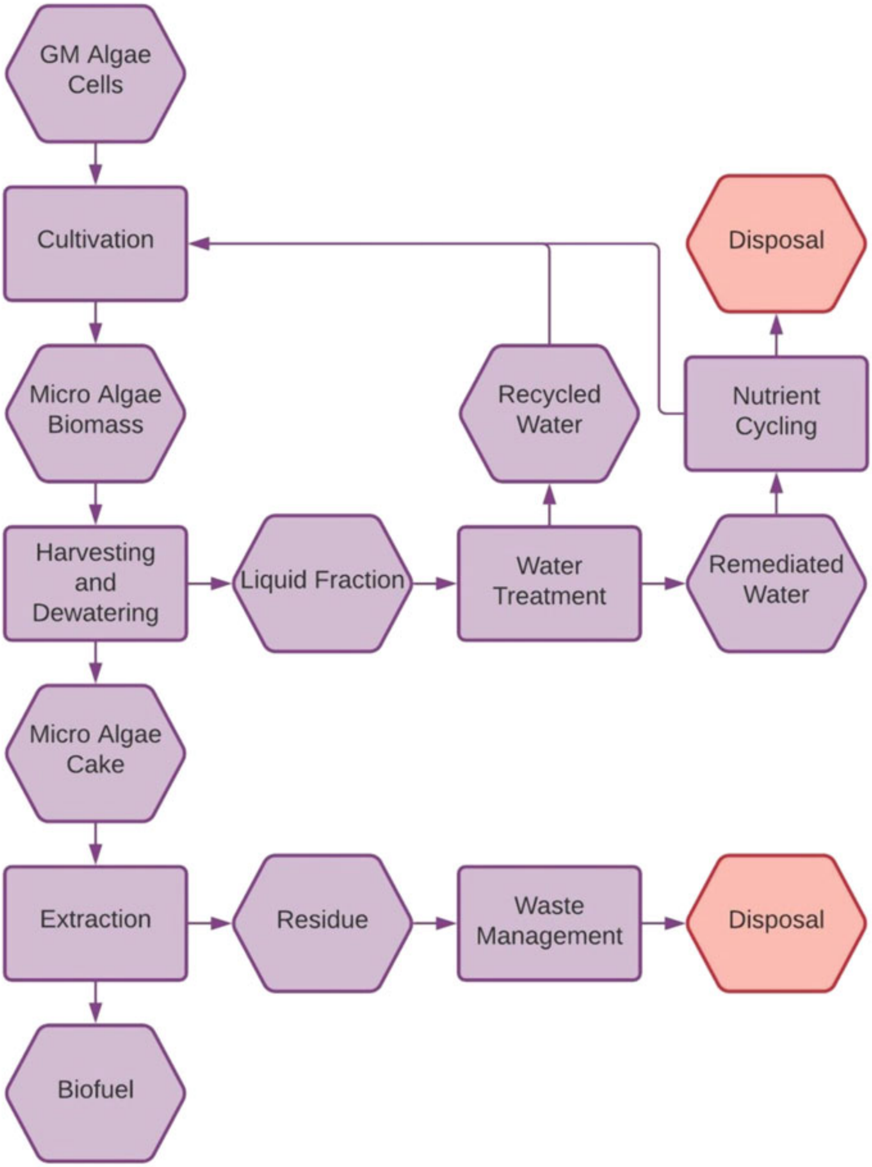

Fig. 9.4 Flow diagram of the production of fourth-generation biofuel

9.1.3.2 Strategies Used to Increase the Production Rate of Lipid

Different kinds of strategies are used to increase the rate of lipid production in algae, they are transcription, mechanical, and genetic methods. The effective production of lipids depends on the selection of types of algae which is being used in the experiment. In the biochemical strategy, during cultivation, the environmental stresses are being applied on the algae as well as other factors are also being monitored like nutrient, mineral, chemical, and physical, respectively. In genetic strategy, gene knockout, gene suppressing, and gene overexpression strategies are being used to increase the lipid production rate. With the help of these strategies, we can study the mechanism and also control it very efficiently. But the drawback of this strategy is that the complex transgenic, available genomic data is not complete and it is a little difficult to create balance between energy and metabolic pathway accurately. In transcription strategies, the study is not just limited to the single pathway while it is the study of complete cells (Shokravi et al. 2019).

9.1.3.3 Strain Selection

To select an appropriate strain of algae for biofuel production, some parameters should be in considered:

1. Growth rate
2. Digestibility
3. Chemical composition
4. It should be resistant to harsh conditions
5. High lipid content (Peng et al. 2020)

The cell wall of algae contains plastids and polysaccharides which give carbohydrates after the fractionation of the cell wall; it is attractive to the consumer, because they have the least amount of hemicellulose and absence of lignin. Another parameter also plays an important role in the selection of strain which is sugar composition and some strains like *Chlorella*, *Scenedesmus*, and *Chlamydomonas* consist of more than 50% carbohydrate which makes them a good choice for the production of bioethanol and bio-butanol.

Protein 16.7 kJ/g and carbohydrates 15.7 kJ/g are the energy-providing compounds, but their concentration is lower as compare to lipids. Protein is not used due to its deamination hydrolyzing activities. Some of the algae strains used in biofuel production and their chemical composition are given in detail in the Table 9.3 (Shokravi et al. 2019).

9.1.3.4 Methods to Produce Genetically Modified Algae

There are different techniques used to create algal biomass which are highly rich in lipid and production rate; genetically modified techniques like CRISPR/Cas-9, recombinant DNA technology, particle bombardment and markers identification, etc. The CRISPR/Cas-9 technique is used to insert gene and marker MAA7, chLM, and CpSRP43 in *Chlamydomonas reinhardtii* strain to knock-out or knock-in through NHEJ. Other strains like *P. tricornutu*, used FcpB and FcpF promoters; PCR, Southern Blotting, and western blot were used for the detection, and the Particle bombardment method is used to transfer urease gene into it. *Phaeodactylum* strain is modified by the use of particle bombardment of TALEN (Transcription Activator-like effector Nuclease) PtAureola and then PCR, Southern Blotting, and the western blotting for protein analysis. In the last, the detection of phosphorylase is done by the sequence analysis. Zinc-finger nuclease (ZFN) and mega-nuclease methods are also used for the cultivation of genetically modified algae. CRISPR is widely used due to its simplicity and efficiency in highly complex mutagens. In Cholera Vulgaris, nitrogen level is lower down, and in return, it increases 40% of lipid content. In spirulina, there is also a decrease in the level of nitrogen and temperature which increases the lipid level three times more than the normal range (Shokravi et al. 2019).

9.1.3.5 Cyanobacteria

Cyanobacteria is a gram-negative bacteria (Farrokh et al. 2019) and plays an important role in every human being's life for the availability of oxygen in the atmosphere. It has a wide range of applications like wastewater treatment, agricultural bio-fertilizers, and nutrition sources, and it can belong to genera Anabaena or genera Gloethece and Trichodesmium. Cyanobacteria can be unicellular, such as Synechococcus, Gloethece, and some are attached to surface like Oscillatoria and Lyngbya. Alcohols, fatty acids, and hydrocarbons are the chemicals synthesized by cyanobacteria (Shokravi et al. 2019) (Fig. 9.5).

9.2 Types of Biofuel

9.2.1 Bio-ethanol

Sugar cane provides a yield of ethanol in higher developing countries with the highest yield of approximately 7000 l/ha year in Brazil (Neto et al. 2019). The worldwide average rate of production is 5000 l/ha year. In the United States, the yield produced from corn is approximately 3800 l/ha year; on the other hand, the average rate of production all over the world is 2370 l/ha year. The greenhouse gas reduction from the corn is between 0% and 38% (DOE US 1999). It releases almost 15% fewer greenhouse gases in the environment than gasoline or petrol (Walker 2020) (Table 9.4).

9.2.2 Bio-diesel

Bio-diesel is also known as green diesel and they both are confusing terms because they both are purified forms of vegetable oil or extracted from animal fat. Bio-diesel has a very high boiling point and its color ranges from yellow to dark brown. Another most valuable aspect is that it has no sulfur content (biofuel.org.uk/third-generation-biofuels.html). In Europe, the high production rate of biodiesel is provided by the canola (oilseed rape), while in the United States and Brazil it is produced from soya bean. It gives higher production as compared to palm oil which is produced highly in Indonesia and Malaysia. This raw material used as food by the animals for its high protein concentration and also used in the form of soya meat and

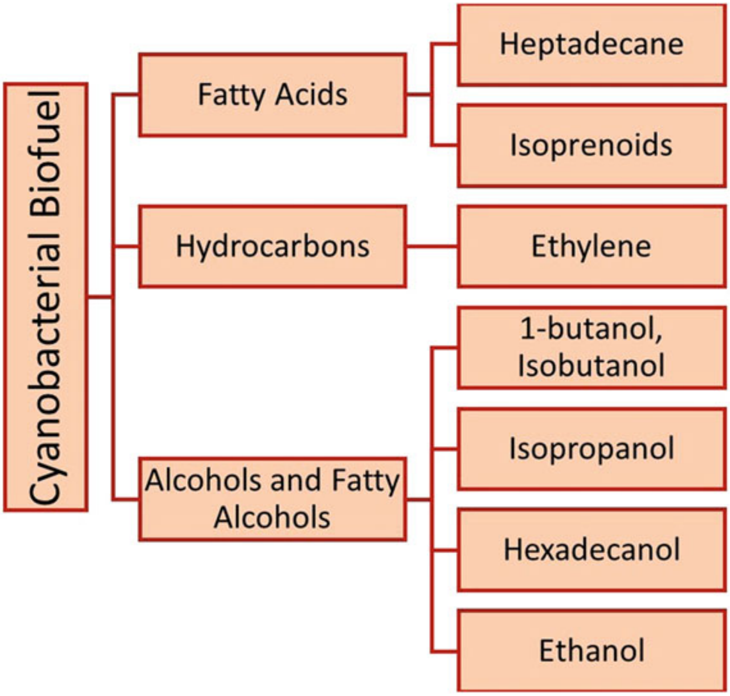

Fig. 9.5 A flow diagram of cyanobacteria for the biofuel production

Table 9.4 A detailed description of the energy balance, productivity, and GHG reduction according to life cycle assessment (LCA) (Macedo et al. 2008; Alckmin-Governor and Goldemberg-Secretary 2004)

Feed stock	Energy balance	Typical productivity (l/ha year)	GHG reduction
Wheat	2.0	2500	16–69%
Corn	1.4	3800	0–38%
Sugar beat	2.0	5500	52%
Sugar cane	9.3	7000	61–69%

soya milk. Palm oil gives a higher value of biodiesel annually approximately 4500 l/ha year. Palm is used on large scale, however, 90% of it is used in many food products as well as in cosmetics, etc. Another plant known as *Jatropha* grows on a minor scale in Tanzania and India but it is a good resource for the production of biodiesel. Castor and *Pongamiapinata* are also used as raw material for the production of biodiesel. But its production rate is not enough to meet the demand of cooking oil as well as for biodiesel, and it is much expensive as compare to others. Thus, it is not preferable. As the production of biodiesel is less than that of bioethanol but its relative demand is increasing outstandingly up to 18.1 billion L in 2010 while it is 3.9 billion L in 2005. In a lot of countries, there are thousands of petrol stations which provide 5% blended diesel. It contains a higher amount of

Table 9.5 Classification of biofuels and their yield with feedstock (Bierbaum et al. 2015; Dutta et al. 2014)

Biofuel	Technology	Feed stock/process	Yield (l/ha year)
Bioethanol	First-generation	Sugar cane/fermentation	5000–8000
		Sweet sorghum/fermentation	2500–3500
Biodiesel	First-generation	Canola	1000–1500
		Jatropha/trans-esterification	1000–1500
		Soya bean/trans-esterification	500–1000

oxygen and hydrogen while a lower amount of carbon content which makes it more ecofriendly; this prevents the unburnt carbon to release carbon into the environment (Kothari and Gujral 2013) (Table 9.5).

9.2.2.1 Biodiesel Production by Trans-esterification

The reaction of fatty acids with alcohols in the presence of catalysts can form NaOH or KOH, which is strongly acidic (Marx 2016). This procedure takes less time (Christopher et al. 2014).

Procedure

In this procedure, different kinds of alcohols like methanol, propanol, and butanol, etc., are used for the production of biodiesel. The most frequently used alcohols are ethanol and methanol as they are cost-effective and also have advantages physically and chemically. Catalysts used in this reaction are acids or alkalies, and lipase enzyme is also used to control this procedure. Three molecules of alcohol react with one molecule of triglyceride to produce biodiesel (methyl esters) and glycerol as a byproduct. To maintain equilibrium conditions in the reactor, the glycerol is removed from the reactor as it is much denser than the biodiesel. The presence of methanol can cause engine failure; therefore, the biodiesel should be washed again and again with water to secure the engine from any harm. The reaction under the acid catalysts is very slow while the reaction is carrying under the alkali catalysts is 4000 times higher than the acid catalysts; sodium hydroxide (NaOH) and potassium hydroxide (KOH) are commonly used in the market. *Scenedesmus* species is used to produce biodiesel with the help of acid or basic catalysts (Fig. 9.6).

Types of Trans-esterification

Extractive Esterification

In extractive esterification, there are several steps to convert biomass into biodiesel, such as cell disruption, biodiesel refining, and drying, etc. If there is high water content in the biomass, then it increases the cost of the whole experiment.

In Situ Esterification

This method does not include the step of oil extraction. Cell membrane porosity level increases due to the presence of alcohol which acts as a reagent and solvent

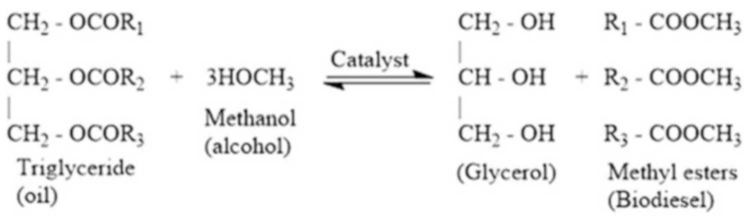

Fig. 9.6 The conversion of triglycerides into biodiesel; R represents the functional group (Behera et al. 2015)

Table 9.6 Comparison between extractive and in-situ esterification (Haas et al. 2006)

Extractive esterification	In-situ esterification
The heating value is low	The heating value is high
Yield is low	Yield is high
Complex and time consuming procedure	Simple and quick procedure
Harmful byproducts	Less harmful byproducts
Cost is very high	Cost-effective
Higher loss of lipid	Less loss of lipid

extraction simultaneously. This produces a higher yield than the conventional route, and it also enhances global warming and smog by releasing solvent extract at the industrial level (Behera et al. 2015) (Table 9.6).

Biodiesel Gelling and Temperature

The determinant of the temperature is the polarity at which a molecule freezes. Biodiesel is more polar than standard diesel due to its high oxygen content. Fuel does not freeze solid and develops small crystals. This is known as gelling and it gels at a temperature of 16 °C. Normal diesel gels at a temperature of −15 to −19 °C.

Effects on Engine

Biodiesel contains alcohols and other solvents that affect normal engine rubbers and makes them dry which leads to cracking; therefore, it is not recommended for standard engines. To use this type of diesel, a specific kind of engine is needed.

9.2.3 Bio-gas

It is known as synthetic natural gas or SNG (Lackner 2017). It is formed by the breakdown of organic compounds into simple compounds in the absence of oxygen and it is called bio-gas because it consists of several chemicals. The organic matter can be waste material of animals and plants. The major component of biogas chemical is methane and others in less composition are carbon dioxide (CO_2), oxygen (O_2), hydrogen sulfide (HS), and nitrogen (N), etc. (Pawlita-Posmyk et al.

2018). These chemicals present in minor amounts could be eliminated by many processes like refining, processing, and in other controlled conditions.

9.2.3.1 Environmental Impacts of Biogas

As it is clear that biogas is produced by the animal wastes, and these wastes mainly consist of nitrogenous oxide (NO) (Chiu and Lo 2016), which is further converted into nitrogen dioxide (NO_2) and it is very effective in trapping heat from the environment as compared to carbon dioxide. It traps up to 310 times more heat than carbon dioxide and also it reduces greenhouse gas emissions by controlling the heating process. The main component of biogas methane is also very efficient in trapping carbon dioxide.

Germany is investing a lot of its money in producing biogas which meets up to 2.3 GW of electricity and it is doing to promote green technology in the world. China, India, and Brazil, etc., are the major biogas producing countries, while Brazil ranks second (UK Biofuel 2020b).

9.2.3.2 Production of Biogas by Algae

Mass production of biogas from algae is gaining popularity worldwide, and it is advantageous due to its less concentration of cellulose, lignin, and higher amount of polysaccharides like carrageenan, mannitol, agar, and alginate, etc., in the cell wall. This is done under anaerobic conditions by the bacteria micro-organisms. The most famous algae strains used by the producers are *Euglena*, *Spirulina*, and *Scendesmus*, etc., Scenedesmus obliquus converts solid biomass into biogas and gives 287 mL/g biogas. Processes such as microwave, thermal, or sound-waves are used to get higher production of biogas.

The process consists of four steps, and in the first step, Clostridia and streptococci release some enzymes to convert inorganic soluble compounds like carbohydrates, lipids, and protein to a soluble form by the hydrolyzed process (Prasad et al. 2014). In the second step, during the conversion of organic compounds into carbon dioxide and ammonia (Guo et al. 2015), the acidogenic bacteria secretes enzymes which release volatile fatty acids (VFAs) as well as alcohol (Prasad et al. 2014), and this process is called acido-genesis. After the third step, the acidogenic compounds convert into acetic acids and hydrogen with the help of aceto-genic bacteria. This conversion process is known as aceto-genesis. In the fourth step, these acetic compounds are finally converted into methane and CO_2 under the controlled conditions of methanogens, and this process is known as methano-genesis (Guo et al. 2015).

It's a drawback for the biogas production that anaerobic organisms could not work well in the presence of sodium ions; so it is suggested by the workers to use salt-bearing micro-organisms.

9.3 Adaptation and Reduction in Climate Change

In 2007, the intergovernmental panel on climate change (IPCC) proposed that the only way to control climate change is to use a combination of reduction and adaptation steps. While in 2012 at Doha, it was decided to modify the Koyoto protocol; after this, the member states entered into an agreement to achieve the target of overcoming the risk of climate change in the period 2013–2020. It was decided to reduce the level of risk up to a maximum of 15% or at least 5%. This goal could be achieved by the industrialized nations if they change their energy source from standard fuel to biofuel. It is estimated by the European Union they could reduce the release of poisonous gases into the environment up to 50% by 2050 and also achieve the target of producing biofuel up to 10% in 2020. India is one of the most populous countries, consuming an energy of 3.8% on large scale. And it produces energy from coal, oil and with less than 1% of the renewable source. India has modified its strategies and policies to play part in the reduction of greenhouse gases from the atmosphere; it also works as an active member of the clean development mechanism (CDM).

9.4 Role of Biomass in Climate Change

Adequately used biomass cannot change the environment and also does not add harmful gases like carbon dioxide into the atmosphere. With its help, the government can also achieve its long-term goals and also use it to maintain a balance between the production and supply of energy. As discussed earlier, IPPC prepared a document to ensure that reduction and adaptation can maintain climate change. It is estimated that in the twenty-second century (2100) the demand for biofuel energy would increase between the ranges of 25–46%. It is also stated that the release of CO is also reduced in 2025 from 6.2 to 5.9 Gt C while in the twenty-second century it would be at 1.8 Gt C. From the study, it is calculated that there is a land of 700 million ha from which we could only use 125 million to achieve our desire demand for energy. Forests residues, municipal and other waste material could be used to solve this problem, and it plays a very important role in the reduction of this alarming situation; it also serves as an alternative approach against fossil fuels. It helps to reduce carbon dioxide in the following ways:

1. Carbon dioxide present in fossil fuel is remained closed in it
2. Carbon dioxide released by different ways is used by the biomass to help in the growth
3. Prevent the release of carbon dioxide with fossil fuels

9.5 Bioethanol and Biodiesel for Climate Change Reduction

Biofuels like biodiesel are used in the transportation industry to save the environment from harmful gases and also make it cost-effective for rural areas. Various policies were developed for production of biofuels in developed countries like the United States, China, Europe, and Brazil, etc., the first prepared biofuel is bio-ethanol, and a lot of efforts were made to promote it. Two main countries Brazil and the United States both work together and produced bioethanol on large scale; they produce it annually at 36 billion L and supplied it globally. On the other hand, Germany produces 3.5 billion L of biofuel annually. Germany covers up to 50% of the production while the United States and Brazil cover 90% production of biofuel. It is studied that the use of land directly or indirectly affects climate change through the use of biofuels. It is estimated by the workers that corn ethanol gives less benefit and required higher energy for the conversion of corn into ethanol fuel. The reduction level done by corn ethanol is 13% as the reduction was done by standard gasoline. The second-generation release less harmful gases like carbon into the environment as compared to the first generation. The lignocellulosic biomass used for the production of oil releases very less amount of carbon and therefore it is called body CONCAWE in the oil industry. It saves climate change by up to 90% while the first generation saves only 20–70%. From the reports, it is estimated that release done by the fertilizers is much higher than the release done by the whole agricultural land so, in the end, it majorly affects the greenhouse gases of palm oil (Prasad et al. 2014).

9.6 The Life Cycle of Carbon

Carbon is part of everything in the environment, it moves from one part of the environment to another like soils, rocks, animals, oceans, and plants. This movement of the carbon cycle between them maintains the balance of nature by releasing or absorbing carbon content. Biodiesel and bioethanol are used as biofuel for transportation purposes and it maintains the carbon dioxide in nature. The carbon dioxide released by vehicles is absorbed by the plants or used by animals which is needed for their growth, in this way, the reduction of greenhouse gases is maintained as the cyclic process continues. So, it is the best way to maintain the natural cycle in its optimum conditions (DOE US 1999).

9.7 Carbon Isolation Methods

The most efficient way to reduce CO_2 in the environment is sequestration or isolation. Carbon isolation deals with capturing and storage of CO_2 from nature; if not then all the released carbon would remain in the environment and increase the greenhouse gas effect day by day. The carbon pool range of the atmosphere is

760 G tons bigger than the soil pool and this pool is 3.3-fold greater. It consists of organic and inorganic soil carbon of 1550 G tons and 950 G tons, respectively.

9.8 Isolation of Carbon in Terrestrial Biomass

The terrestrial plants or biomass absorb carbon dioxide from the atmosphere and converts it into useful carbon by the process of photosynthesis. It is noticed that plants present in Northern Hemisphere isolate carbon up to 0.7 G tons yearly. The carbon biomass is present in four forms:

1. Above-ground biomass
   It consists of branches, foliage and tree stems, etc. The total biomass consumes above ground is 70–90% (Kumar and Mutanga 2017).
2. Under-ground biomass
   It consists of the roots of the plants.
3. Carbon present in the soil
   It consists of micro-size biotic organisms in the soil. It has two to three times more carbon than above-ground soil (Kumar and Mutanga 2017).
4. Dead organic material
   It consists of rotten leaves and wasted woody material.

United Nations took an initiative of afforestation by planting or seeding those areas which are being left uncultivated for the past 50 years. In this way, the carbon sinks in the atmosphere are increased in size by afforestation. And it can be used further for the production of energy and transportation sector (Prasad et al. 2014).

9.9 Conclusion

Biofuels are a good alternative source of energy and oil for transportation. The usage of bio-fuel can make our life easy and the release of carbon dioxide into the environment could be reduced. Algae is a most suitable source for biomass collection on large scale as it is said in the above discussion that it can grow everywhere. This is the best approach to adapt for us to live in a clean environment as we lived in ancient days of life.

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Ayesha Kanwal has research interests to improve the techniques or tools used in genetic engineering and microbiology. She had particular interest to save animals and humans from infectious diseases by using genetic engineering.

Ambreen Ashar is working as Assistant Professor at Government College Women University Faisalabad Pakistan. Her research interests include the use of nanotechnology in water treatment, photocatalysis, Functional fabrics and biomedical application of nanomaterials. She has a special interest in environmental protection.

Zeeshan Ahmad Bhutta has research interests in the field of One Health to improve and save the health of humans and animals by protecting their ecosystem and finding alternative ways to combat the problem of antimicrobial resistance using nanobiotechnology.

Moazam Ali has expertise in molecular biology and diagnostics. His research interests include the use of One Health approach to control the infectious diseases using modern genetic techniques.

Muhammad Shoaib is a veterinary microbiologist having expertise in bacteriology. He is currently working to overcome the problem of multi drug resistant bacteria using alternative ways such as nanotechnology and natural medicinal products.

Muhammad Fakhar-e-Alam Kulyar has broad and acute interest about latest diagnostic tools, nutritional management, quarantine management, and disease control systems. He has abilities in gene expression studies especially related to clinical diseases in veterinary sector.

Wangyuan Yao has expertise in molecular biology, clinical research, and diagnostics. His research interests are in the development and validation of advanced technologies for cell and tissue interfacing especially chondrocytes cells in related to bone diseases.

Third-Generation Hybrid Technology for Algal Biomass Production, Wastewater Treatment, and Greenhouse Gas Mitigation

10

Ashwani Kumar, Pavithra Acharya, and Vibha Jaiman

Abstract

Greenhouse gas accumulation and climate change impact reduction requires widespread utilization of green technology. However, escalating demand for crops as a food source coupled with the finite availability of arable land makes cultivation of biofuel crops unsustainable. Algal biomass can be grown using non-arable areas such as lakes, oceans, or deserts, thus avoiding the current problem of land use competition with the food supply chain. Third-generation biofuels mainly consist of algal biofuels. However recently, hybrid use of algae for production of biofuels and also treating the wastewater for greenhouse gas reduction is gaining ground. Algae have the potential to produce valuable substances for the food, feed, cosmetic, pharmaceutical, and waste treatment industries. Microalgae mass cultures using solar energy and concentrated CO_2 sources can be used to produce renewable fuels such as methane, ethanol, biodiesel, oils and hydrogen and for other fossil fuel sparing products and processes. Recently developed hybrid technologies include biomass production, wastewater treatment, and GHG mitigation for production of prime products as biofuels. This also helps in atmospheric pollution control such as the reduction of GHG (CO_2 fixation) and bioremediation of wastewater microalgae growth. However, the selection of efficient strain, cultivation systems, microbial metabolism, and biomass production are important steps of viable technology for microalgae-based biodiesel production and phytoremediation. This chapter will

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discuss the latest developments in area of selection, production, and accumulation of target bioenergy carrier's strains, as well as the third-generation biofuels and hybrid technology for development of oil, biodiesel, ethanol, methanol, biogas production, and GHG mitigation.

Keywords

Biodiesel · Hybrid technology · biogas production · GHG mitigation · Ethanol · Microalgae

10.1 Introduction

Anthropogenic carbon dioxide emissions largely from fossil fuels in energy and transportation sectors have raised the concentration of CO_2 from pre-industrial levels of 280 ppm to about 415 ppm today, and they will continue to raise to about 570 ppm by the twenty-second century (Aaron and Tsouris 2005). This will have disastrous consequences on human health, climate, and agriculture (Kumar 2013, 2018; Kumar et al. 2018, 2020). In general, the use of photosynthetic organisms as a feedstock mitigates ever-increasing anthropogenic CO_2 emissions. High photosynthetic carbon sequestration efficiencies and carbon capture percentages of 90% make algae very well suited as a source for biofuels and bioproducts (Demirel 2018b). Algae are capable of eliminating 513 tons of CO_2 and producing up to 100 tons of dry biomass per hectare per year (Bilanovic et al. 2009).

The first generation of biofuels from food crops or their derivatives require farmland or wasteland but irrigation and fertilizer use improved their growth and productivity. Thus, the debate is focussed on food vs. fuel. It also posed a severe threat to food security triggering food versus fuel dilemma (Kumar 2018; Kumar et al. 2018, 2020; Nazneen and Kumar 2014).

The second-generation biofuels derived from lignocellulosic biomass were largely extracted from agricultural waste, lignocellulosic materials, food waste, etc. Although they addressed the above-mentioned problems, their processing technology and biofuel production has not been perfected for large-scale utilization (Anindita and Kumar 2013; Kumar and Gupta 2018).

10.2 Third-Generation Biofuels

Photosynthetic organisms include filamentous and unicellular macro- and microalgae, and cyanobacterial species. Microalgae have the potential to produce valuable substances for the food, feed, cosmetic, pharmaceutical, and waste treatment industries. Commercial culture of microalgae has >40 years history with some of the main species grown being *Spirulina* for health food, *Dunaliella salina* and *Haematococcus pluvialis* for carotenoid production, and several species for aquaculture (Lee 1997; Borowitzka 1999; Carvalho et al. 2006; see review Behera et al.

2019). Biorefinery approach with integrated biology, ecology, and engineering could lead to a feasible algal-based technology for a variety of biofuels and bioproducts (Allen et al. 2018).

The environmental consequence of global warming has led to a paradigm shift towards renewable fuels (Maity et al. 2014). Algal biofuel is an example of third-generation biomass which has the potential to replace fossil fuels and animal feeds (John et al. 2011; Sharma and Singh 2017; Gajraj et al. 2018; Kumar et al. 2018, 2020). Macro and microalgae transform solar energy into the carbon storage products, leading to lipid accumulation, including those that can be transformed into biodiesel, bioethanol, and biomethanol (Kraan 2013; Maity et al. 2014). Maity et al. (2014) reported that microalgae provide mainly TAG (triacylglycerols), a potential source of biofuel at large scale.

Although wastewater treatment and CO_2 removal by microalgae have been studied separately for a long time, there is no detailed information available on combining both processes (Craggs et al. 2011; Li et al. 2017; Kumar 2018; Kumar et al. 2019, 2020).

10.3 Wastewater Treatment

Discharge of untreated/partially treated brewery wastewater leads to environmental problems, such as water scarcity, excessive growth of undesirable microbes that cause loss of aquatic lifeforms (Okolo et al. 2018), and health-related problems in communities around the discharge areas (Norman 1997). Wastewater is a complex mixture of natural organic and inorganic materials, as well as man-made compounds. Different sources of pollutants include "Discharge of either raw or treated sewage from towns and villages; dis-charge from manufacturing or industrial plants; run-off from agricultural land; and leachates from solid waste disposal sites" these sites of pollution have problems so that a solution is sought (Gray 1989; Horan 1990; Showkat and Najar 2019). Three quarters of organic carbon in sewage are present as carbohydrates, fats, proteins, amino acids, and volatile acids. The inorganic constituents include large concentrations of sodium, calcium, potassium, magnesium, chlorine, sulphur, phosphate, ammonium salts bicarbonate, and heavy metals (Talbot et al. 1990; Horan 1990; Lim et al. 2010). Scarcity of water, the need for energy and food are forcing us to explore the feasibility of wastewater recycling and resource recovery (De la Noue and De Pauw 1988).

Microbiological composition of sewage wastewater environment is an ideal media for a wide range of microorganisms, especially bacteria, viruses, and protozoa. The majority is harmless and can be used in biological sewage treatment, but sewage also contains pathogenic microorganisms. However algae have been used in hybrid technology which produces biofuels along with removing the heavy metals and pollutants from wastewater. In conventional wastewater treatment system, the removal of biochemical oxygen demands (BOD) suspended solids, nutrients (NO_3^--N, NO_2^--N, NH_4^+-N and PO_4^{3-}-P), coliform bacteria, and toxicity are the main goal for getting purified wastewater (Abdel-Raouf et al. 2012; Lim et al. 2010; Amenorfenyo et al. 2019; Kumar et al. 2020).

10.3.1 Hybrid Technologies

Molazadeh et al. (2019) reviewed microalgae-based CO_2 biofixation, various microalgae cultivation systems, as well as concept of integration of CO_2 biofixation process and wastewater treatment. This helps in photosynthetic biomass production, wastewater treatment, and GHG mitigation. Thus, hybrid technology offers atmospheric pollution control such as the reduction of GHG (CO_2 fixation) coupling wastewater treatment with microalgae growth (Weissman and Goebel 1988; Abdel-Raouf et al. 2012; Maity et al. 2014) (Figs. 10.1 and 10.2). Sharma and Singh (2017) made systematic analyses of energy demand and GHG emission statistics of various nations, as well as all the steps involved in overall process from algal strain selection to biodiesel production. Microalgae including eukaryotic algae and cyanobacteria, as well as macro-algae, have demonstrated to be an environmental-friendly and sustainable alternative to energy-intensive and conventional biological treatment processes (Mohsenpour et al. 2021).

Fig. 10.1 Hybrid technology: Microalgae and wastewater treatment. (*Source*: Abdel-Raouf, N., Al-Homaidan, A. A., and Ibraheem, I. B. (2012). Microalgae and wastewater treatment. *Saudi J. Biol. Sci*. 19, 257–275. doi: https://doi.org/10.1016/j.sjbs.2012.04.005. Open access: Reprinted with Licence no. 4880801372566 dated 2nd Aug 2020 RightsLink)

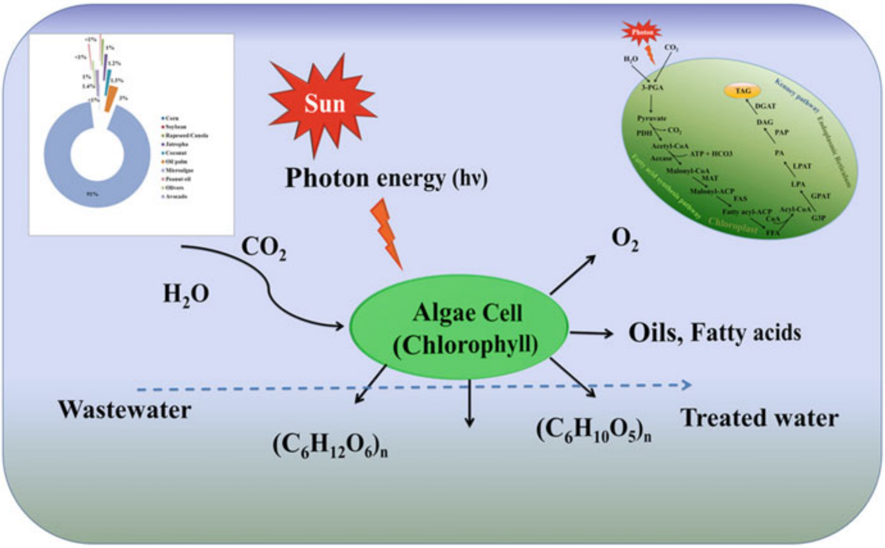

Fig. 10.2 Microalgae for third-generation biofuel production, mitigation of greenhouse gas emissions, and wastewater treatment. (*Source*: Maity, J. P., Bundschuh, J., Chen, C.-Y., & Bhattacharya, P. (2014). Microalgae for third-generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. *Energy*, *78*, 104–113. https://doi.org/10.1016/j.energy.2014.04.003. Reproduced with licence no 5064180534847 dated 8th May 2021)

Currently, algae wastewater treatment and its biomass use are attracting attention worldwide (Simate et al. 2011). Liu et al. (2013) highlighted the use of microalgae to address the problems of wastewater treatment. The current progress of hybrid technologies (biomass production, wastewater treatment, GHG mitigation) for production of prime products as biofuels offer atmospheric pollution control, such as the reduction of GHG (CO_2 fixation) coupling wastewater treatment with microalgae growth (Maity et al. 2014).

Wang et al. (2017) reported reuse of secondary municipal effluent from wastewater treatment plants to alleviate freshwater resource shortage. Wastewater treatment (WWT) for biofuel production provides additional incentives (Milano et al. 2016). This integrated approach (as illustrated in Fig. 10.5) is postulated to provide resource recovery-based monetary benefits, as well as 800–1400 GJ/ha/year energy which can be a source of energy at the community level (Mehrabadi et al. 2015). Furthermore, algae-based strategies for the removal of toxic minerals, such as As, Br, Cd, Hg, Pb, Sc, and Sn ions, have also been reported individually or in combination (Abdel-Raouf et al. 2012; Amenorfenyo et al. 2019). Olajire (2020) reviewed some of these challenges with a focus on key issues: water consumption and waste generation, energy efficiency, emission management, environmental impact of brewing process, and best environmental management for breweries. The potential for remediation of inorganic nitrogen and phosphorus from wastewater by microalgae is well documented (Shi et al. 2007; Abhinandan et al. 2015; Whitton et al. 2015). Abhinandan et al. (2015) studied cultivation of microalgae species namely *Chlorella pyrenoidosa* and *Scenedesmus abundans* in rice mill effluent (i.e., paddy soaked water) for nutrient removal.

10.3.2 Steps of Wastewater Treatment

10.3.2.1 Cultivation

Cultivating microalgae under photosynthetic conditions on wastewater with low cost resources and easy separation of the biomass from the treated wastewater and high value products can be obtained in an eco-friendly manner. It can also be used to decontaminate pesticides and heavy metals. Singh et al. (2016) presented a hypothetical model of cyanobacteria in sustainable agriculture and environmental management (Fig. 10.3). Cyanobacteria could play a potential role in the enhancement of agriculture productivity and mitigation of GHG emissions (Singh 2011). Cyanobacteria are also useful for wastewater treatment and are an effective bio-fertilizer source and have the ability to degrade the various toxic compounds, even the pesticides (Cohen 2006). They also help in ecological restoration of degraded lands (Singh 2014, 2015a, b) (Fig. 10.4). Wastewater treatment, however, can also be organized or categorized by the nature of the treatment process operation being used, for example, physical, chemical, or biological. Li et al. (2020) reported that coupling algal growth on wastewater with hydrothermal liquefaction (HTL) is regarded as an environment-enhancing pathway for biomass amplification, wastewater management, sustainable energy generation, and value-added products generation. They proposed a paradigm shift involving enhanced algal wastewater treatment and bioenergy production for field application (Fig. 10.3).

Cyano bioremediation using cyanobacteria is a green clean tool for decontamination of synthetic pesticides from agro- and aquatic ecosystems (Kumar and Singh 2017) (Figs. 10.4 and 10.5).

Microalgal production inside industrial premises using effluent rich flue gas and wastewater would produce biomass for meeting the ever increasing energy demands, with the added benefits of wastewater treatment (WWT) and emission control (Milano et al. 2016). The algal biomass can be converted into various types of renewable biofuels including bioethanol, biodiesel, biogas, photo biologically produced biohydrogen, and further processing for bio-oil and syngas production through liquefaction and gasification, respectively (Kraan 2013). This integrated approach of microalgal wastewater treatment with resource recovery for maximizing the derivable products is illustrated in Fig. 10.6 (Behera et al. 2019).

10.3.2.2 Physical Treatment

Physical wastewater treatment, a pretreatment stage, has been used generally to reduce suspended solids, as well as grease and oil from wastewater through sedimentation by gravitational force (Jayanti and Narayanan 2004).

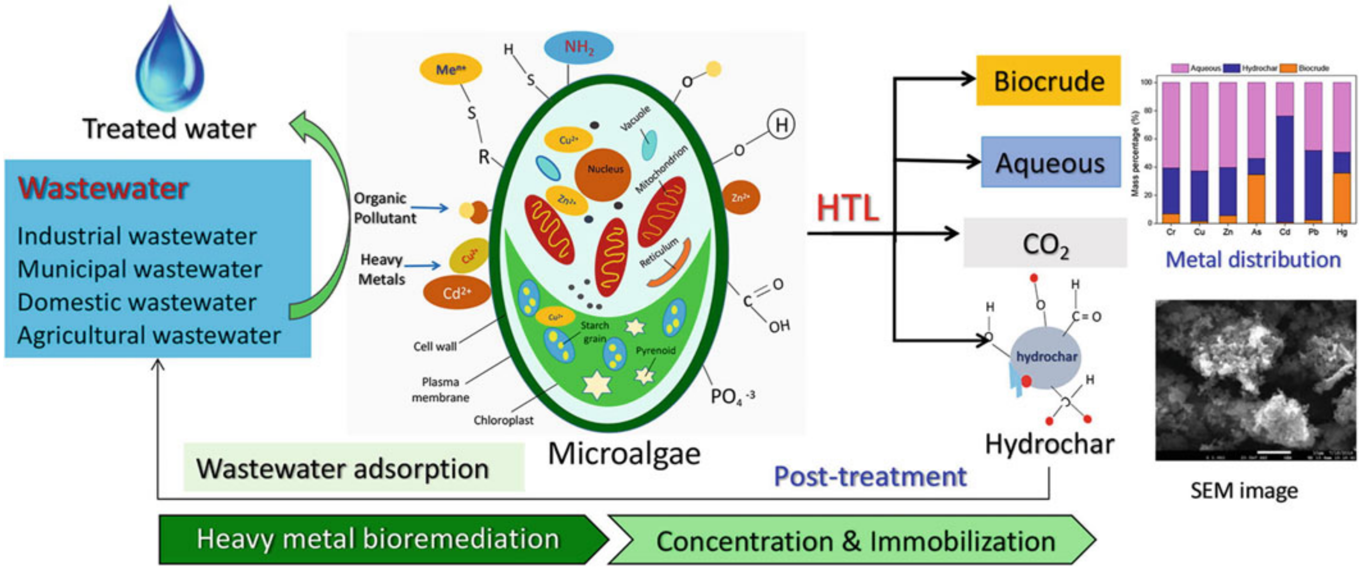

Fig. 10.3 Algal growth on wastewater with hydrothermal liquefaction (HTL). (*Source*: Li H, Watson J, Zhang Y, Lu H, Liu Z. Environment-enhancing process for algal wastewater treatment, heavy metal control, and hydrothermal biofuel production: A critical review. Bioresour Technol. 2020 Feb; 298: 122421. doi: https://doi.org/10.1016/j.biortech.2019.122421. Epub 2019 Nov 14. PMID: 31767428. Licence no 5065350239998 dated 10th May)

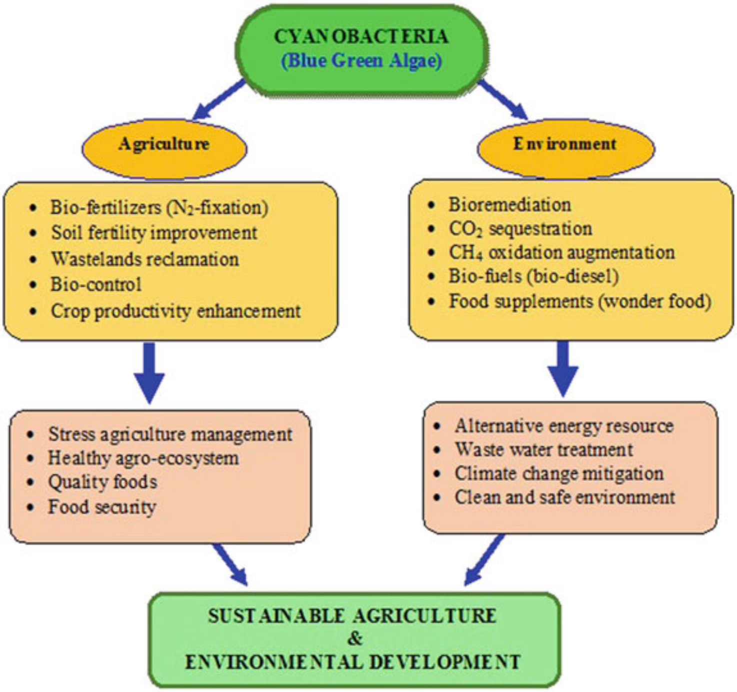

Fig. 10.4 A hypothetical model exhibiting the potential roles of cyanobacteria in sustainable agriculture and environmental management. (Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A Precious Bio-resource in Agriculture, Ecosystem, and Environmental Sustainability. *Frontiers in microbiology*, *7*, 529. https://doi.org/10.3389/fmicb.2016.00529. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY))

10.3.2.3 Chemical Treatment

Chemical treatment processes involve pH adjustment or coagulation/flocculation by adding different chemicals to the effluent to alter its chemistry (Simate et al. 2011). Flocculation involves stirring/agitation of chemically treated effluent to induce coagulation that improves sedimentation performance by increasing particle size, thereby increasing settling efficiency (Simate et al. 2011).

10.3.2.4 Use of Consortia of Bacteria Cyanobacteria/Microalgae

From an environmental friendly perspective, bacteria, cyanobacteria, and microalgae, and the consortia have been largely considered for biological treatment of wastewaters (Perera et al. 2019). Perera et al. (2019) highlighted the use of specific molecular techniques of proteomics, genomics, transcriptomics, metabolomics, and genetic engineering to develop more stable consortia of bacteria and cyanobacteria/microalgae with their improved biotechnological capabilities in wastewater treatment.

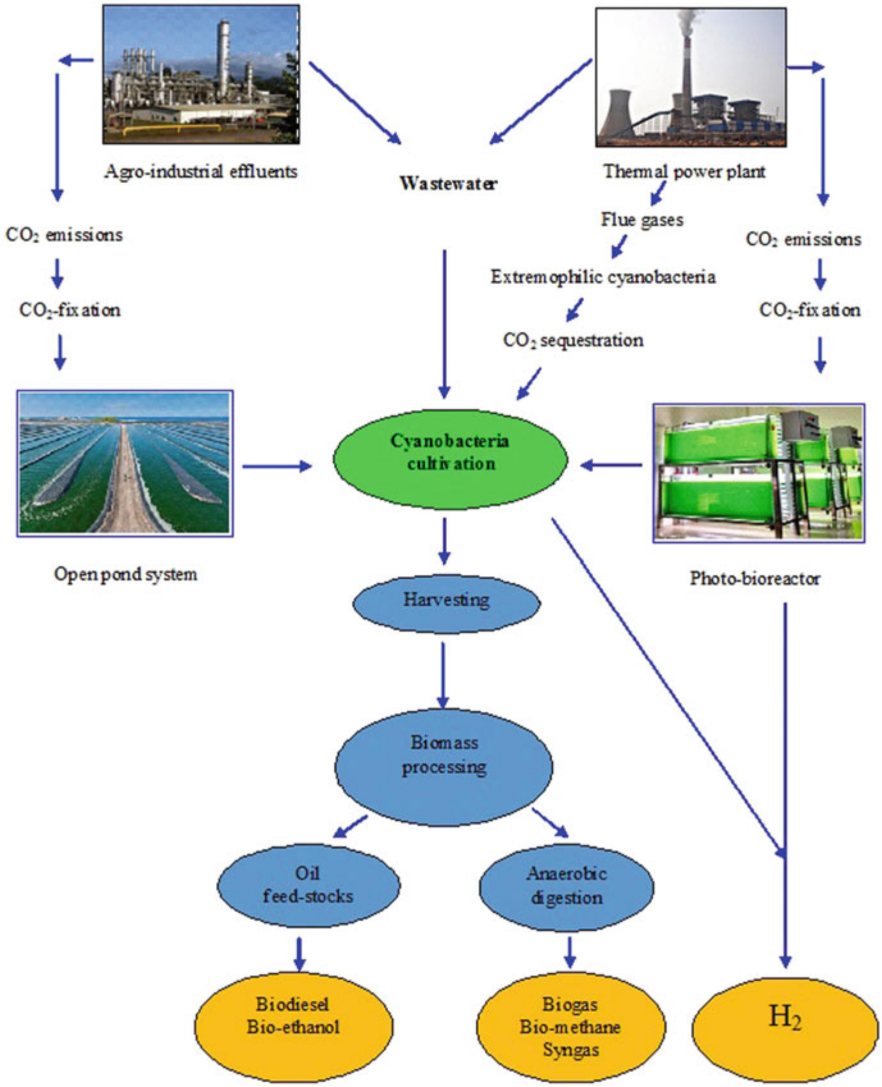

Fig. 10.5 A model showing the technologies used for production of biofuels from the mass cultivation of cyanobacteria. (Source: Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A Precious Bio-resource in Agriculture, Ecosystem, and Environmental Sustainability. *Frontiers in Microbiology*, *7*, 529. https://doi.org/10.3389/fmicb.2016.00529. Reproduced under CC-BY license which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and the source are credited)

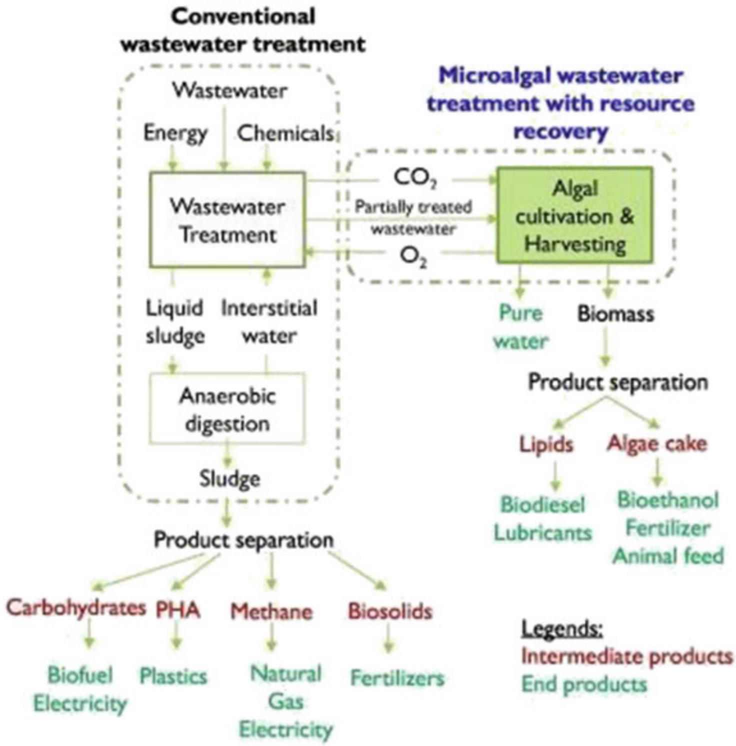

Fig. 10.6 Integration of microalgal wastewater treatment with resource recovery for maximizing the derivable products. (*Source*: Behera, B., Acharya, A., Gargey, I. A., Aly, N., & P, B. (2019). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. *Bioresource Technology Reports*, *5*, 297–316. https://doi.org/10.1016/j.biteb.2018.08.001. Reproduced under Licence number 4906420744566 dated 12th September)

10.3.2.5 Overview of Microalgal Nutrient Remediation Mechanisms

Direct remediation is the most commonly discussed mechanism of remediation and is achieved through interconnected biochemical pathways for the uptake of the target nutrients into the biomass for storage (Seco and Ferrer 2015) or assimilation into nucleic acids and proteins for biomass growth (Cai et al. 2013).

Whitton et al. (2015) described that nutrient remediation with microalgae occurs through one of two pathways. A nitrogen source is required for the synthesis of proteins (Powell et al. 2008) or assimilation into nucleic acids and proteins for biomass growth (Cai et al. 2013; see also Whitton et al. 2015). The microalgae can be utilized for total nitrogen (TN) removal (nitrification and denitrification), with NO_3^- assimilation observed following the near-complete exhaustion of NH_4^+ (Eixler et al. 2006) from the source wastewater. Phosphate, in the preferred form of $(H_2PO_4)^2$ and $(HPO_4)^2$, is transported across the cell membrane via energized transport and assimilated into nucleotides following phosphorylation for the synthesis of ribosomal RNA (Beuckels et al. 2015; see Whitton et al. 2015).

10.4 Algal CO_2 Fixation

According to Benemann and Pedroni (2003), approximately 1.8 tons of CO_2 can be fixed through photosynthesis by producing 1 ton algal biomass in high rate algal pond (HRAP)-based wastewater treatment (see Craggs et al. 2014). Raeesossadati and Ahmadzadeh (2015) reported CO_2 fixation rates of several microalgae and cyanobacteria species under different CO_2 concentrations and culture conditions for biomass production (see also Moheimani et al. 2015). According to Allen et al. (2018), some of the best biofuel microalgae strains are *Nannochloropsis* spp., *Chlorella vulgaris*, *Chlorella minutissima*, *Chlorella protothecoides*, *Botryo-coccus braunii*, *Spirulina platensis*, *Chlorella emersonii*, *Spirulina maxima*, *Phaeodactylum tricornutum*, *Scenedesmus obliquus*, *Chlorococcum* spp., *Dunaliella tertiolecta*, *Crypthecodinium cohnii*, *Dunaliella salina*, *Schizochytrium* spp., *Chlamydomonas reinhardtii*, and *Microcystis aeruginosa* in terms of high growth rate and lipid contents. These algae usually require light, nutrients, and carbon dioxide, to produce high levels of polysaccharides such as starch and cellulose. These polysaccharides can be extracted to fermentable sugars through hydrolysis and further fermentation to bioethanol and separated through distillation (Allen et al. 2018; Rodionova et al. 2017).

10.5 Algal Ccultivation for Biomass Production

High carbon capture percentages of 90% paired with the ability to perform high photosynthetic carbon sequestration efficiencies and harvesting and using the totality of the biomass make algae a suitable source for biofuels and bioproducts (Demirel 2018b).

Microalgae mass cultures can use solar energy for the biofixation of power plant flue gas and other concentrated CO_2 sources into biomass Gajraj et al. (2018). Owing to the presence of low lignin and hemicellulose content in algae in comparison to lignocellulosic biomass, the algal biomass has been considered more suitable for the bioethanol production (Chen et al. 2013).

Macro- and microalgae are a promising new source of biomass that may complement agricultural crops. They are found in diverse environments, some species thriving in freshwater, others in saline conditions and sea water (Benemann 1993; Carlsson et al. 2007; Schenk et al. 2008). As demonstrated here, microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Chisti 2007). There are three types of macroalgae, brown, green, and red algae approximately 40 kg wet biomass/m^2 of gulf-weed (*Sargassum muticum*), compared to 2.3 kg/m^2 and 6.6 kg/m^2 of green laver (*Ulva lactuca*) and agar weed (*Gelidium amansii*), respectively. The brown algae such as sea mustard (*Undaria pinnatifida*) and kelp (*Saccharina japonica*) are one of the promising biomass for biofuel production because cultivation productivity based on area size is the highest among three types of macroalgae (Clarens et al. 2010; Eshaq et al. 2011; Slade and Bauen 2013; Rajkumar et al. 2014; Directive 2009/28/EC). Silambarasan et al. (2021) studied use of *Chlorella* sp., *Scenedesmus* sp., and their consortium for the biorefinery approach. Moreover, deoiled algal biomass (DAB) waste used as a biofertilizer combined with inorganic fertilizer resulted in the greater improvement of Solanum lycopersicum (Silambarasan et al. 2021).

Currently, cultivating microalgae has gained large momentum among researchers due to their photosynthetic rate of CO_2 fixation and its versatile nature to grow in various wastewater systems (Abhinandan and Shanthakumar 2015). However, the efficiency of wastewater treatment differs species to species. Abhinandan and Shanthakumar (2015) reviewed the use of microalgae group *Chlorophyta* for industrial wastewater treatment, domestic wastewater treatment, and nutrient removal.

Several physicochemical factors govern algal growth such as carbon concentration, medium pH, and bubbling depth, on absorption and utilization of supplied CO_2 (Yin et al. 2019). They reported that increasing CO_2 bubbling depth and keeping higher carbon concentration and higher pH in microalgae culture can improve CO_2 absorption ratio, which will optimize the bio fixation of CO_2.

Applications of aquatic ecology to algal cultivation systems, in optimizing nutrients designing and constructing biotic communities, can help to maximize algal biomass yields (Shurin et al. 2013; Bartley et al. 2016; Smith and Mcbride 2015). Algal biofilms in natural ecosystems represent three-dimensional, multispecies, and multi-layered structures which involve consortia of heterotrophic and photoautotrophic prokaryotic and eukaryotic organisms (Berner et al. 2015; Gross et al. 2015).

Some microalgae can be grow under saline conditions in desert zones near the ocean when freshwater supply is not feasible (Mussgnug et al. 2010). According to Demirel (2018a), microalgae can fix CO_2 10–50 times more efficient than other energy plants making them a very well-suited resource for biofuels and bio products.

Kargupta et al. (2015) studied growth of *Chlorella pyrenoidosa* and *Scenedesmus abundans* in a tubular batch photo bioreactor with provision for continuous flow of 10% CO_2 enriched air through the headspace. They reported that CO_2 sequestration and growth rate were comparable at height/diameter ratio of 8 and 16.

10.6 Bioreactors

Whitton et al. (2015) summarized different types of bioreactors with sub-categories, either open to the environment or enclosed, which include suspended and non-suspended systems (see also Christenson and Sims 2011) (Fig. 10.7).

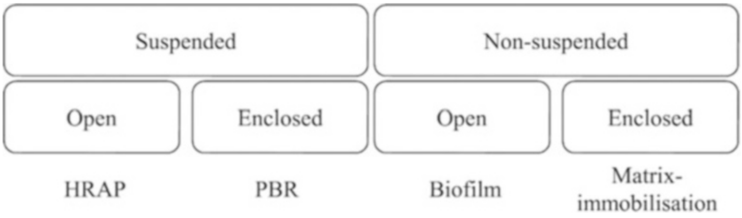

Fig. 10.7 Categories of microalgal bioreactors for wastewater remediation. (*Source*: Whitton, R Francesco Ometto, Marc Pidou, Peter Jarvis, Raffaella Villa & Bruce Jefferson (2015) Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment, Environmental Technology Reviews, 4: 133–148, DOI: https://doi.org/10.1080/21622515.2015.1105308. This is an open access article distributed under the terms of the Creative Commons CC BY license)

10.6.1 Suspended Type Bioreactors

10.6.1.1 Suspended Open High Rate Algal Pond (HRAP)

High rate algal ponds (HRAPs) are open shallow raceway ponds about 30–40 cm deep, with a paddle wheel that are used for low energy wastewater treatment (Mehrabadi et al. 2017a, b). It is a raceway-configured open pond mixed via a paddle wheel to circulate the algal culture and prevent settlement where sunlight is the primary method of irradiation, and as such, culture depths of 20–60 cm are typical (García et al. 2017). High rate algal ponds (HRAP) also provide more efficient natural disinfection. HRAP performance can be further enhanced by bubbling CO_2 into the pond during the day to promote algal growth when it is often carbon-limited. Craggs et al. (2014) presented the design and operation and performance of HRAP systems and their application for economical, low-energy upgrade of conventional wastewater treatment ponds combined with energy recovery and biofuel production. Currently, the HRAP-based wastewater treatment is the most economical and environmental approach to produce algal biomass for conversion to biofuels. Kumar et al. (2010) developed technology for efficient use of microalgal CO_2 fixation integrated with wastewater treatment (Kumar et al. 2018; Gajraj et al. 2018). Currently, by producing 1 ton algal biomass in HRAP-based wastewater treatment approximately 1.8 tons of CO_2 can be fixed through photosynthesis (Benemann and Pedroni 2003). However, there is a critical need for a cost-effective alternative upgrade option for pond systems. This can include:

1. Solids are removed and digested in covered anaerobic digester ponds (CADP) anaerobically.
2. Aerobic treatment by sunlight-powered algal growth on the supernatant;
3. Removal of algal growth and subsequent conversion to biofuel; and
4. Further polishing of the treated effluent as required.

The HRAP supports a symbiotic community of microalgae and bacteria for the assimilation of nutrients and organic matter (Park and Craggs 2014). It can be found

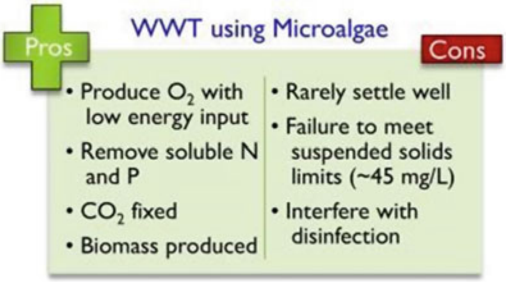

Fig. 10.8 Pros and cons of utilizing microalgae in WWT HRAPs. (*Source*: Behera, B., Acharya, A., Gargey, I. A., Aly, N., & P, B. (2019). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. *Bioresource Technology Reports*, *5*, 297–316. https://doi.org/10.1016/j.biteb.2018.08.001. Reproduced under Licencenumber 4906420744566 dated 12th September)

operational at full scale with a demonstration plant located in New Zealand with individual pond footprints of 1.25 ha (Sutherland et al. 2014). The major drawback in integrating waste water treatment (WWT) with algal cultivation among others (as shown in Fig. 10.8) (Behera et al. 2019) is the low lipid content of the biomass due to the presence of bacteria (lipid content $<10\%$) available in HRAPs that reduces the biomass energy (Mehrabadi et al. 2015).

The concept of industrial symbiosis, with synergistic effects of achieving WWT and biofuel production, is well-known (see Behera et al. 2019). The algal growth rate is dependent on the complex interaction of various environmental, operational, and biological factors. Different parameters influence microalgal growth and associated energy production in WWT HRAPs (Behera et al. 2011) (Fig. 10.9).

10.6.1.2 Suspended Closed Photobioreactor (PBR)

Bioprocess engineers have developed photobioreactors (PBRs) aiming for mass culture of microalgae. Photobioreactors are devices that are optimized to convert light energy into biomass energy and offer the highest levels of experimental control for developing optimal microalgae production systems (Chisti 2007). Photobioreactors (Hulatt et al. 2017) obtained light conversion efficiency up to 0.70 g biomass per mol of PAR for *Nannochloropsis* sp. (Davis et al. 2011; Ehimen et al. 2011).

Allen et al. (2018) reported some possible algal-based processes for biofuel and by-product productions: OP open pond, PBR photobioreactor (Fig. 10.10).

Behera et al. (2019) extensively reviewed the design considerations, mass transfer characteristics, economic and energy consideration for increasing the performance of closed PBRs.

A PBR is an example of a closed, suspended system available in various configurations, including horizontal or vertical tubular photo bioreactor (TPBR) (Fig. 10.11) (Abdel-Raouf et al. 2012; Molina et al. 2001; Hulatt and Thomas

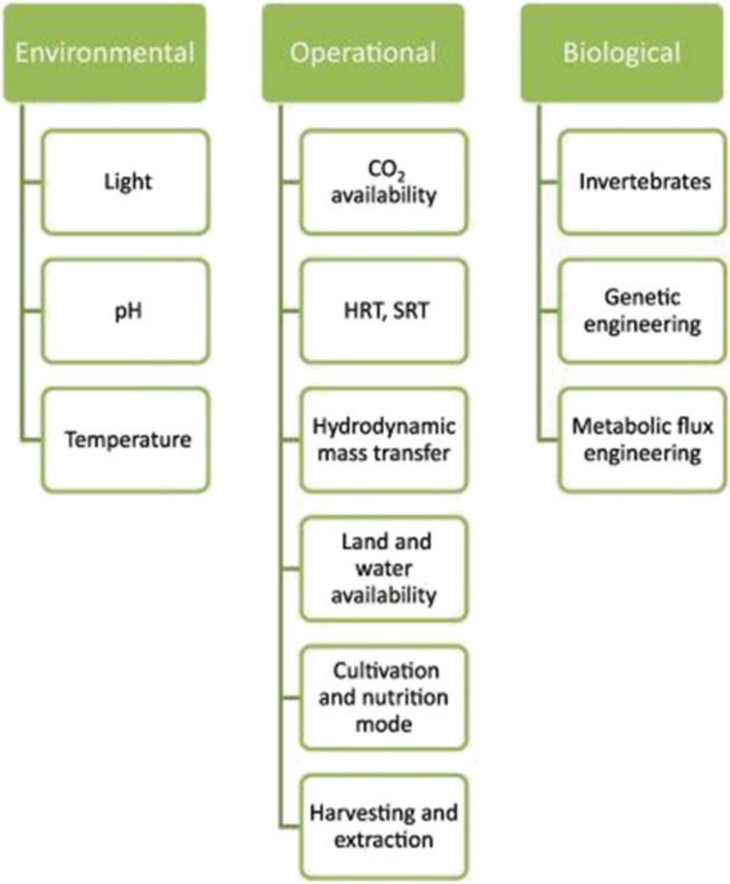

Fig. 10.9 Parameters influencing microalgal growth and associated energy production in WWT HRAPs. (Source: Behera, B., Acharya, A., Gargey, I. A., Aly, N., & P, B. (2019). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. *Bioresource Technology Reports*, *5*, 297–316. https://doi.org/10.1016/j.biteb.2018.08.001. Reproduced under licence number 4906420744566 dated 12th September)

2011; Bechet et al. 2013) or flat panel reactors (Hu et al. 1998; Ugwu et al. 2008; Sierra et al. 2009) and bio-film (Blanken et al. 2014).

Flat-plate photo bioreactors with short light path lengths as they have a high surface area to volume ratio and consume less energy than tubular systems (Zou et al. 2000; Jorquera et al. 2010; Vejrazka et al. 2012).

Some species of microalgae synthesize very long chain fatty acids (carbon chains 20+ in length), including eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) (Guiheneuf and Stengel 2013). *Nannochloropsis* is a genus of robust, oleaginous microalgae that synthesizes EPA during balanced growth and is a promising candidate for commercial applications

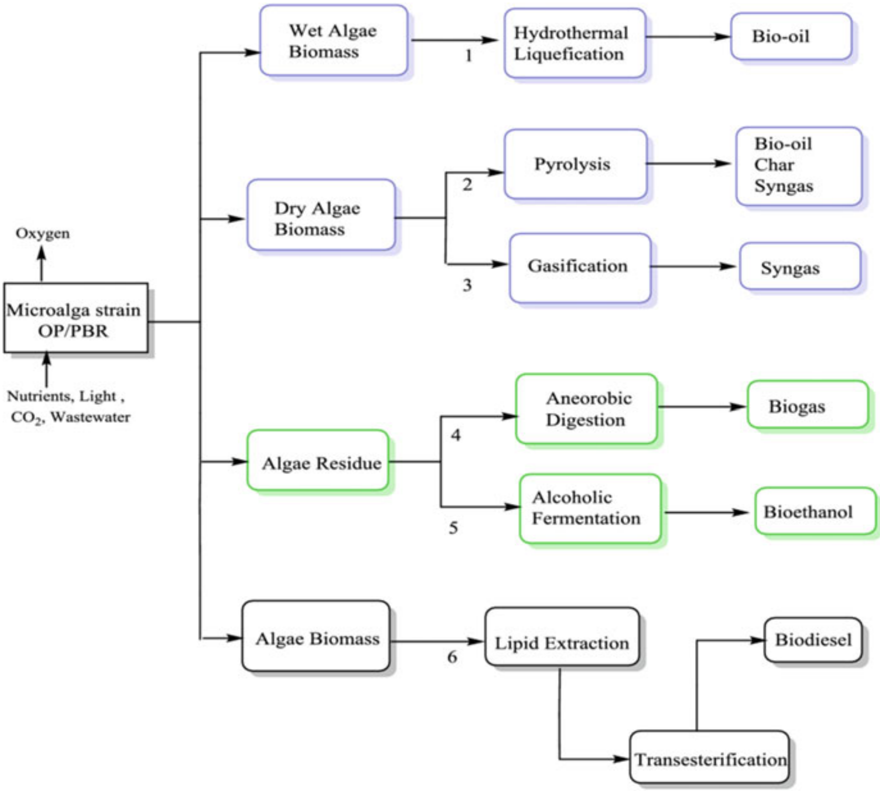

Fig. 10.10 Some possible algal-based processes for biofuel and by-product productions. *OP* open pond, *PBR* photobioreactor. (Source: Allen, J., Unlu, S., Demirel, Y. et al. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. *Bioresour. Bioprocess*. **5**, 47 (2018). https://doi.org/10.1186/s40643-018-0233-5. This is an open access article distributed under the terms of the Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited)

(Sharma and Schenk 2015). Hulatt et al. (2017) examined changes in the biochemical composition of *Nannochloropsis* sp. cultivated in optimized flat-plate photo bioreactors as a potential feedstock for aqua feeds (Fig. 10.12) (Yustinadiar et al. 2020).

10.6.2 Non-suspended Open Algal Biofilms

Microalgae may grow in suspension, but also in biofilms. Gross et al. (2015) summarizes the state of the art of different algal biofilm systems in terms of their design and operation. Microalgae biofilms represent an alternative to the suspension-based systems and could be used as a production platform for microalgae biomass

Fig. 10.11 Schematic photobioreactor design that follows a horizontal tube. (*Source*: Abdel-Raouf N., Al-Homaidan, A. A., and Ibraheem, I. B. (2012). Microalgae and wastewater treatment. *Saudi J. Biol. Sci*. 19, 257–275. doi: https://doi.org/10.1016/j.sjbs.2012.04.005. Open access Reprinted with Licence no. 4880801372566 dated 2nd Aug 2020 RightsLink)

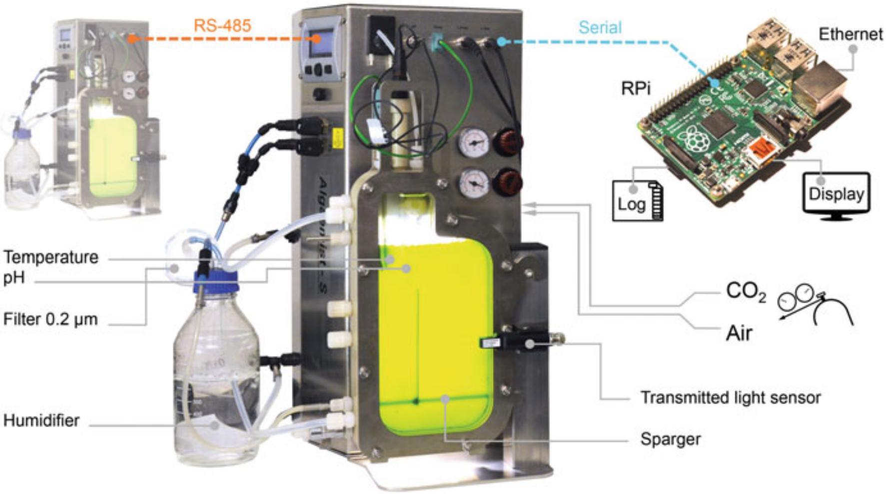

Fig. 10.12 Configuration of the flat-plate photobioreactor systems. Photobioreactors used a 14 mm light path length with illumination by warm white LED lights. Photobioreactors were set up, monitored, and data logged using a custom program running on a Linux single board computer. (This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. Hulatt CJ, Wijffels RH, Bolla S, Kiron V (2017) Production of Fatty Acids and Protein by *Nannochloropsis* in Flat-Plate Photobioreactors. PLoS ONE 12(1): e0170440. https://doi.org/10.1371/journal.pone.0170440)

(Blanken et al. 2014). The advantage of algal biofilm systems is that algae can be simply harvested through scraping and thus avoid the expensive harvesting procedures used in suspension-based harvesting, such as flocculation and centrifugation. A biofilm looks as a slimy, green layer and consists of large numbers of microalgae entrapped in a gel-like matrix (Miranda et al. 2017) (Fig. 10.13).

Miranda et al. (2017) isolated and characterized a number of natural microalgal biofilms from freshwater, saline lakes, and marine habitats (Fig. 10.13). Structurally, these biofilms represent complex consortia of unicellular and multicellular, photosynthetic and heterotrophic inhabitants, such as microalgae, bacteria, cyanobacteria, diatoms, and fungi. Symbiotic microalgal–bacterial biofilms can be very attractive for municipal wastewater treatment (Boelee et al. 2014). Boelee et al. (2014) described that microalgae remove nitrogen and phosphorus and simultaneously produce the oxygen that is required for the aerobic, heterotrophic degradation of organic pollutants. However, attempts are on to obtain a balanced system where no additional oxygen is required.

10.6.3 Non-suspended Enclosed Immobilized Cell System

Abdel-Raouf et al. (2012) reported that one of the major problems in the utilization of microalgae for the biological tertiary treatment of wastewater is their recovery from the treated effluent. However, cell immobilization allows high cell density of immobilized cells, improves the product yield and the volumetric productivity of bioreactors. Immobilization appears to offer several advantages in comparison with batch or continuous fermentation where free microorganisms are used.

It has been reported that *Phormidium laminosum* immobilized on polymer foam has the potential to remove nitrate in a continuous flow system with uptake efficiencies above 90% (Sawayama et al. 1998).

Matrix immobilization is a variant of the attachment theme of reactors through the entrapment of living microalgae cells within a natural or artificial resin, e.g., alginate or carrageenan beads (Mallick 2002; Mehta and Gaur 2005). Shi et al. (2007) reported removal of nitrogen and phosphorus from wastewater by two green microalgae (*Chlorella vulgaris* and *Scenedesmus rubescens*) using a novel method of algal cell immobilization, the twin-layer system. In the twin-layer system, microalgae are immobilized by self-adhesion on a wet, microporous, ultrathin substrate (the substrate layer) (Fig. 10.14).

10.7 Harvesting

Harvesting of the microalgae requires the separation of a low amount of biomass consisting of small individual cells from a large volume of culture medium. The microalgal cell surface is negatively charged, and thus harvesting typically involves dosing a positively charged metal coagulant to neutralize the surface charge, allowing the cells to aggregate together, creating flocs (Henderson et al. 2008a).

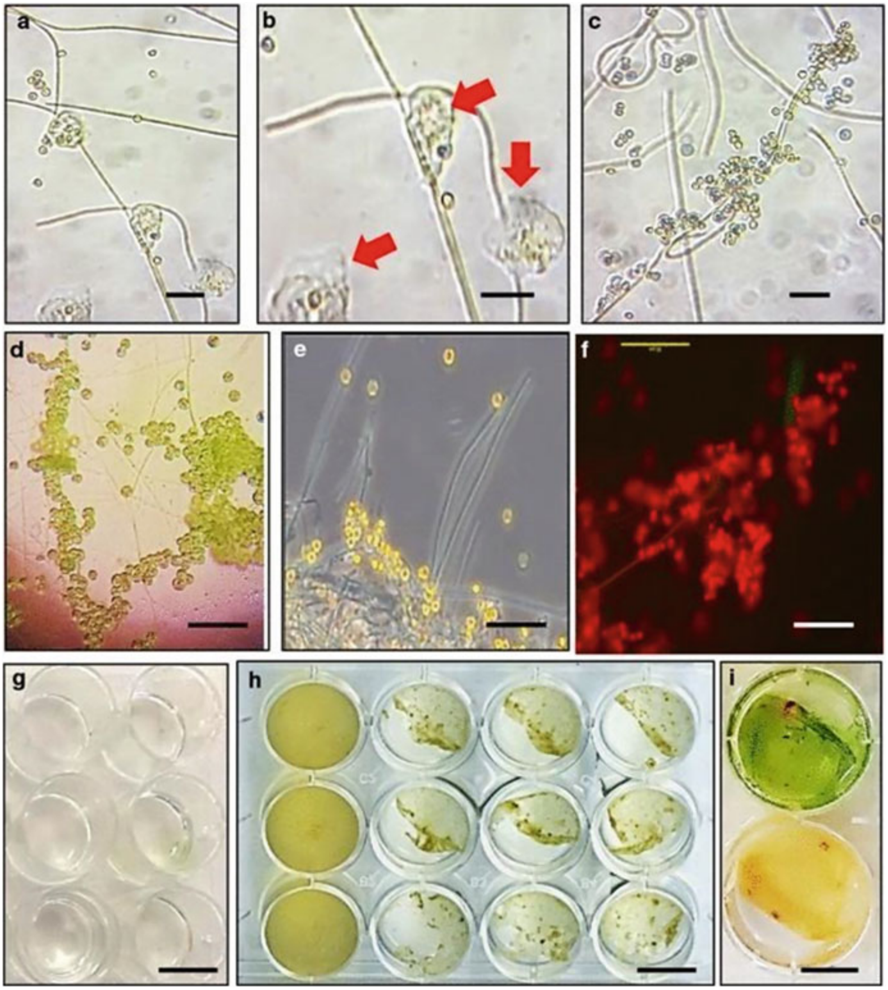

Fig. 10.13 Bio-flocculation of *Isochrysis* sp. cells by BAPS-52-2 filaments. (**a**–**f**) Attachment of *Isochrysis* sp. cells to BAPS-52-2 filaments. Secreted EPS shown by *red arrows*. (**g**) *Isochrysis* cells (*left* wells, controls) and *Isochrysis* cells mixed with BAPS-52-2 filaments (*right* wells) at day 0 and day 10 (**h**). Green pigmentation produced by biofilm produced by monocultured BAPS-52-2 filaments at day 10 ((**i**) *upper* well) and Biofilm #102 at day 10 ((**i**) *bottom* well). *Scale bars* (**a**–**f**) 20 μm; (**g**–**i**) 1 cm. (Source: Miranda, A.F., Ramkumar, N., Andriotis, C. *et al.* Applications of microalgal biofilms for wastewater treatment and bioenergy production. *Biotechnol Biofuels* 10, 120 (2017). https://doi.org/10.1186/s13068-017-0798-9 (http://creativecommons.org/licenses/by/4.0/))

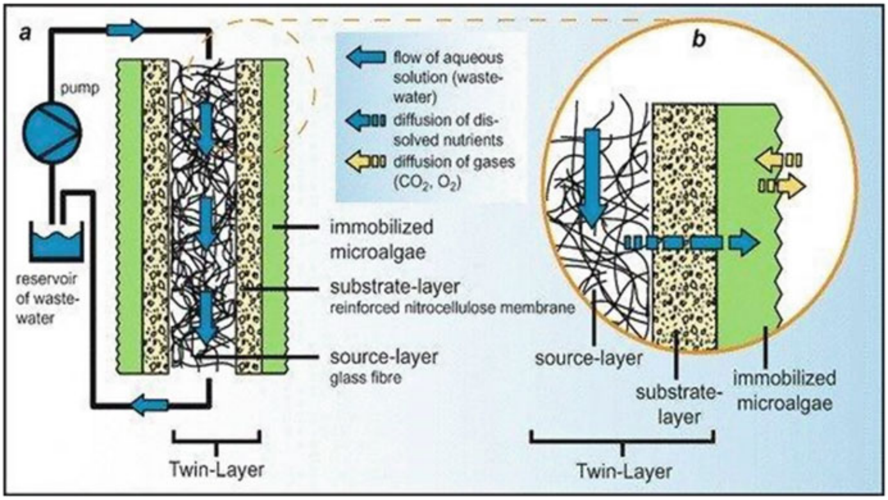

Fig. 10.14 Configuration of twin-layer wastewater treatment system. (*Source*: Shi, J., Podola, B. & Melkonian, M. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *J Appl Phycol* **19**, 417–423 (2007). https://doi.org/10.1007/s10811-006-9148-1. Reproduced with Licence no 4881430070033 dated 3rd August 2020)

These flocs are removed through filtration, sedimentation, centrifugation, or dissolved in air flotation (DAF) (Henderson et al. 2008b). DAF utilizes the natural tendency of algae to float by raising the biomass to the surface, where it is skimmed off and recovered.

10.7.1 Extraction Methods

Various methods, including autoclaving, bead-beating, microwaves, sonication, and a 10% NaCl solution, have been tested to identify the most effective cell disruption method. The total lipids from *Botryococcus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. were extracted using a mixture of chloroform and methanol (1:1) (Lee et al. 2010; Mercer and Armenta 2011) Harun et al. (2013) presented approximate mass balance for biomass to biodiesel and fertilizer/animal feed supply chain (Fig. 10.15).

However, unicellular algae are difficult to harvest, resulting in recent research into non-planktonic algae, especially filamentous species such as *Oedogonium* sp. and *Tribonema* sp. (Roberts et al. 2013). For instance, Wang et al. (2013) reported that for the filamentous algae trialled, *Cladophora* sp. was most efficient under low N:P ratio wastewater whilst *Pseudanabaena* sp. was better for removing nitrogen from high N:P ratio wastewater.

Harun et al. (2013) presented approximate mass balance for biomass to biodiesel and fertilizer/animal feed supply chain (Fig. 10.15).

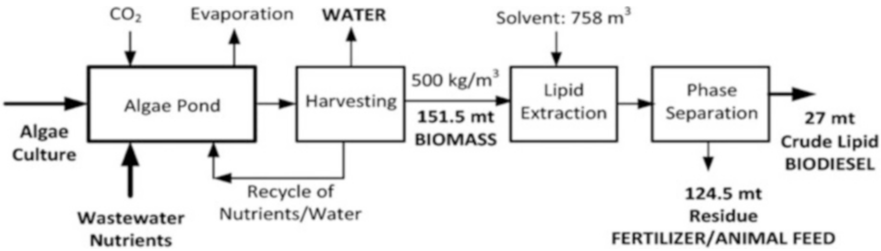

Fig. 10.15 An approximate mass balance for biomass to biodiesel and fertilizer/animal feed supply chain. (*Source*: Harun R, Doyle M, Gopiraj R, Davidson M, Forde GM, Danquah MK (2013) Process economics and greenhouse gas audit for microalgal biodiesel production, in: advanced biofuels and bioproducts. Springer, New York, pp 709–744): *Source*: Allen, J., Unlu, S., Demirel, Y. et al. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. *Bioresour. Bioprocess*. **5**, 47 (2018). https://doi.org/10.1186/s40643-018-0233-5. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)

10.8 Algal Biofuel Production

Demirbas (2006) investigated microalgal biomass for bio-oil production and found the quality superior to the wood. Microalgal biomass with the diversified biochemical content of lipids, carbohydrates, and proteins can be processed via a variety of thermal and biochemical conversion routes into biodiesel, biohydrogen, biomethane, bio-oil, bio-crude oil, etc. (Behera et al. 2019). Behera et al. (2015) discussed the importance of the algal cell contents, many strategies for product formation through various conversion technologies.

Recently, attempts have been made for bioethanol production through fermentation process using algae as the feed stock (Singh 2011; Nguyen and Vu 2012; Chaudhary et al. 2014; Kumar et al. 2018). Sunil Kumar and Buddolla (2019) reported that many species of microalgae can be induced to accumulate significant quantities of lipids, thus contributing to an elevated oil yield, which is later converted into biodiesel by a process called transesterification. Rodionova et al. (2017) presented a scheme for production of biofuels from the hybrid technology (Fig. 10.16).

10.9 Pyrolysis

Pyrolysis is the thermochemical process involving the application of heat (300–700 °C and higher) at atmospheric pressure under anoxic (the total absence of oxygen) conditions (Kazemi et al. 2019). Porphy and Farid (2012) produced bio-oil from pyrolysis of algae (*Nannochloropsis* sp.) at 300 °C after lipid extraction, which is composed of 50 wt% acetone, 30 wt% methyl ethyl ketone, and 19 wt%

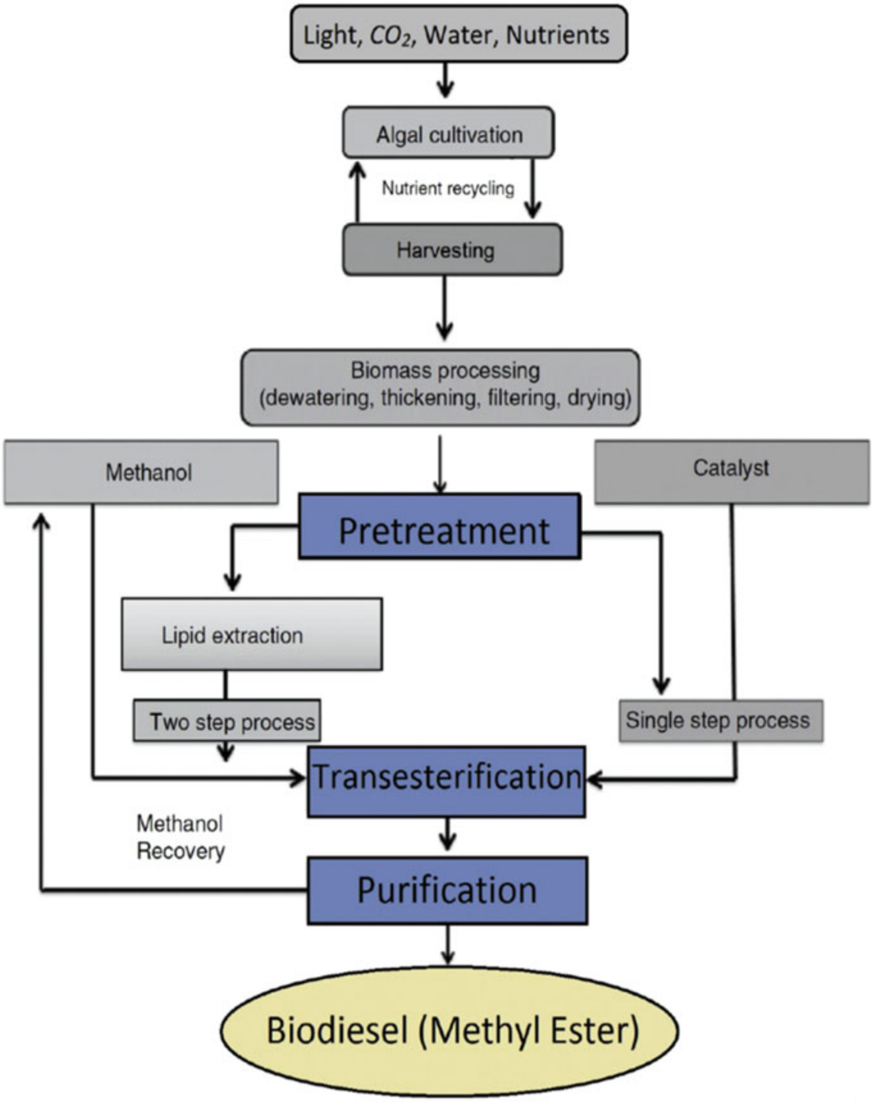

Fig. 10.16 Algal cultivation for biofuel production. (*Source*. Rodionova, M. V, Poudyal, R. S., Tiwari, I., Voloshin, R. A., Zharmukhamedov, S. K., Nam, H. G., Allakhverdiev, S. I. (2017). Biofuel production: Challenges and opportunities. *International Journal of Hydrogen Energy*, *42*(12), 8450–8461. https://doi.org/10.1016/j.ijhydene.2016.11.125. License Number 4883421498494 dated Aug 07, 2020)

aromatics, such as pyrazine and pyrrole. Similarly, Choi et al. (2014) carried out pyrolysis study on a species of brown algae *Saccharina japonica* at a temperature of 450 °C and obtained about 47% of bio-oil yield. They reviewed thermochemical conversion of microalgae into bio-crude oil through pyrolysis and hydrothermal liquefaction technologies. Kazemi et al. (2019) presented HTL process of microalgae biomass (Fig. 10.17).

Allen et al. (2018) biorefinery approach with integrated biology, ecology, and engineering has been presented in Fig. 10.18.

10.10 Discussion

Anthropogenic emissions of excessive atmospheric CO_2 is leading to climate change causing cyclones never heard of, like Tauktae which was responsible for large number of deaths in India (https://economictimes.indiatimes.com/news/india/168-deaths-98-tauktae-hit-hospitals-resume-work/articleshow/82809054.cms). The frequency and fury of cyclones has been increasing to devastating levels due to heating of oceans which results in formation of cyclones. The world's largest iceberg, dubbed A-76, has calved from Antarctica. The animation in the link shows a giant slab of ice breaking off from the Ronne Ice Shelf, lying in the Weddell Sea (https://www.nbcnews.com/science/science-news/worlds-largest-iceberg-just-broke-antarctic-ice-shelf-rcna974). This is also outcome of global warming.

The Paris Accord commits to reduction of global emissions to zero by 2050 in order to achieve 1.5 °C (Kumar et al. 2020). Biodiesel derived from oil crops is a potential renewable and carbon-neutral alternative to petroleum fuels (Behera et al. 2015; Kumar 2018; Kumar et al. 2019, 2020). According to Chisti (2007), commercial production of biodiesel or fatty acid methyl ester (FAME) usually involves alkaline-catalysed transesterification of triglycerides found in oilseed crops (mainly rapeseed in Europe and soybean in the United States) with methanol. However, escalating demand for these crops as a food source coupled with the finite availability of arable land makes their cultivation for biofuels unsustainable (Chisti 2007).

Appropriation of the metabolic products of microorganisms as sources of energy and specialized organic compounds represents a growing form of agriculture (Shurin et al. 2013).

Many microalgae species have been adapted to grow efficiently in wastewater. Thus, the cost of production may be decreased due to the simultaneous use of wastewater and cultivation of specific nutrient-rich microalgae. Therefore, microalgae-mediated CO_2 bio-mitigation can be more economic, cost-effective, and eco-friendly, when it is incorporated into a wastewater treatment infrastructure (Kuo et al. 2016; Collotta et al. 2018).

According to Molazadeh et al. (2019), three major methods are being taken into action in order to remove excess CO_2 which is the largest contributor to the greenhouse effect: (1) transforming CO_2 to organic matters through photosynthesis. The use of third-generation biofuels does not require land and water like crops which compete with food crops (Kumar 2018; Kumar et al. 2010, Chap. 3). (2) Using chemical reactions including chemical/physical solvent scrubbing, adsorption, cryogenics, and membranes, (3) The storage of CO_2 emitted underground or into the ocean. According to Miranda et al. (2017), next generation of bioenergy feedstocks should meet key selection criteria: (1) low freshwater requirement; (2) high growth

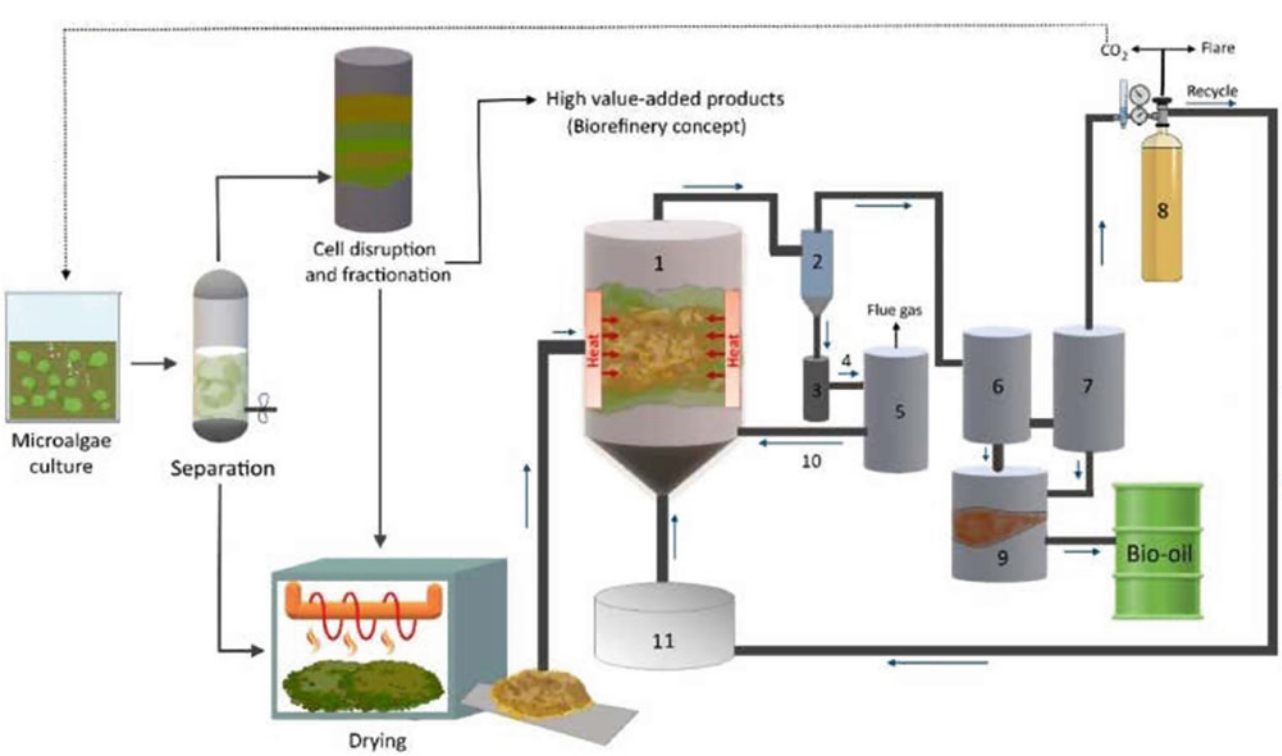

Fig. 10.17 Schematic diagram of HTL process of microalgae biomass. (1) Slurry reservoir (2) high-pressure pump (3) reactor (4) gas cylinder (5) heat exchanger (6) condenser (7) separation vessel. (*Source*: Kazemi Shariat Panahi, H., Tabatabaei, M., Aghbashlo, M., Dehhaghi, M (2019). Recent updates on the production and upgrading of bio-crude oil from microalgae. *Bioresource Technology Reports*, *7*, 100216. https://doi.org/10.1016/j.biteb.2019.100216. Licence 4906430857910 dated 12th September 2020)

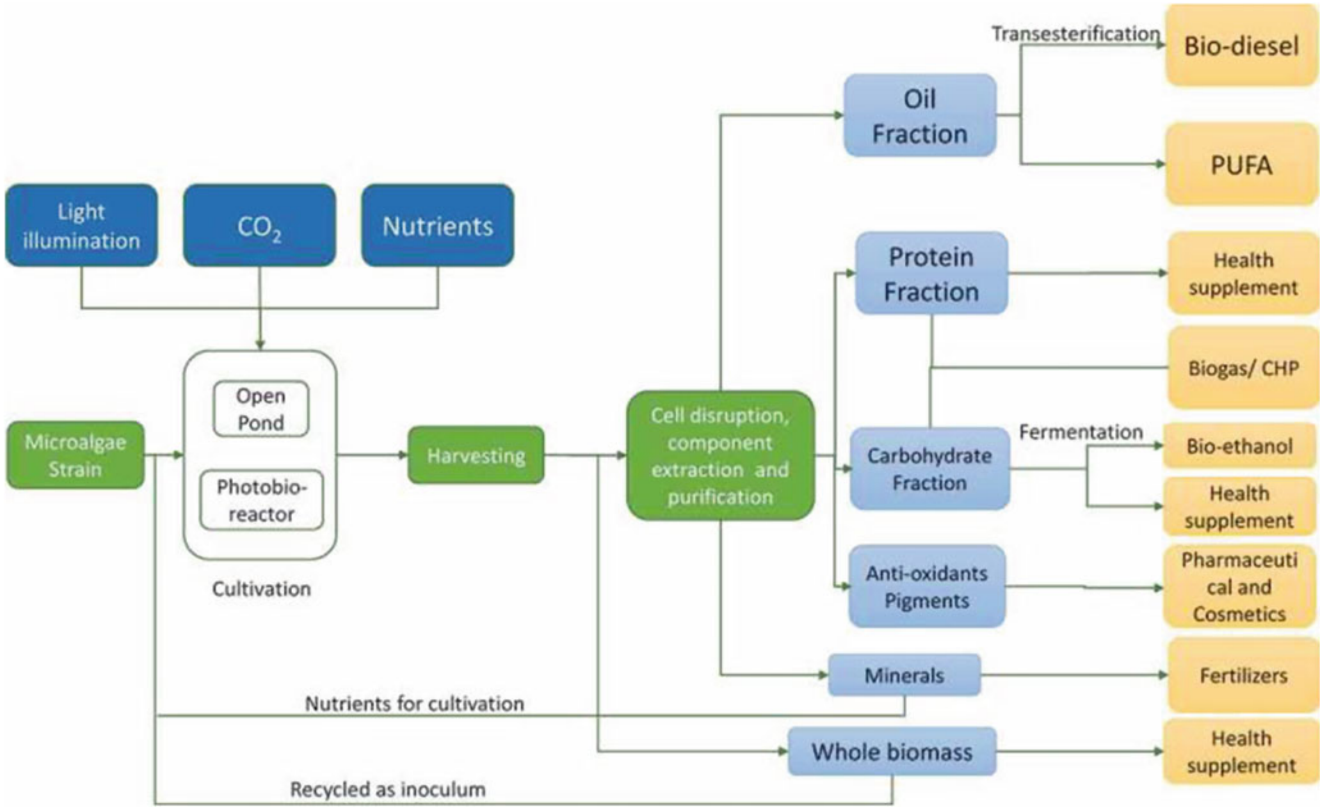

Fig. 10.18 Microalgae biorefinery model. (Koyande AK, Show PL, Guo R, Tang B, Ogino C, Chang JS. Bio-processing of algal bio-refinery: a review on current advances and future perspectives. Bioengineered. 2019 Dec;10(1):574-592. https://doi.org/10.1080/21655979.2019.1679697. This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited)

rates and biomass production; (3) high harvesting index in short rotation period; (4) high content of bioenergy-producing molecules (5) ability to grow on marginal lands and lack of competition with agricultural crops for arable lands; (6) low costs for growth and harvest; and (7) production of high-value co-products (Henry 2010).

However, the mitigation through biological CO_2 fixation is an economically practical and environmentally sustainable technology which has achieved much attention as an alternative method in the long term (Kumar et al. 2010, 2018, 2020; Kumar 2013, 2018).

Microalgae are autotrophic organisms that capture CO_2 present in the air for oxygenic photosynthesis. Because of high tolerant capability to elevated levels of CO_2 in the atmosphere, microalgae are preferred over other carbon sequestration methods (Behera et al. 2019).

The relative supplies of different mineral nutrients and light can have strong phytoplankton and their value as biofuel feedstocks. Large supplies of P relative to N may favour competitive dominance by heterocystous N_2-fixing cyanobacteria (Schindler et al. 2008), which have low cellular lipid contents. Several species of eukaryotic algae accumulate lipid in their cells under conditions of N-starvation (Shurin et al. 2013).

There are three main sources of wastewater, municipal (domestic), agricultural, and industrial wastewater, which contain a variety of ingredients (Chiu et al. 2015). Industrial waste from metal processing industries as well as textile, leather, tannery, and electroplating, have varying amounts of toxic metal ions. Likewise heavy metal ions such as Cd, Cr and Zn, as well as organic chemical toxins such as surfactants, hydrocarbons, and biocides are all present in industrial wastewaters (Ahluwalia and Goyal 2007).

Water from domestic wastewater treatment plants or livestock operations offers a potential source of cheap nutrients that might otherwise be discharged into surface waters (Craggs et al. 2011; Sturm et al. 2012). Recent development of hybrid technologies (biomass production, wastewater treatment, GHG mitigation) for production of prime products as biofuels offer atmospheric pollution control such as the reduction of GHG (CO_2 fixation) coupling wastewater treatment with microalgae growth (Maity et al. 2014). Marine microalgae constitute a useful form of biomass to overcome environmental problems as they can be grown on saline water. However, the availability of innovative approaches for maximizing the treatment efficiency, coupled with biomass productivity, remains the major bottleneck for commercialization of microalgal technology (Abinandan et al. 2018). However in addition to algae culture and growth, it is also essential to develop cost-effective technologies for efficient biomass harvesting, lipid extraction, and biofuels production (Safi et al. 2014). As single-celled molecular factories, microalgae can also be cultivated on marginal land unsuitable for agriculture, using waste streams or saline water supplies (Clarens et al. 2010; Marjakangas et al. 2015). *Nannochloropsis* is a genus of robust, oleaginous microalgae that synthesizes eicosapentaenoic acid (EPA) during balanced growth and is a promising candidate for commercial applications (Davis et al. 2011; Kilian et al. 2011; Radakovits et al. 2012; Sharma and Schenk 2015).

Although some components in the wastewater, such as nitrogen and phosphorus, act as nutrients for microalgae, algal growth rates are lower in different industrial wastewaters, and thus potential for the large-scale treatments of industrial wastewaters containing high levels of heavy metal ions for algal culture are not so promising at the moment. The main challenges are not limited to: industrial-scale capturing of carbon dioxide using microalgal species, the tolerances of specific algal strains grown in a wide variety and concentration of heavy metal ions in industrial wastewaters but optimization of several parameters like simultaneous removal of CO_2 and treatment of wastewaters containing heavy metal ions (Molazadeh et al. 2019).

10.11 Conclusion

Renewable energy plays a critical role in addressing issues of energy security and climate change at global and domestic scales. Biomass offers only means of absorption of global carbon dioxide the major cause of global warming. However, first- and second-generation biofuels compete mostly for land and water with arable lands. Third-generation biofuels derived from algae (or a consortium of microbes) offer triple benefits like algal biomass production, wastewater treatment, and greenhouse gas mitigation. This is receiving increasing attention worldwide as an alternative and renewable source for energy production. Algal biomass can be grown using non-arable areas such as lakes, oceans, or deserts, thus avoiding the current problem of land use competition with the food supply chain. The potential for remediation of inorganic nitrogen and phosphorus from wastewater by microalgae is considered an environmental approach to nutrient polishing. It also has benefits of (a) sequestering of CO_2 from the atmosphere during photosynthesis (b) oxygenating the treated effluent and biofuel production. The chapter presented some of these processes in detail.

Acknowledgements Authors acknowledge with thanks the authors of the papers and figures used in this chapter with permission: Figure 10.1 *Source*: Abdel-Raouf, N., Al-Homaidan, A. A., and Ibraheem, I. B. (2012). Microalgae and wastewater treatment. *Saudi J. Biol. Sci*. 19, 257–275. doi: https://doi.org/10.1016/j.sjbs.2012.04.005. Open access: Reprinted with Licence no. 4880801372566 dated 2nd Aug 2020 RightsLink. Figure 10.2 Maity, J. P., Bundschuh, J., Chen, C.-Y., & Bhattacharya, P. (2014). Microalgae for third-generation biofuel production, mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives – A mini review. *Energy*, *78*, 104–113. https://doi.org/10.1016/j.energy.2014.04.003. Reproduced with licence no 5064180534847 dated 8th May 2021. Figure 10.3 Li H, Watson J, Zhang Y, Lu H, Liu Z. Environment-enhancing process for algal wastewater treatment, heavy metal control and hydrothermal biofuel production: A critical review. Bioresour Technol. 2020 Feb; 298: 122421. doi: https://doi.org/10.1016/j.biortech.2019.122421. Epub 2019 Nov 14. PMID: 31767428. Licence no 5065350239998 dated 10th May. Figure 10.4 Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A Precious Bio-resource in Agriculture, Ecosystem, and Environmental Sustainability. *Frontiers in microbiology*, *7*, 529. https://doi.org/10.3389/fmicb.2016.00529. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). Figure 10.5 Kumar A., Singh J.S. (2017)

Cyano Remediation: A Green-Clean Tool for Decontamination of Synthetic Pesticides from Agro- and Aquatic Ecosystems. In: Singh J., Seneviratne G. (eds) Agro-Environmental Sustainability. Springer, Cham. https://doi.org/10.1007/978-3-319-49727-3_4. Reproduced with RightsLink licence number 5071420183103 dated 17th May 2021. Figure 10.6 Integration of microalgal wastewater treatment with resource recovery for maximizing the derivable products. (Source: Behera, B., Acharya, A., Gargey, I. A., Aly, N., & P, B. (2019). Bioprocess engineering principles of microalgal cultivation for sustainable biofuel production. *Bioresource Technology Reports*, *5*, 297–316. https://doi.org/10.1016/j.biteb.2018.08.001. Reproduced under Licence number 4906420744566 dated 12th September). 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Open access Reprinted with Licence no. 4880801372566 dated 2nd Aug 2020 RightsLink. Figure 10.12 Yustinadiar, N., Manurung, R. & Suantika, G. Enhanced biomass productivity of microalgae Nannochloropsis sp. in an airlift photobioreactor using low-frequency flashing light with blue LED. Bioresour. Bioprocess. 7, 43 (2020). https://doi.org/10.1186/s40643-020-00331-9. This article is licensed under a Creative Commons Attribution 4.0 International License http://creativecommons.org/licenses/by/4.0/. Figure 10.13 Miranda, A.F., Ramkumar, N., Andriotis, C. et al. Applications of microalgal biofilms for wastewater treatment and bioenergy production. *Biotechnol Biofuels* **10**, 120 (2017). https://doi.org/10.1186/s13068-017-0798-9 (http://creativecommons.org/licenses/by/4.0/). Figure 10.14 Shi, J., Podola, B. & Melkonian, M. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *J Appl Phycol* **19**, 417–423 (2007). https://doi.org/10.1007/s10811-006-9148-1. Reproduced with Licence no 4881430070033 dated 3rd August 2020. Figure 10.15 Harun R, Doyle M, Gopiraj R, Davidson M, Forde GM, Danquah MK (2013) Process economics and greenhouse gas audit for microalgal biodiesel production, in: advanced biofuels and bioproducts. Springer, New York, pp 709–744. Allen, J., Unlu, S., Demirel, Y. et al. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. *Bioresour. Bioprocess*. **5**, 47 (2018). https://doi.org/10.1186/s40643-018-0233-5. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). Figure 10.16 Rodionova, M. V, Poudyal, R. S., Tiwari, I., Voloshin, R. A., Zharmukhamedov, S. K., Nam, H. G., Allakhverdiev, S. I. (2017). Biofuel production: Challenges and opportunities. *International Journal of Hydrogen Energy*, *42*(12), 8450–8461. https://doi.org/10.1016/j.ijhydene.2016.11.125. License Number 4883421498494 dated Aug 07, 2020. Figure 10.17 Kazemi Shariat Panahi, H., Tabatabaei, M., Aghbashlo, M., Dehhaghi, M (2019). Recent updates on the production and upgrading of bio-crude oil from microalgae. *Bioresource Technology Reports*, *7*, 100216. https://doi.org/10.1016/j.biteb.2019.100216. Licence 4906430857910 dated 12th September 2020. Figure 10.18 Allen et al. Integration of biology, ecology and engineering for sustainable algal-based biofuel and bioproduct biorefinery. *Bioresour. Bioprocess*. **5**, 47 (2018). https://doi.org/10.1186/s40643-018-0233-5.

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Advances in Biological Nitrogen Removal 11

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Abstract

Inefficient nitrogen removal during wastewater treatment is undesirable as it affects human health adversely and causes eutrophication triggered oxygen depletion in the receiving water bodies. The main conversion pathway (aerobic autotrophic nitrification followed by anoxic heterotrophic denitrification) in traditional aerobic treatment systems necessitates an additional anoxic unit process to meet the nitrogen discharge standards resulting in cost escalation and biomass acclimatization problems. The discovery of alternative nitrogen conversion routes like sulphur oxidizing autotrophic denitrification (SOAD), heterotrophic nitrification aerobic denitrification (HNAD), and ANAMMOX processes have opened unforeseen avenues that may contribute significantly to total nitrogen removal under specific environmental conditions. SOAD can carry out denitrification in carbon-limiting conditions utilizing reduced sulphur compounds as source of electrons and energy, with a concomitant reduction in the total greenhouse gas (N_2O) emission. HNAD couples the reducing power generated during heterotrophic nitrification to aerobic denitrification in a single reactor and can convert nitrate nitrogen efficiently at low DO levels. ANAMMOX bacteria can anaerobically convert ammonium ion to nitrogen directly utilizing nitrite—generated either by nitritation or by coupled nitrification—partial denitrification. Suitable process controls allow generation of anoxic conditions in biofilms or fluidized systems of many existing aerobic technologies facilitating maintenance of ANAMMOX bacteria. These systems can potentially become energy self-sustainable by diverting the organic matter saved through reduced consumption in ANAMMOX process to methane generation. This chapter discusses some

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recent developments in the microbiology of such specialized bacteria and identifies their possible role in enhancing the performance of nitrogen removal.

Keywords

ANAMMOX · Heterotrophic nitrification aerobic denitrification · Sulphur oxidation aerobic denitrification

11.1 Introduction

Wastewater treatment systems are designed to ensure safe discharge of treated wastewater into water bodies. The primary design criterion in these systems is directed towards efficient removal of organics and faecal coliforms to a good extent, but they may not perform nitrogen removal optimally. Inefficient nitrogen removal from wastewaters generated through anthropogenic activities leads to undesirable effects in the ecosystem (Fig. 11.1). The contamination of surface water with excess nitrogenous compounds leads to eutrophication with subsequent death of aquatic organisms due to oxygen depletion (Ghafari et al. 2008; Erisman et al. 2013). Nitrogen in the form of nitrate is highly soluble and mobile in nature and can easily percolate through soil to reach groundwater resulting in its contamination (Wellman and Rupert 2016). This is of particular concern in areas where groundwater is the

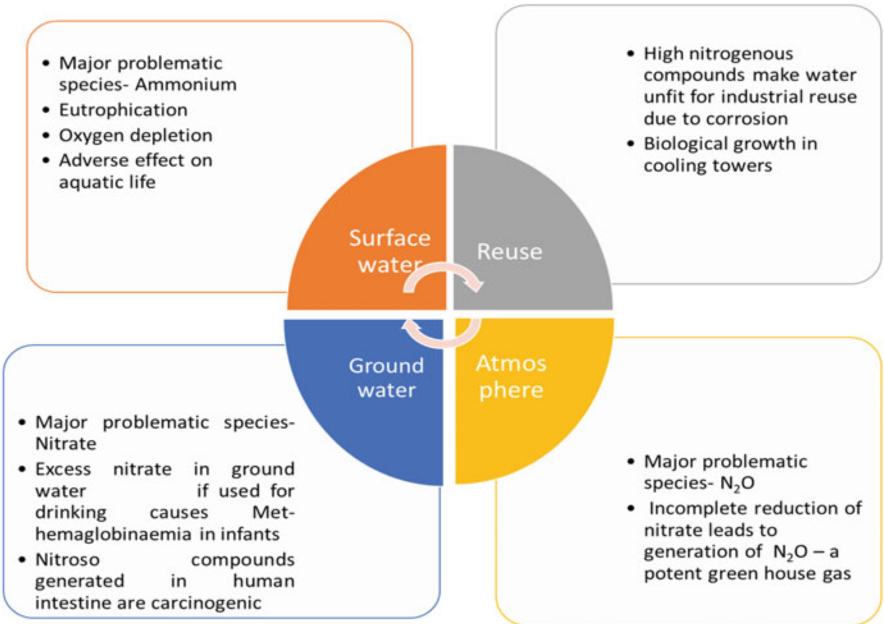

Fig. 11.1 Effect of anthropogenic nitrogen compounds on the ecosystem

main source of potable water. Consumption of water rich in nitrate may lead to undesirable health effects in humans, especially in infants (Methemoglobinemia/ blue baby syndrome). Furthermore, the research on nitrate toxicity has also implicated it for recurrent stomatitis, recurrent respiratory tract infections, and recurrent diarrhoea giving deeper insight to pathophysiology of nitrates and the possible defence mechanism in human body through adaptation of cytochrome b_5 reductase enzyme (Bednarek et al. 2014; Gupta et al. 1999a, b, 2000, 2001). The nitrate ingested with drinking water can also be converted to carcinogenic N-nitroso compounds endogenously by bacteria present in the GI tract (Van Maanen et al. 1996). These compounds can react with nitrogenous bases in DNA and may lead to cancer (Weyer et al. 2001). Nitrate has also been linked to birth defects, tumours, and respiratory problems (Rajta et al. 2020). The nitrate-rich water is also unfit for industrial reuse as it leads to the problems of corrosion and microbial growth in cooling towers (Goldstein and Casana 1982). Improper management of conditions during advance nitrogen removal processes can lead to the release of large amounts of N_2O as undesirable by-product (Guo et al. 2018). N_2O is a potent greenhouse gas and is 300 times more potent than carbon dioxide in its global warming potential (Montzka et al. 2011). Thus, it is imperative to treat wastewaters effectively for removal of nitrogenous compounds to prevent nitrate contamination in water bodies and to minimize greenhouse gas emission.

Physico-chemical methods such as ion exchange, reverse osmosis, and electro dialysis have been applied in treating nitrate-rich drinking water, but these methods are economically non-viable for wastewater applications (Rittmann and McCarty 2012). Contrarily, biological methods of nitrate removal utilizing heterotrophic denitrifiers are efficient subject to availability of readily biodegradable organic matter in the form of methanol, ethanol, or volatile fatty acids (Manconi et al. 2007).

11.2 Conventional Nitrogen Transformation Processes and Their Limitations

The most widely understood pathway of nitrogen transformation in wastewater treatment systems is the conventional autotrophic nitrification followed by anoxic denitrification. The two processes are carried out by microorganisms differing in their oxygen requirements. Nitrification is carried out in two steps. The first step: oxidation of ammonia to nitrate is mediated by ammonium-oxidizing bacteria (AOB) and more recently recognized ammonium-oxidizing archaea (Offre et al. 2013), whereas the second step: oxidation of nitrite to nitrate is mediated by nitrite oxidizing bacteria (NOB). Both the categories of bacteria are aerobic in nature. Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria are slow growing chemolitho autotrophic organisms belonging to genera Nitrosomonas and Nitrosococcus (AOB) and Nitrobacter and Nitrospina (NOB) that carry out nitrification in the mesophilic range of temperature and at neutral to alkaline pH (Rajta et al. 2020). Ammonia oxidizing archaea, however, possess the enzyme ammonia monooxygenase and are known to carry out ammonification under harsh conditions like low DO; temperature and pH extremes; and high salinity (Erguder et al. 2009).

Denitrification refers to stepwise reduction of nitrate to nitrogen gas and is carried out by denitrifying bacteria under anoxic conditions. Certain archaea, e.g., *Haloferaxdenitrificans* and *Pyrobaculumaerophilum*, are also known to carry out denitrification up to dinitrogen. But reports of enrichment of archaea in actual wastewater treatment systems are limited (Yin et al. 2018).

The differences in the oxygen and carbon source requirements of bacteria involved in conventional nitrification and denitrification reactions lead to procedural complexity. The aerobic and anoxic conditions can be generated by alternation. Alternation can be tank, spatial, or temporal (Henze 1991). Tank alternation involves separate oxic and anoxic tanks and incurs additional design and operational cost on the treatment system. Alternatively, nitrified wastewater may be recirculated for denitrification by controlling the aeration pattern within the tank spatially or temporally. The maintenance of aerobic and anoxic conditions is critical and requires trained personnel.

Another limitation of conventional nitrification—denitrification—is encountered in wastewaters containing low carbon (CODc:N ratio <2.86 COD/g N stoichiometric value for complete heterotrophic denitrification) where incorporation of additional organic matter is inevitable to carry out heterotrophic denitrification post nitrification (Cui et al. 2019). Filtered raw wastewater is sometimes utilized as internal carbon source (Rittmann and McCarty 2012), but clogging of filters is the major practical problem in this case. The carbon source can also be generated 'in-plant' by hydrolysis.

Other limitations of the traditional method involve autotrophic nitrifying bacteria that are susceptible to variations in concentration of ammonium and organic matter in the wastewater. The rate of nitrification is extremely low (Rittmann and McCarty 2012). However, heterotrophic denitrifiers are fast-growing bacteria but in the process they generate large quantities of excess sludge adding to the treatment and disposal cost (Sheik et al. 2014).

N_2O generated as a by-product of advanced nitrogen removal processes during nitrification and denitrification (Francis et al. 2007) is a greenhouse gas, which has 300 times higher global warming potential than CO_2.

The major drawbacks of the conventional system are depicted in Fig. 11.2. Overall, the conventional nitrogen treatment process involving nitrifiers and denitrifiers is considered to be environmentally unsustainable due to sludge generation, energy consumption, and greenhouse gas emission (Sheik et al. 2014). These drawbacks limit the use of traditional nitrification–denitrification for advanced tertiary treatment of nitrogen in wastewater and highlight the need to develop alternate strategies to remove nitrogen contaminants from wastewater.

Discovery of novel microbial routes for nitrogen transformation in the recent times have led to development of new nitrogen removal processes by tweaking the process parameters in favour of these microorganisms. These include use of aerobic denitrifiers (Robertson et al. 1985), sulphur oxidizing autotrophic denitrifiers (Schmidt et al. 2003), and ANAMMOX bacteria (Jetten et al. 1997). The following

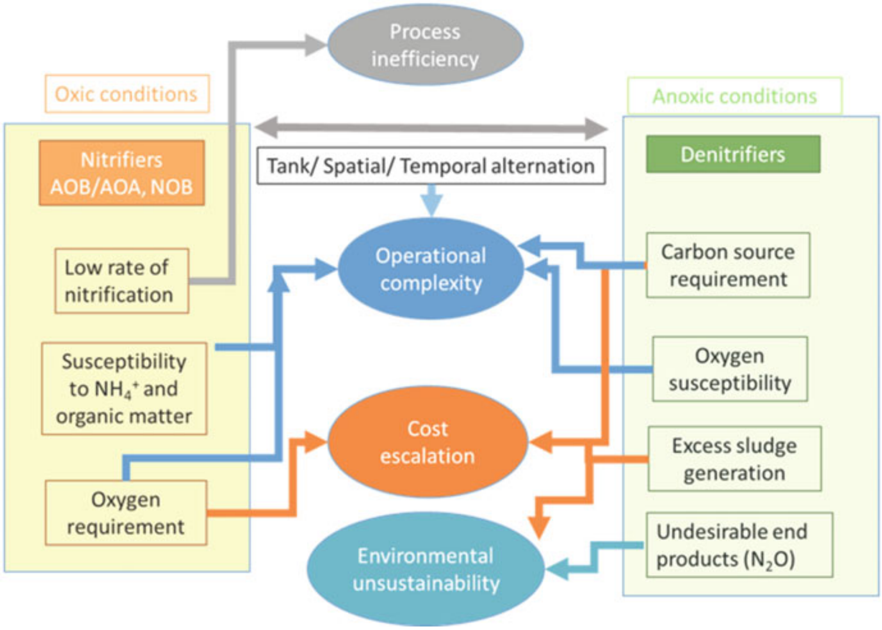

Fig. 11.2 Limitations of traditional nitrification–denitrification reaction

sections discuss the role of these microorganisms in advanced wastewater treatment processes for nitrogen removal. As these processes bypass the requirement of organic matter necessary for conventional denitrification step, the organic matter can be used for energy generation by methanogenic bacteria by incorporating appropriate anaerobic digestion steps. The overall reduction in the aeration cost and energy generation due to increased biogas production can potentially make the treatment system energy-neutral or energy-positive (Siegrist et al. 2008).

11.3 Sulphur Oxidizing Autotrophic Denitrification (SOAD)

SOAD bacteria are autotrophic, carbon dioxide fixing bacteria that can utilize reduced sulphur compounds like sulphide, thiosulphate, and elemental sulphur for reduction of oxidized nitrogenous species like nitrite and nitrate (Fig. 11.3) (Cardoso et al. 2006). SOAD can carry out denitrification in carbon-limiting conditions utilizing reduced inorganic sulphur compounds as source of electrons and energy, with a concomitant reduction in the total greenhouse gas (N_2O) emission if sufficient sulphur is provided (Yang et al. 2016). The biomass yield coefficient (gram biomass generated per gram of nitrate nitrogen denitrified) of SOAD is much lower (0.15–0.57-g) than the heterotrophic denitrification process (0.71–1.2-g), thus reducing the sludge volume considerably (Manconi et al. 2007). As these bacteria are

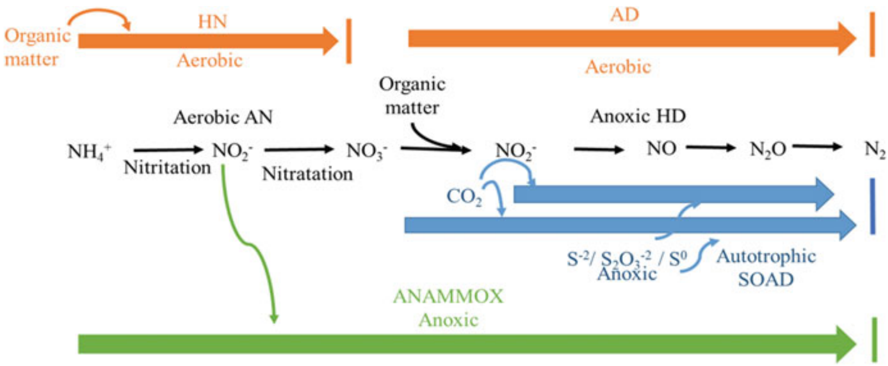

Fig. 11.3 Various alternate nitrogen transformation processes. *AN* autotrophic nitrification, *HD* heterotrophic denitrification, *HN* heterotrophic nitrification, *AD* aerobic denitrification, *SOAD* sulphur oxidizing autotrophic denitrification

autotrophic in nature, that is, external supplementation of organic matter is not required.

These bacteria also offer an additional benefit of simultaneous sulphur removal in addition to nitrate removal. Reduced sulphur compounds like H_2S—a corrosive, toxic gas with obnoxious odour—is converted to sulphur or sulphate by autotrophic denitrification by SOAD bacteria that can be discharged into water bodies (Manconi et al. 2007). Therefore, autotrophic denitrification aided by SOAD bacteria offers a cost-effective and environmental-friendly solution to the nitrate problem in wastewaters.

The major genera of SOAD bacteria that can carry out autotrophic denitrification in monoculture or co-culture mode include *Thiobacillus*, *Sulfurimonas*, *Paracoccus*, and *Thioalkalivibrio*. The major genes involved in the SOAD process are sulphur-oxidizing (Sox) enzymes, sulphide-quinoneoxido reductase (SQR), and flavocytochrome c (FCC) (Wang et al. 2020). Both the genes involved in sulphur oxidation as well as nitrate reduction are indispensable for SOAD process. The presence of sulphur-oxidizing enzymes and nitrate-reducing enzymes in the periplasm ensures efficient coupling of the two reactions without the accumulation of nitrite (Pan et al. 2013).

SOAD can be incorporated into municipal treatment systems using three species as drivers—elemental sulphur, thiosulphate, or sulphide. This process may be used as an addition to the existing system, for example activated sludge system may be dosed with reduced sulphur compounds to serve as supplementary electron donors or as a separate post-denitrification unit. These bacteria are generally used in a biofilm reactor to maintain the sulphur oxidizing denitrifying bacterial growth. These include packed bed bioreactor, up-flow anaerobic sludge blanket reactor; Moving bed biofilm reactor, fixed film reactor. They have also been used in continuous stirred tank reactors and fluidized bed bioreactors (Cui et al. 2019).

The major factors affecting SOAD include temperature, pH, dissolved oxygen, and S/N ratio. Dissolved oxygen content in the wastewater greatly influences denitrification rate as the denitrification enzymes are sensitive to oxygen level. Nitrous oxide reductase is inhibited in the presence of 0.1–0.3 mg O_2/L (Gu et al. 2004), whereas denitrification is completely inhibited if it is higher than 1.6 mg O_2/L. Optimal pH of the SOAD reaction depends upon the sulphur species being utilized as electron donor. For SOAD involving thiosulphate and elemental sulphur, optimum pH is 6.5–8, whereas for sulphide as electron donor, optimum pH is 7.5–9.0. The pH of the reactor also varies during the reaction. When sulphide is used as an electron donor, protons are consumed during denitrification, leading to increase in pH and alkalinity. However, when sulphur or thiosulphate is used as electron donor, proton is generated leading to decrease in pH and reduction in alkalinity consumption (Cui et al. 2019). The operating temperature for all the three processes is 25–35 °C (Shao et al. 2010; Fajardo et al. 2014; Koenig and Liu 2004). A nitrate concentration of more than 670 mg N/L is inhibitory for SOAD reaction. S/N ratio of 2.9 g S/g N is required for SOAD utilizing sulphide as electron donor (Gadekar et al. 2006).

11.4 Heterotrophic Nitrification Aerobic Denitrification (HNAD)

Aerobic denitrification, also called co-respiration, refers to the denitrification carried out by aerobic denitrifiers in the presence of oxygen, where oxygen and nitrate are simultaneously utilized as electron acceptors due to a bottleneck in the electron transport system that leads to excess electrons being transferred to denitrifying enzymes. They are characterized by the presence of a periplasmic nitrate reductase enzyme (Philippot 2002). Aerobic denitrifiers can conduct an aerobic respiratory process in which nitrate is converted gradually to N_2 ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$), using nitrate reductase (Nar or Nao), nitrite reductase (Nir), nitric oxide reductase (Nor), and nitrous oxide reductase (Nos). The complete denitrification to nitrogen gas requires presence of all the four enzymes in the active state in cells (Yang et al. 2020).

The process offers several advantages (Huang and Tseng 2001). The denitrification performed under aerobic conditions eliminates the requirement of tank separation from nitrification reaction. The amount of sludge generated is also less as compared to the conventional nitrification–denitrification reaction. The alkalinity generated in the denitrification reaction partly compensates for the alkalinity consumed in nitrification, thus reducing requirement of alkalinity compensation (Gupta and Gupta 1999). Many aerobic denitrifiers are also able to perform heterotrophic nitrification (Rajta et al. 2020). This greatly reduces the process complexity as both nitrification and denitrification can be performed in similar conditions in the same tank simultaneously (Seifi and Fazaelipoor 2012; Chen and Ni 2012).

Factors Affecting HNAD

1. Choice of carbon source varies from strain to strain. Solid phase denitrification utilizing biodegradable solid substrate is being explored.

2. C/N ratio: An optimum electron donor: acceptor (C/N) is very important for an effective HNAD process. This ratio has been determined to be 8:10 (Joo et al. 2006). Species that can remove nitrogen species at very high C/N ratio are ideal for use in wastewater containing high organic load (Rajta et al. 2020).
3. Dissolved oxygen: the effect of dissolved oxygen concentration on nitrate removal using HNAD is largely dependent upon the particular species involved, for example the maximum oxygen tolerance of *P. denitrificans* is only 2.2 mg/L (Wilson and Bouwer 1997), whereas for *Agrobacterium* DO of 7–8 mg/L becomes limiting for denitrification (Chen and Ni 2012). In actual wastewater treatment plants the optimal DO for nitrogen removal has been found to be 0.15–0.35 mg/L in membrane bioreactor based process (Hocaoglu et al. 2011) and 1.5 mg/L in aerobic granular sludge reactor (Winkler et al. 2012).
4. Temperature: Although most HNAD bacteria are capable of efficient denitrification in mesophilic temperature range, both in psychrophilic (He et al. 2018) and thermophilic (Takenaka et al. 2007), HNAD bacteria have been identified that can efficiently eliminate nitrogen at low and high temperatures, respectively.
5. pH: Neutral to alkaline pH (7–8) is optimal for most HNAD bacteria. As removal of nitrate generates alkalinity and removes H^+, certain species are known to carry out efficient aerobic denitrification at pH as high as 11 (Chen et al. 2014).

Besides these factors, it is important to study the major end product during HNAD process as incomplete denitrification can lead to sequestration of N_2O, a potent greenhouse gas. Some studies have focussed on this issue and have identified strains that generate low levels of N_2O during aerobic nitrogen treatment process (Qing et al. 2018).

HNAD bacteria have been utilized for simultaneous nitrification and denitrification in existing wastewater treatment plants. The operational modes include continuous stirred tank reactors (Patureau et al. 1997) or sequencing batch reactors (He et al. 2018), as well as in membrane processes (Gupta and Gupta 1999; Liu et al. 2018).

11.5 Anammox process and Its Combination with Partial Nitritation

ANAMMOX bacteria are chemolithotrophic bacteria capable of fixing atmospheric CO_2 to carry out bypass conversion of ammonium to nitrogen gas utilizing nitrite. The nitrite ion required for ANAMMOX reaction can be generated in two ways

1. Nitritation—conversion of ammonia to nitrite carried out by ammonium-oxidizing bacteria (Cao et al. 2015). These chemolithotrophic bacteria possess membrane bound ammonia monooxygenase and hydroxylamine oxidoreductase which is rate limiting.

2. Nitrification followed by partial denitrification (Du et al. 2019) carried out by ammonium-oxidizing bacteria, nitrite-oxidizing bacteria, and denitrifiers

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^- \rightarrow NO_2^-$$

The first route, that is nitritation followed by ANAMMOX process, is completely autotrophic process, and thus does not require organic matter. Nitritation–ANAMMOX process can save aeration cost by up to 60% apart from the 100% cost reduction of carbon source. The volume of sludge generated is 90% less as compared to conventional nitrification–denitrification (Morales et al. 2015). The second route, that is partial denitrification–ANAMMOX, reduces oxygen demand and organic matter requirement by 45% and 79%, respectively. The biomass production is also relatively less decreasing the overall cost of sludge treatment and disposal. In the second step, the nitrite generated can act as electron acceptor leading to generation of N_2 by anaerobic ammonium-oxidizing microbes that use ammonium as the electron donor (Xu et al. 2015). The organic matter left after the ANAMMOX reaction can be utilized for methanogenesis (Kartal et al. 2010).

Bacteria having the ability for ANAMMOX reaction belong to the following five genera within order Planctomycetales: *Candidatus Brocadia* (freshwater), *Candidatus Kuenenia* (freshwater), *Candidatus Jettenia* (freshwater), *Candidatus Scalindula* (marine), and *Candidatus Annamoxoglobus*. These ANAMMOX bacteria contain a special organelle called 'Anammoxosome' within which the conversion of ammonium and nitrite to nitrogen gas occurs via the generation of highly toxic intermediate—hydrazine. A unique lipid—ladderane—found in these bacteria is thought to aid in keeping these toxic molecules inside the organelles carrying out ANAMMOX reaction (Damsté et al. 2002).

The major factors affecting presence of ANAMMOX include (Ma et al. 2016)

1. Substrate concentration—The reaction is not inhibited by up to 500 mg/L of ammonium and nitrate species. However, nitrite species inhibits at concentration of >20 mg/L (Lotti et al. 2012). This is of no major concern in STPs as activated sludge is recycled therefore diluting the nutrients.
2. Temperature—ANAMMOX takes place at temperature of 6–45 °C, but the rate of reaction drops at temperatures less than 15 and more than 40 °C (Zhu et al. 2008).
3. DO—although primarily thought to occur in anoxic conditions, the ANAMMOX bacteria are able to remain alive even in the aerobic tanks of STPs at DO concentrations of >2 mg/L (Wang et al. 2015).
4. Organic matter: High organic matter inhibits ANAMMOX; however, maintaining low organic matter in STPs is impossible. The only solution is maintenance of mixed communities. The major bottleneck in this case is how

to make mixed system stable as denitrifying bacteria have a higher growth rate and cell yield than ANAMMOX bacteria (Ma et al. 2016).

5. Sludge retention time—The SRT should be greater than the ANAMMOX doubling time which is 15–30 days in ANAMMOX reactor. Although ANAMMOX bacteria are retained at the SRT of 3 days, the volumetric-specific removal rate of nitrite decreases considerably (Lotti et al. 2015); therefore, maintenance of appropriate SRT is critical for efficient nitrogen removal using ANAMMOX bacteria.
6. Biofilm—If the SRT is high and loading rate is also high, biofilm formation is enhanced—ANAMMOX bacteria form granular sludge. At low temperatures, owing to the increase in viscosity, the decrease in sedimentation velocity results in loss of ANAMMOX granular sludge as the high EPS (extra polymeric substances) content blocks gas release, increasing buoyant forces, making sludge float and washed away in a continuous process.

The ANAMMOX process requires warm waters and high ammonical nitrogen (500–1500 mg/L), but sewage typically has $<$100 mg/L ammonical nitrogen. Therefore, the probability of ANAMMOX bacteria developing spontaneously is low. Nevertheless, ANAMMOX bacteria have been detected in various units of STP. The challenges in development of stable ANAMMOX reaction in the STPs include maintenance of partial nitrification (nitritation) and co-culture of nitritation and ANAMMOX bacteria. The lack of efficient strategies to suppress the growth of nitrite-oxidizing bacteria and maintain stable partial nitrification is a major bottleneck in the full-scale application of ANAMMOX process (Le et al. 2019). The strategies to enrich AOBs against NOB include intermittent aeration, alternating anoxic conditions with oxic conditions, step feeding, and prompt SRT control (Gu et al. 2018). The first two strategies are based on the fact that NOB takes longer to recover when conditions shift from anoxic to oxic (Kornaros et al. 2010). Further as dissolved oxygen has higher affinity for AOB than NOB (Blackburne et al. 2008), limiting the DO content using intermittent aeration favours AOB over NOB. However, low DO also triggers N_2O production which is a major drawback (Massara et al. 2017).

Furthermore, the co-culture of nitritation and ANAMMOX bacteria also presents problems in sewage treatment plants with regard to low retention of biomass. This limitation has been overcome by use of granular sludge of co-cultures which are compact and fast-settling granules that provide large surface area for mass transfer and high volumetric conversion rates (Kartal et al. 2010). Kartal et al. have suggested use of activated sludge process for removal of organic matter, followed by flocculation of biomass that can be used for biogas generation. The effluent of anaerobic digester is then combined with ASP effluent and treated in granular sludge ANAMMOX reactor to remove residual nitrate. This process can reverse the energy equation of wastewater treatment plants and can convert them to energy-yielding processes (Siegrist et al. 2008).

Partial nitritation–ANAMMOX process efficiently removes nitrogenous compounds from wastewaters that are ammonium-rich like anaerobic sludge digester effluents, but its application in relatively low ammonium waters, like full scale STPs, would require further exploration (Kartal et al. 2010; Kuypers et al. 2018). ANAMMOX process can be applied as a mainstream or side stream process. In side stream ANAMMOX, the partial nitritation and ANAMMOX reactions can either be carried out in separate or in same tanks. The former process compartmentalizes AOB bacteria and ANAMMOX bacteria on the basis of their oxygen requirement, whereas the latter utilizes bacterial biofilms that have oxic zone as the surface and anoxic zones in the core (Winkler and Straka 2019). Full scale side stream ANAMMOX process has been successfully used to treat ammonia-rich (500–1500 mg N/L) and COD-limited (COD/N ratio of less than 1 g COD/g N) warm waters (more than 25 °C) from anaerobic digestors (Lackner et al. 2014).

The application of ANAMMOX as mainstream treatment has gained a lot of interest in recent years due to energy savings in aeration and carbon source requirement. However, there are some challenges in realizing this process (Cao et al. 2017). As the COD/N ratio is much higher in the secondary treated water, the heterotrophic bacteria out-compete the slow-growing ANAMMOX bacteria. The low ammonium load also acts against the growth of AOB and ANAMMOX bacteria. The nitrogen in effluent from the mainstream ANAMMOX process needs to be much lower than that from a side-stream process. The full-scale application of mainstream ANAMMOX have been limited and are based on using intermittent aeration (Wett et al. 2015) or step feeding combined with alternate oxic and anoxic conditions (Cao et al. 2013) to suppress NOB. Few reports have suggested the use of algae to reduce the effluent concentrations (Cao et al. 2017).

The discovery of microorganisms performing anaerobic methane oxidation in a nitrite (*Methylomirabilis* spp.) and nitrate (*Methanoperedens* spp.) dependent reaction (Haroon et al. 2013) have paved way for novel treatment systems using ANAMMOX bacteria. If these lab based studies can be successfully converted to full scale, the co-culture of these nitrite and nitrate reducing methanotrophs and ANAMMOX bacteria could carry out simultaneous removal of ammonium, nitrate, and methane (Haroon et al. 2013).

A comparison of nitrogen transformation processes discussed in this chapter is given in Table 11.1.

Table 11.1 Summary of practical aspects of different nitrogen removal processes in wastewater treatment

Property	ND	SOAD	HNAD	Partial nitritation—ANAMMOX
Requirement of oxic and anoxic zones	Yes	Yes	No	Yes/No
Sludge generation	High	Low	Low	Low
Aeration cost	High	High	Low	Very low
Carbon source supplementation	Yes	No	No	No
Buffer requirement	High	low	Low	
N_2O production	High	• Low if sufficient reduced sulphur compounds are provided	Low	low
Other benefits		• Simultaneous sulphide removal	• Less acclimatization problems • High rate of nitrification-denitrification	Lower production of CO_2
Major issues	• Requirement of adequate amount of readily biodegradable substrates (C/N 7–9)	• May increase hardness of wastewater • S/N ratio critical • Sulphide levels need to be critically controlled to avoid inhibition of denitrification • Low biomass retention	• Selection pressures to be maintained through skilled operation	• Suppression of NOB • Maintenance of co-cultures of AOB and ANAMMOX bacteria

11.6 Conclusion

The alternative nitrogen removal processes like SOAD, HNAD, and ANAMMOX-based processes offer significant advantages over conventional nitrification–denitrification for wastewater treatment. With some design and process modifications, the existing systems can be used for efficient nitrogen removal along with the BOD removal at minimal additional cost. These processes can provide cost effective and environmentally sustainable solutions to the wastewater management and are likely to be adopted at the full scale in the coming years.

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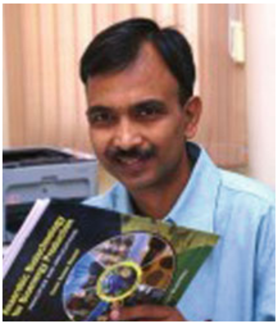

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Application of Microbial Enzymes: Biodegradation of Paper and Pulp Waste

12

Kamlesh Kumar R. Shah, Sutaria Devanshi, Gayatriben Bhagavandas Patel, and Vidhi Dhirajbhai Patel

Abstract

The paper and pulp industry is the sixth largest polluting industry (after oil, cement, leather, textile, and steel industries) releasing a range of gaseous, liquid, and solid wastes into the environment. The paper and pulp industry processes huge numbers of chemicals in pulping, bleaching, and deinking of waste paper. Several pollutants are released as effluent due to the utilization of several chemicals. The paper and pulp industry is associated with pollution-related to high BOD, COD, toxicity, AOX, suspended solids, lignin, and its derivatives and chlorinated compounds. Several of the pollutants are naturally occurring wood extractives like tannins, resin acids, stableness, and lignin. It is well-recognized that many contaminants are acute or even chronic toxins. Chlorinated organic compounds like dioxins and furans can bring genetic changes in exposed organisms. This has resulted in a growing concern about the potential adverse effects of genotoxicants on aquatic biota and public health through the contamination of drinking water supplies, fun water, or edible organic species. Although biotechnological approaches are used to remove COD, BOD, color, etc. from paper and pulp effluents. Various microorganisms or enzymes like cellulases, xylanases, and lignin-degrading enzymes have been used as cost-effective and eco-friendly technology to reduce the utilization of chemicals and reduce waste material. Enzymes producing microbial strains, *Streptomyces*, *Nocardia*,

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Nocardiopsis, *Thermobifidafusca*, *Phanerochaete chrysosporium*, *Coprinuscinereus Bacillus* spp., and *Arthromycesramosus*, etc., have been concerned to degrade the xenobiotic compounds in paper and pulp effluent.

Keywords

Paper and pulp · BOD · COD · Genotoxicants · Lignin · Cellulase · Xylanase

12.1 Introduction

Paper and pulp industry is labeled as a central sector industry; it is the fifth-largest contributor to industrial water pollution. The production in 2005–2006 was 5.9 million tons. The present paper consumption is low at 6 kg/capita. The paper and pulp industries of India; extremely water-intensive use 100–250 m^3 freshwater/ton paper, as a result, wastewater generation is also high 75–225 m^3 wastewater/ton paper. As per the Ministry of Environment and Forest (MoEF), Government of India, the paper and pulp sector is in the "Red Category" list of 17 industries which is marked for huge polluting potential due to its serious pollution threat. Paper and pulp mills have to follow suitable standards set by Central Pollution Control Board (CPCB). Pulping and bleaching, two main processes, are involved in the manufacturing of paper. The effluent produced at the pulping stage, dark brown (black liquor) due to dissolved lignin, and its degradation products such as hemicelluloses, resins, acids, and phenols. The effluent generated at the bleaching stage has absorbable substance organic halogens (AOX), peroxides, and derivatives of lignin and hemicelluloses. It is estimated that 1 ton of paper released 150 m^3 of effluent which is very toxic (Pokhrel and Viraraghavan 2004).

The use of microbial enzymes in the paper and pulp industry has gradually increased with the growing understanding of environmental issues to minimize the adverse effects on the environment. Enzyme-use decreases processing time, energy consumption, and the number of chemicals used in processing. By increasing biological oxygen demand (BOD) and chemical oxygen demand (COD), enzymes are also used to boost deinking and bleaching in the paper and pulp industry and waste management (Srivastava and Singh 2015). Xylanases and ligninases are used to improve the value of the pulp by extracting lignin and hemicelluloses in the paper and pulp industries (Maijala et al. 2008). Amylases are used in these industries for starch coating, deinking, improving paper cleanliness, and improving drainage. Lipases are used to deink and increase pitch control, while cellulases are used to deink, improve softness, and improve drainage (Kirk and Jeffries 1996). Cellulase has also been used for the bioprocessing of used printed papers for recycling purposes. The use of laccases is an alternative to the use and requirement of large amounts of chlorine in the process of chemical pulping, thus minimizing the amount of waste causing ozone depletion and acidification (Fu et al. 2005). Besides, mannases are used to degrade glucomannan to increase luminosity in the paper industry (Clarke et al. 2000).

Chlorine-free bleached pulp has increased demand in the market throughout the last decade, chiefly due to its appropriateness toward customer contentment (Sharma et al. 2015). However, eco-friendly bioleaching and other applications, such as pitch removal and deinking of paper waste, are feasible by the use of potent biocatalysts, such as xylanases and laccases. In the beginning, the enzyme application for paper and pulp technology was not measured technically and economically feasible as the need for easy availability of biocatalysts. Commercial-scale production and application of enzymes was a cost-intensive and burdensome process in the long run. Furthermore, enzymes like laccases need mediators for their bleaching action and bear enzyme stability problems (Singh and Chen 2008). Fortunately, research efforts by scientific institutions and enzyme producers have led to the development of biocatalysts that proposed significant benefits to the paper and pulp industry. In the absence of enzymes, available options are (1) bleaching of pulps with oxygen and extended cooking, (2) hydrogen peroxide, and (3) ozone treatment. But most of these methods are highly capital rigorous for process change. Therefore, applying an alternative and cost-effective method of enzymes has afforded an extremely simple and economic way to reduce the use of chlorine and other bleaching chemicals (Bhoria et al. 2012). Xylanase bleaching technology is nowadays used in numerous industries worldwide. This technology has been productively transferred to a full-scale industry in recent times. The main energetic factors, the economic and environmental compensation of biocatalysts, can convey to the pulp-bleaching plant. After xylanases, numerous researchers have recognized laccase as one of the multipurpose biocatalysts that have the likelihood to modify the pulping and paper making procedure. It plays a vital role in the delignification and brightening of pulp which has also further been renowned for the elimination of lipophilic extractives, liable for pitch deposition, from both wood and nonwood pulps. Laccases are competent to improve physical, chemical, and mechanical properties of pulp by forming reactive radicals with lignin with functionalizing ligno cellulosic fibers. Laccases target the colored and toxic complex mixture out as effluents from pulp industries and render them nontoxic during polymerization and depolymerization reaction (Virk Antar et al. 2012).

Enzymes, biocatalysts formed by biological cells to cause specific biochemical reactions usually form a variety of metabolic activity of the cells and are crucial to the maintenance of life. Enzymes are greatly specific to substrates and frequently different enzymes are required to carry a sequence of metabolic reactions completed by living cells. Microorganism produces a large number of enzymes which are involved in hydrolyzing, oxidizing, or reducing the metabolic reaction. Microbial enzymes are recognized as metabolic catalysts, ensuring their use in a range of industrial applications. The end-use of industrial enzyme markets is immensely well-known among diverse industrial commercial applications.

12.2 Use of Various Microbial Enzymes in Paper and Pulp Industry

Enzymes are biocatalysts produced by living organisms. To induce complex biochemical reactions in cells, various metabolic processes usually form part of the cells and are important for life care and operation. Microorganisms have very precise enzymatic activity on substrates; there are many different enzymes, as well as many that are required to carry out metabolic sequence reactions that living cells encounter. Everyone a large strain of a microorganism generates a large number of enzymes that can be considered hydrolyzing, oxidizing, or declining and reducing in nature, metabolic. Microbial enzymes are known to play a crucial role in metabolic processing.

12.2.1 Application of Various Enzymes

12.2.1.1 Microbial Oxidoreductases

The detoxification of toxic organic compounds by various bacteria and fungi and higher plants (Gianfreda et al. 1999) through oxidative coupling is mediated with oxidoreductases.

12.2.1.2 Oxygenases

Oxygenases belong to the enzyme group of oxidoreductases and participate in the oxidation of reduced substrates by transferring oxygen from molecular oxygen (O_2) as a co-substrate using FAD/NADH/NADPH. In the metabolism of organic compounds, these enzymes play an important role as they increase their reactivity or solubility in water or allow the aromatic rings to cleave. In association with multifunctional enzymes, oxygenases also mediate the dehalogenation reactions of halogenated methane, ethane, and ethylene (Susanne and Lingens 1994).

12.2.1.3 Monooxygenases

These enzymes catalyze the oxidative reactions of alkane substrates to complex molecules, such as steroids and fatty acids, and for their function, need only molecular oxygen. These enzymes use only molecular oxygen and use the substrate as a reducing agent for their activities. Monooxygenases are used to catalyze the desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, biotransformation, and biodegradation of various aromatic and aliphatic compounds.

12.2.1.4 Microbial Dioxygenases

These dioxygenases catalyze enantiomers specifically the oxygenation of wide range of substrates. Aromatic compounds are primarily oxidized by dioxygenases, reflecting the applications of dioxygenases in environmental remediation. The catechol dioxygenases are found in the soil bacteria and involved in the transformation of aromatic precursors into aliphatic products.

12.2.1.5 Microbial Laccases

Laccases (*p*-diphenol: dioxygenoxidoreductase) constitute a family of multicopper oxidases produced by certain plants, fungi, insects, and bacteria that catalyze the oxidation of a wide range of reduced phenolic and aromatic substrates with concomitant reduction of molecular oxygen to water. Many microorganisms produce intra and extracellular laccases capable of catalyzing the oxidation of ortho and paradiphenols, aminophenols, polyphenols, polyamines, lignins, and aryl diamines, as well as some inorganic ions (Arora 2010).

12.2.1.6 Microbial Peroxidases

In the presence of a mediator, these enzymes catalyze the oxidation of lignin and other phenolic compounds, at the cost of hydrogen peroxide (H_2O_2). Lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) were the most studied due to their high ability to degrade toxic substances in nature.

12.2.1.7 Microbial Lipases

These enzymes, such as hydrolysis, intersterification, esterification, alcoholysis, and aminolysis, can catalyze several reactions. Lipase has many potential applications in the food, pharmaceutical, detergent, cosmetic, and papermaking industries, along with its diagnostic use in bioremediation, but its production cost has limited its industrial use (Sharma et al. 2011; Prasad and Manjunath 2011).

12.2.1.8 Microbial Cellulases

Cellulose is degraded by the cellulases during enzymatic hydrolysis to eliminate sugars that can be fermented into ethanol by yeasts or bacteria. Cellulases cause the removal of microfibrils of cellulose that are formed during washing and cotton-based clothing usage. Cellulase is used in the paper and pulp industry for the removal of ink during paper recycling.

12.2.1.9 Microbial Proteases

In an aqueous environment, proteases belong to a group of enzymes that hydrolyze peptide bonds and synthesize them in a non-aqueous environment. In the food, leather, detergent, and pharmaceutical industries, proteases have a broad range of applications (Beena and Geevarghese 2010).

12.2.1.10 Industries and Pollutants

One of the major groups of contaminants contains aromatic compounds, including phenols and aromatic amines, and is strictly regulated in many countries. They are present in the waste water of a wide range of industries, including the conversion of coal, oil refining, resins and plastics, wood preservation, metal painting, dyes and other chemicals, textiles, mining and dressing, and pulp and paper. The Kraft process leaves 5–8% (w/w) of residual changed lignin in the pulp, which is commonly used in wood pulping. The residue is responsible for the pulp's distinctive brown color and is commercially eliminated by the use of bleaching agents such as chlorine and oxides of chlorine. Bleaching processes create dark brown effluents containing toxic and mutagenic chlorinated products that pose an environmental risk. A variety of experiments have been performed, including the use of peroxidases and laccases for bleaching effluent treatment. Pesticides, which include herbicides, insecticides, and fungicides, are widely used for crop protection around the world today, and this use is expected to continue to increase. The possible adverse effects that the pesticide industry may have on the environment stem from the disposal of waste produced during pesticide manufacturing and formulation, the detoxification of pesticide containers and spray tanks, and the pollution of pesticide runoff from surface and groundwater and also in various manufacturing processes, including the processing of chemical intermediates, synthetic fibers, rubber. In various industrial processes, including the manufacture of chemical intermediates, synthetic fabrics, rubber and pharmaceuticals, as well as ore leaching, coal processing, and metal plating, it is estimated that 3 million tons of cyanide are used annually throughout the world. Enzymes could be used by enzymatic processing to eliminate food waste in order to produce higher value by-products and to help in the clean-up of sources of food waste. There has been a growing interest in the enzymatic hydrolysis of cellulose over the past decade. This interest stems from the advantages provided by such a method, namely the conversion of lignocellulosic and cellulosic waste through the processing of sugars, ethanol, biogas, or other energetic end products into a useful energy source (Anders and Chen 1993). Dangerous pollutants present in pulp effluents are heavy metals such as arsenic, copper, cadmium, lead, and chromium, among others.

12.3 Pulping

Pulping is the method of creating paper-making fibrous raw material that is accomplished by breaking the bondage in wood or other raw materials such as non-wood, grass, and agro-residues. Chemical pulping, mechanical pulping, and a mixture of chemical and mechanical pulping are three types of pulping. Lignin is an aromatic polymer that consists of units of phenyl propane arranged into a broad three-dimensional network structure (Kumar et al. 2016a). In the process of paper making, lignin is assumed to be eliminated as far as possible. Chemical pulping produces cellulosic fibers by solubilizing lignin from the plant cell wall with a variety of chemicals. To transform the raw materials into a fibrous mass, called pulps, they are roasted at a high temperature and pressure in an aqueous solution with appropriate chemicals. Solubilize the lignin current in the cell walls of raw materials. Chemicals such as sodium hydroxide (NaOH) and sodium sulfide are used to solubilize and remove lignins in the Kraft (sulfate) pulping process, which creates stronger pulps (Na_2S). To remove lignin, sulfuric acid (H_2SO_3) is used in sulfite pulping (Pokhrel and Viraraghavan 2004). Pulping is the primary step in the entire papermaking industry for the processing of contaminants.

12.3.1 Biopulping

The environmental-friendly alternative to conventional techniques of pulping is bio. The process for the delignification of cellulosic raw materials is cost-effective. Prior to pulping, pretreatment of the raw material with white rot fungi is called biopulping. White rot fungi produce enzymes that destroy lignin, such as lignin peroxidase, laccase and manganese peroxidase during the process of biopulping. Biopulping is the target for the removal or loosening of lignin in a wood matrix without cellulose decomposition. During the process, various enzymes destroy biopulping, lignin and hemicelluloses.

Forests are broken down into chips or other raw materials in the first phase of biopulping. Small bits are chopped into products such as agro-residues and grasses. Chips of wood cells and spores of various bacteria and fungi are usually infected. These unwanted microorganisms would compete with biopulping fungus and would have a negative effect on them influencing the production of biopulping fungus. The development of these unwanted microorganisms into cellulases can degrade the cellulose and adversely affect its strength. Decontamination of wood chips and other raw materials is therefore necessary, and steam is crucial. The method of choice for decontamination is sterilization. Within the second stage is the inoculation and incubation of the desirable fungus into the substrate. Aerated conditions for 1–4 weeks at suitable temperatures depend upon the climatic conditions. The method of raw material fungal pretreatment is executed under conditions of solid-state fermentation. The substrate's moisture content in a range of 50–80% is maintained during fermentation. In warm and damp conditions, the surface of wood chips or other raw materials and lignin degrading fungi will colonize the interior is penetrated by wood chips or other raw materials.

12.3.2 Effect of Biopulping on Chemical Composition of Raw Materials

The lignocellulosic paper-making raw materials consist primarily of cellulose, hemicellulose, and lignin. Selective removal of lignin and hemicellulose is the purpose of biopulping. Enzymes which change or dissolve lignin are produced during fungal pretreatment of raw materials. The lignin-degrading enzymes that are produced by white rot fungi are lignin peroxidase (LiP, EC 1.11.1.14), laccase (Lac, EC 1.10.3.2), and manganese peroxidase (MnP, EC 111113) (Millati et al. 2011). Lignin peroxidase is a prostetic group comprising Fe. It is a high oxidation ability hema protein that can oxidize phenolic and non-phenolic substrates. MnP is an H_2O_2-dependent glycoprotein that requires Mn^{2+} to oxidize monoaromatic phenols. Lac is a multicopper oxidase that catalyzes the reduction of O^2 to H_2O, and the aromatic amines are oxidized (Bonugli-Santos et al. 2010). Various white rot fungi, such as Phanerochaete chrysosporium, Pleurotus ostreatus, Trametes Versicolar, T., develop LiP, Lac and MnP. Cervina, Meruliaceae spp., Coriolaceae spp., Bjerkandera spp., Polyporaceae spp., Coprinellus spp. During the biopulping of the raw substrate, lignin is degraded by fungal enzymes, such as LiP, Lac, and MnP, and the percentage of lignin in the substrate that requires less pulping chemicals is decreased. Hemicelluloses are also extracted through the action of white rot fungi produced by xylanase. The colonization of white rot fungi dissolves lignin and hemicelluloses, leaving the cellulose largely intact (Singh et al. 2010). The biological treatment of poplar chips with Polyporus varius was performed by Lei et al. in 2011, and the lignin content decreased from 23.15% to 17.56% after 20 days of incubation. Conducted an analysis of Phanerochaete chrysosporium NRRL 6370 on biopulping of banana fruit stalk and found improvement in holocellulose and alpha-cellulose by 6.5% and 15.3%, while the content of Klason lignin and hemicellulose decreased by 7.6% and 8.8% after 30 days of fungal pretreatment.

12.3.3 Environmental Consequences

Biopulping is an environmentally sustainable solution compared to pulping in which previous fungal pretreatment minimizes the use of pulping chemicals. Owing to the removal of lignin and hemicellulose after fungal pretreatment, raw material becomes more sensitive to chemicals and can be processed into pulp with lower pulping chemicals. White rot fungi absorb some of the pitch enclosed in the lignocellulosic material during biopulping, reducing both the toxicity and biological oxygen demand (BOD) content of mill process water. In addition, biopulping pulps are more prone to oxidative and reductive bleaching chemicals. The operation of fungi during pretreatment relies on a well-grown stage of mycelium. Saad et al. (2008) reported that pretreatment of Ceriporiopsis subvermispora sugar cane straw had a positive impact on lignin decomposition during pulping of acetosolv. The findings indicate that this fungus is selective for the degradation of lignin. The results showed that this fungus is selective for the degradation of lignin, causing structural changes in the components of lignin and other straw components, which may result in decreased cooking times and the need for pulping chemicals (Kang et al. 2007), prior to Kraft studied the biopulping of Poplar wood chips with the white-rot fungus Phanerochaete chrysosporium KCCM 34740 and reported an increase in screened pulp yield and a decrease in kappa (residual lignin content) after fungal pretreatment of the hardwood pulps. Improved optical and strength properties with reduced active alkali (from 17% to 15%) and sulphidity were observed during biopulping (20–15%).

12.4 Pulp Bleaching

Pulp is defined as the fibrous material obtained after pulping, consisting primarily of cellulose. Cellulose and hemicellulose are white in nature and do not contribute to any color. Chromophoric groups found in lignin primarily contribute to the color of the pulp. While most lignin is extracted during the bulk delignification step of chemical pulping processes, during multi-stage bleaching sequences, residual lignin associated with secondary wall layers of fibers (residual lignin) is removed. The full removal of residual lignin from pulp is carried out by the use of various oxidative agents for the manufacture of white paper varieties (Fu et al. 2005; Ibarra et al. 2006). In general, as optical properties are less significant, the paper used for wrapping and packaging purposes and certain varieties of absorbent grades do not need bleaching.

12.4.1 Biobleaching

The use of microbial enzymes, such as xylanases and laccases, is considered a simple, economical, and environment-friendly choice for pulp bleaching. The use in the paper industry of biological agents, such as enzymes or whole microorganisms in the bleaching process of pulp, is known as biobleaching (Kumar et al. 2016b). Pretreatment of xylanase pulp is referred to as “prebleaching” or “bleach boosting” since it increases the penetration of bleaching chemicals through splitting the xylan network, which helps to extract the trapped lignin from the pulp fibre instead of directly extracting lignin or targeting the chromophores dependent on lignin (Bajpai 1997; Gangwar et al. 2014). For the paper industry, the use of xylanase in bleaching is a cost-effective option that provides different bleaching benefits. Xylanase is very effective in reducing bleaching chemicals, such as chlorine or chlorine dioxide, and can also minimize AOX by up to 25% in effluent discharge (Atik et al. 2006; Gangwar et al. 2014; Manji 2006; Nie et al. 2015). A significant role in the bleaching of pulp has been played by cellulase-free xylanases. Xylanase preparations contaminated with cellulase will increase the degradation of cellulose, which adversely affects the pulp’s viscosity and power. Therefore, cellulase-free xylanases are ideal for pre-bleaching of pulp. Xylanase should also be active at high temperatures and at a wide pH range (alkaline range) to improve its bleaching applicability. Because of the high temperature and alkaline pH of the method of pulp bleaching, it is desirable for the enzyme to be able to work at alkaline and high temperatures with an incubation time of up to 3/5 h (Gautam et al. 2015; Techapun et al. 2003). Cellulase-free xylanases from different microorganisms including bacteria and fungi have been successfully used in biobleaching of pulp. By hydrolyzing xylan, xylanase helps to extract lignin and promotes the release of lignin from the pulp, which improves brightness, improves the properties of the pulp, decreases the amount of kappa, and thereby decreases the use of chlorine as a bleaching agent. During the bleaching process, xylanase pulp pretreatment greatly reduces the chlorine demand from 10% to 50% (Kumar et al. 2016b). In 2015, Sharma et al. performed Kraft pulp biobleaching using xylanase from *Bacillus halodurans* FNP135 via submerged (SmF) and solid-state fermentation (SSF) and recorded a 35–30% decrease in kappa volume, a 5.8% and 4.3% increase in brightness levels, and a 20% and 10% decrease in chlorine demand for inoculated pulp compared to uninoculated pulp for SmF and SSF. Zheng et al. (2012) carried out a study on the biobleaching of cotton stalk pulp by using xylanase and observed 50% reduction in chlorine dioxide with 3.65% improvement in brightness by xylanase pretreatment.

12.5 Deinking of Waste Paper

Deinking is characterized as the separation of dirt and ink particles from waste paper and is the most important step in the recycling of waste paper. The recycling of waste paper is an environmentally sustainable and economically beneficial process, because it decreases the use of raw materials dependent on forests and reduces the amount of recycled paper entering landfills. Recycled fiber substitution of virgin pulp saves green plants and lowers water and electricity per ton of pulp per ton of pulp (Makinen et al. 2013). The main aim of recycling paper is to eliminate ink and other contaminants while preserving the optical and strength properties of fibers (Bajpai 2010). Compared to virgin fibre, recycling of waste paper requires 23–70% less energy consumption. In addition, large quantities of chemical agents, such as sodium hydroxide, sodium carbonate, diethyleneetriaminepentacetic acid (DTPA), sodium silicate, hydrogen peroxide, and surfactants are needed by most traditional deinking techniques, resulting in costly wastewater treatment. Alternatively, the use of enzymes to extract toner particles from fibers has been documented as an effective and less polluting approach to solve this problem of disposal (Marques et al. 2003; Pathak et al. 2011). Cellulases are the best option in most situations, but other enzymes, such as hemicellulases, amylases, and lipases, may help to optimize the process depending on the paper and ink form (Bajpai 2010). In their studies, Dutt et al. (2012), showed that concoctions of cellulase, xylanase, and amylase-containing enzymes are very effective in deinking sorted office paper.

12.5.1 Traditional Method

Significant amounts of surface-active chemicals are used for the detachment of ink particles from the fiber surface in traditional deinking processes. Sodium hydroxide, hydrogen peroxide, sodium silicate, surfactants, and chelating agents are the primary chemicals used in deinking (EDTA, DTPA). In deinking process, these chemicals play various roles: subsequent fiber swelling, ink particle wetting, flocculation, agglomeration, and chromophore oxidation/reduction. During the pulping of waste paper, sodium hydroxide retains an alkaline pH range of 9.5–11.0. This action breaks the connection between the fiber and the ink particle, so the ink particle does not swell. It causes fiber swelling. It also enhances fiber flexibility, which helps to extract ink particles by flexing the motion of the pulper. In addition, sodium hydroxide will return the binders (resins) in ink to their constituent components to saponify or hydrolyze. The resin breakdown method allows the pigment to differentiate from the fibre. The process of breaking down of resin assists pigment to separate from fibre (Behin and Vahed 2007; Lassus 2000). Depending on the printing method and ink formulations, various types of paper grades exhibit distinct deinkability in the deinking process. It is easy to deink newspaper grades printed with oil-based inks through traditional deinking processes. It is more difficult to deink non-impact printed papers usually created by photocopy and computer printing (Bajpai and Bajpai 1998; Lee et al. 2011) because it is also difficult to extract cross-linked inks used in offset lithography. In traditional flotation deinking, water-based flexographic newsprint grades of paper can trigger problems. The most daunting recycled paper to drink is MOW. Using photocopiers and laser printers that fuse the ink with the fibres, a significant portion of these fibers are printed, making it impossible to extract ink using traditional methods. MOW is a huge, practically untapped source of high-quality fibers that can be used if the deinking process can be improved for fine papers and many other items (Bajpai and Bajpai 1998). Many chemicals have also been used for bleaching deinked pulp. Chlorine-free bleaching of secondary fibre is more common. Hydrogen peroxide, oxygen, and ozone are used as oxidative bleaching agents for post-bleaching of deinked pulp (Pratima 2014).

12.6 Enzymatic Process of De-inking

In the deinking process, different enzymes are used and can be divided into three separate classes based on the attack on their substrates.

12.6.1 Ink Degrading Enzymes

Lipases and esterases are enzymes that can dissolve ink based on vegetable oil and are efficient in decoding waste paper printed with ink based on soy bean oil. In addition, lipases are amphiphilic in nature and may have a surfactant effect that allows waste paper decoding (Verma et al. 2012). Cellulases, xylanases, pectinases, and laccases may attack the fiber surface and modify the fiber surface or bonds in the vicinity of ink particles, which facilitates the removal of ink by washing and flotation (Bajpai and Bajpai 1998). By increasing fibrillation or extracting surface layers of individual fibres, enzymatic pulp treatment most likely weakens the bonds. The most studied enzymes for the deinking process are cellulases and xylanases. Cellulases peel the fibrils from fibre surfaces, leaving the fragments of ink-free for removal (Bajpai and Bajpai 1998; Bolanca and Bolanca 2004).

12.6.2 Starch Degrading Enzymes

Amylases are the enzymes that can act on starch and assist cellulases in the detachment of ink particles from the surface of fibre. Starch is present in MOW as a surface sizing agent and dry-strength additive, the degradation of starch may be helpful in ink particle removal from fibre surface (Dutt et al. 2012; Elegir et al. 2000).

12.7 Enzymatic Catalysis and Biotransformation

Prior to mechanical processing, enzymatic treatment of pulp was successfully used to minimize energy consumption in the refining process. Cellulases and xylanases, for the most part, are the enzymes used in the refining process. The treatment of cellulose fibers with xylanases allows the degradation of hemicelluloses or xylans, which enables the penetration into spaces inside cellulose fibers of water molecules that can break down a part of the hydrogen bonds linking the cellulose chains. The three-dimensional structure of cellulosic fibers becomes loose and more flexible due to breakage of hydrogen bonds between cellulosic fibers, resulting in a more compact paper structure with higher physical strength properties (Buzała et al. 2016). Cellulases hydrolyze β-1 in the cellulosic chain with a 4-glycosidic bond. Exoglucanase, endoglucanase, and β-glucosidase are composed of cellulases. Exoglucanase is the crystalline component and endoglucanase is the amorphous component of the cellulosic chain. Enzymatic pulp treatment must not, however, result in the degradation of the strength properties of paper. Hence, one of the most critical requirements is the selection of appropriate enzyme preparations for refining. The cost of the enzyme during the selection enzyme is another consideration (the total cost of the process must not increase) (Buzała et al. 2016). In fibre surface modifications, enzymes are found to be effective. This may influence the bonding of fibre, and consequently the paper strength properties of paper. Fibrillation, durability, and collapsibility are enhanced by enzymatic therapy, leading to improved sheet consolidation and network packing. Cellulases and xylanases have been used individually or in combination to minimize energy consumption in mechanical processing for enzyme-assisted pulp refining (Mansfield and Dicksont 2001; Singh et al. 2014). Cellulases and xylanases from different fungal sources are effective, along with improved drainage, in reducing energy consumption. In 2015, Singh et al. conducted enzymatic pre-treatment of bleached mixed hardwood pulp with biocatalyst cellulase and processed pulp treated with cellulase in the PFI mill. This procedure produced a reduction of approximately 29% in processing energy (specific energy consumption decreased from 1.33 to 0.94 kW h/kg pulp) at a dosage of 0.07 IU/g OD pulp after a reaction period of 90 min with improved pulp consistency. Buzała et al. (2016) studied the impact of pretreatment with cellulases and xylanases on Kraft pulp refining and observed that treatment with cellulase significantly improved the sensitivity of the pulp to refining, increased water retention value (WRV) and fines content while significantly decreased the weighted average fiber duration. Such changes in pulp parameters have caused the properties of paper strength to deteriorate. Xylan treatment partially hydrolyzed xylan, small amounts of which are associated with cellulose fibers that slightly loosened the structure of fibers. Therefore, treatment with xylanase greatly reduced refining energy and enhanced the paper's static strength properties. Gil et al. (2009), using two industrial enzyme preparations, Celluclast® 1.5 L (cellulase mixture) and Viscozyme® L (arabinase, cellulases, β-glucanases, hemicellulases, and xylanase), conducted the refining of a bleached Eucalyptus globule Kraft pulp. The pulp drainage rate and the degree of hydration of the fibres were improved by enzymatic pretreatment before refining. In the case of cellulase, this effect was more pronounced, possibly because most of these enzymes destroy the surface cell wall. Apparently, this oxidation exposed a greater surface area for bonding with the water molecules, leading to higher hydration. After enzymatic treatment, the strength properties of the pulp were positively impacted, although some degradation of the fibers was noted in the case of cellulase (Gil et al. 2009).

12.8 The Delignification of Pulp

Historically, the first-time application of enzymes to change pulp properties was reported by Paice and Jurasek in 1984, and later on, by Viikari et al. (1986) who reported that a similar enzyme treatment decreased the need for chemicals to bleach the pulp. In the paper and pulp industry, xylanases have been widely tried since they demonstrated lignin extractability from Kraft pulp by depolymerizing xylan in the plant cell wall closely associated with lignin. Commercial xylanase treatment of eucalyptus pulp, such as Novozyme 473 and Cartazyme HS-10, decreased chlorine intake by 31% and improved final brightness by 2.1–4.9 points. When used at 10 U/g pulp, Xylanase P (a commercial enzyme) increased Kraft pulp brightness by 5.6 points and induced a 10% decrease in chlorine use (Madlala Andreas et al. 2001). The use of xylanases will minimize 5.0–7.0 kg of chlorine dioxide per ton of Kraft pulp and a decrease in the kappa number (KN) of pulp by an average of 2.0–4.0 units (Polizeli et al. 2005). Xylanase from *Bacillus megaterium* reported an improvement in brightness and viscosity of 8.12% and 1.16%, a decrease in KN of 13.67%, and a decrease in chlorine intake of 31% (Indu et al. 2006). Xylanase by B. Stearothermophilus SDX decreased the intake of chlorine by up to 15%, while its combination with pectinase resulted in a reduction of 20% (Dhiman et al. 2008). *Bacillus pumilus* SV-85S alkali stable and thermo-tolerant xylanase showed a decrease in KN by 1.6 points and a rise in brightness by 1.9 points (at pH 9.0 at 2.0 h at 55 °C). Pulp pretreatment with xylanase resulted in a chlorine consumption reduction of 29.16% while retaining the same brightness as in the control (Nagar et al. 2013).

12.8.1 Xylanases Mechanism in Delignification of Pulp

Senior et al. (1999) studied the xylanase polymer hydrolyses xylanase that occurs within pulp fibers. Xylan is closely related to cellulose and lignin, so it follows that their separation during bleaching is impaired by the xylan backbone condition. Xylanase increases the fiber wall swelling and in turn increases the speed of diffusion through the walls (Clark et al. 1991). In addition, it was suggested that if lignin, covalently bound to xylan, was made smaller by enzyme use, it would be easier to extract. Another theory that came from the analysis was that the xylanase enzymes catalyze the xylan hydrolysis that has reprecipitated on the fibers during alkaline pulping. Xylanase is a bleaching aid rather than a true delignification agent since lignin is not specifically degraded by the enzyme (Woolridge 2014).

There is also a growing interest in the production of new xylanases and product formulations to enhance their compatibility for industrial applications (Serradella and Degreve 2009). Xylanases are generated by using microbes that are either naturally free of cellulase-producing capacity or have been mutated or genetically engineered to remove the cellulases. The most important development is the processing of xylanase, such as Ecopulp TX-200C, which works at alkaline pH and high temperatures. Advances in enzyme-processing technology have lowered production costs dramatically, making enzymes a fair economic option for environmentally sustainable bleaching (Senior et al. 1999).

12.8.2 Application of Laccases in the Delignification of Pulp

Laccases are oxidative enzymes that have been influenced by the paper and pulp industry by their distinct merits than any other bleaching enzyme. Laccases, together with mediators, can delignify the pulps through an oxidation chain reaction that leads to lignin oxidation without the degradation of cellulose. Laccases without mediators are not oxidized by lipophilic groups and non-phenolic lignin compounds (Singh et al. 2015). According to reports in the literature, over 100 compounds were investigated as potential laccase mediators for their ability to oxidize lignin or lignin models by selective oxidation of their benzyl/hydroxyl groups (Cañas Ana and Camarero 2010; Virk Antar et al. 2012). Synthetic mediators minimize the KN (residual lignin content calculation or bleachability of wood pulp) that increases after the L stage (laccase enzyme treatment stage) and decreases after the E stage (alkaline extraction stage). In the case of natural mediators, the KN increases and brightness decreases during the L stage. Synthetic mediators are likely to induce the degradation and/or oxidation of carbohydrate chains in cellulose. Natural mediators oxidize carbohydrate chains in cellulose into carbonyl groups during the L process, thus making the pulp susceptible to degradation by the heavy alkaline medium used in the bleaching step (Fardin and Durán 2004). The possibility of expanding the laccase range of oxidation by redox mediators provides the essential biotechnological potential for pulp bioleaching. It is of particular merit to using such compounds as these radicals can continue to oxidize other compounds until they are oxidized to stable radicals by laccases, including those that are not directly used as enzyme substrates.

12.8.3 Delignification of Pulp with Fungal Laccases

A 20–27% decrease in KN resulted in a Trametesvillosalaccase-HBT method for biobleaching of eucalyptus pulp. An alkaline extraction stage (E), however, raised delignification to 41–45%, far greater than that obtained without enzymes in the control (16–23%). The laccase-HBT device treatment of the pulp decreased the amount of hydrogen peroxide required for subsequent alkaline bleaching by a factor of 3–4 compared to the control factor (Moldes and Vidal 2008). Recombinant laccases were also tested to determine their delignification effectiveness. For the biobleaching of softwood Kraft pulp, *pycnoporus cinnabarinus* laccase fused with the C-terminal linker and carbohydrate-binding module of *Aspergillus niger* cellobiohydrolase B was used in the presence of HBT (Holy et al. 2009). The first proof of natural phenols (syringaldehyde, SA; acetosyringone, AS) to mediate eucalyptus pulp delignification with *P. cinnabarinus* laccases at pH 4.0 and 50 °C was demonstrated by Susana et al. (2007).

When laccase mediators were tested for sinapic acid, ferulic acid, coniferyl aldehyde, and sinapyl aldehyde, they showed lower bleaching efficiency for sisal pulp, because these phenolic compounds appear to bind to pulp fibers (Elisabetta et al. 2009). As natural mediators for laccase from *P. cinnabarinus* at pH 4.0 and 50 °C to bleach flax fibers, Amanda et al. (2010) evaluated SA, AS, and *p*-coumaric acid (PCA). The performance of these three was contrasted with that of HBT in terms of the stability of laccases. In the absence of pulp, HBT and PCA were found to inactivate laccases. After subsequent alkaline treatment with hydrogen peroxide, all-natural mediators resulted in a reduced KN. In general, natural mediators have been found to increase KN, decrease brightness, and change the optical properties of the pulp after the L stage, indicating that during a laccase-mediator procedure, natural mediators appear to pair with fibers (Glòria and Vidal 2011).

12.8.4 Delignification of Pulp with Bacterial Laccases

The majority of laccases assessed for lignin degradation belong to fungi, while widespread in bacterial genomes, bacterial laccases are not so widely characterized. Bacterial laccases with mediators are also capable of biobleaching various pulps. In the presence of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), Streptomyces cyaneuslaccase reduced KN and increased brightness by 2.3 U and 2.2%, respectively (Arias et al. 2003). To assess its bleaching ability, an alkalophilic cellulase-free laccase from γ-proteobacterium JB was applied to wheat straw-rich soda pulp by optimizing the conditions using surface methodology based on central composite nature. The model was used by selecting laccase units, the concentration of ABTS, and pH as model variables.

The findings of second-order factorial design experiments showed that the brightness and KN of laccase-treated pulp had a major effect on all three independent variables. 20 nkat/g of pulp, 2 mM ABTS, and pH 8.0 were ideal conditions for biobleaching of pulp with laccase preparation (specific activity, 65 nkat/mg protein), which increased brightness by 5.89% and decreased KN by 21.1% within 4 h of incubation at 55 °C without further alkaline pulp extraction (Singh and Chen 2008). Laccases have an advantage over other lignin-oxidizing enzymes such as lignin peroxidases (Lip), in that with a decrease in the pH of the reaction environment, Lip's redox potential increases. Such data is not available for laccases, as logically alkali-tolerant laccases would be the best option for pulp bleaching where alkaline conditions are needed under laboratory scale (Singh et al. 2009; Cañas Ana and Camarero 2010).

12.8.5 Synergistic Effects of Enzymes Involved in Biobleaching of Pulp

The mechanisms of delignification of xylanases and laccases differ because xylanases help increase delignification by making pulp more prone to attack by bleaching chemicals, whereas laccases act directly on lignin and cause pulp extraction (Virk Antar et al. 2012). In parallel therapies, the combination of xylanase and the laccase-mediator bleaching method results in enhanced pulp brightness and decreased KN. The competitiveness of enzyme-based, environmentally sustainable processes over current methods may be increased by such an outcome (Kapoor et al. 2007). For biobleaching of mixed wood pulp, mixed-enzyme preparation of xylanase and laccase was evaluated. The enzymes were produced under solid-state fermentation through the co-cultivation of mutant *Penicillium oxalicum* SAU (E)-3.510 and Pleurotus ostreatus MTCC 1804. Compared to xylanase alone, whitening of pulp with mixed-enzyme preparation resulted in a notable decrease in KN and increased brightness. A study of bleaching conditions suggested that the preparation of a mixed enzyme (xylanase:laccase, 22:1) resulted in increased delignification when bleaching was carried out for 3 h at 10% pulp consistency (55 °C, pH 90) (Dwivedi et al. 2010). Up to 10 kg substratum level, xylanase and laccase were cost-effectively to develop and evaluated in elemental chlorine-free bleaching of eucalyptus Kraft pulp. ClO_2 savings were higher with the sequential treatment of xylanase followed by laccase (35%) on a laboratory scale compared with pulp prebleached with xylanase (15%) or laccase (25%) individually. When applied on a pilot scale (50 kg pulp), the sequential enzyme treatment resulted in improved pulp properties (50% decreased post color number, 15.71% increased the tear index) and decreased levels of organochlorine compound (measured as AOX) (34%) in bleach effluents (Sharma et al. 2015).

12.9 Role of Enzymes in Pitch Control

Pitch is made up of fatty acids, sterols, resin acids, fatty acid glycerol esters and other fats and is generally considered to be the methylene soluble wood portion. It is less than 10% of the wood's total weight, but it causes serious issues. These deposits have detrimental effects, such as altered pulp water absorption, hole forming, and paper tearing due to sticky deposits on dryer rolls and imparting discoloration and hydrophobic spots on the paper. High pitch content was present in certain types of wood pulps, including sulfite pulps and various mechanical pulps, especially from pines (Gutiérrez et al. 2001). As an effective biotechnological process, pitch extraction is possible with enzymes. Enzymatic pitch management helps to reduce pitch-related issues to a sufficient degree. It reduces paper web defects as well as the frequency of paper machine cleaning pitch deposits. It has been reported that the laccase-mediator system works selectively on lignin, but its function in removing lipophilic compounds that cause pitch deposition in woody and non-woody paper pulps has recently been identified (Gutiérrez et al. 2006). The role of lipases in pitch modulation, such as the commercially available lipase, Resinase A 2X (Novo Nordisk AG), which is a recombinant lipase expressed in A, has been demonstrated *Oryzae*. In a pine (Pinus densiflora) mechanical pulp, resinase hydrolyzed 95% of the triglycerides. Resinase treatment also reduced the number of deposits, decreased the number of paper spots and cracks, reduced the dose of talc to manage pitch deposition, and allowed higher volumes of fresh wood to be used. For enzymatic regulation of pitch, lipidase 10,000 (American Lab. Inc.) and Candida and aspergillus lipases have been investigated (Virk Antar et al. 2012).

12.10 Conclusion

The continuously growing pulp and paper industry utilizes many chemicals that are released into the effluent in the papermaking process. In different stages of the papermaking process and degradation, this chapter discusses the use of microbial enzymes or whole microorganisms, which mitigates the consumption of hazardous chemical compounds in the pulp and paper industry. Cellulase-free xylanases in biobleaching are effective in reducing the use of chlorine compounds to make the process environmen-friendly. Cellulases, xylanases, and amylases are utilized to increase the quality of the paper and decrease the demand for deinking chemicals in the process of deinking of recycled fiber. The use of white-rot fungi in biopulping and using enzymes such as cellulases and xylanases in enzymatic refining can minimize mechanical energy consumption. From the environmental viewpoint, due to their biodegradability, enzymes are more appropriate. The enzyme receives one or more electrons from the substrate and donates these electrons to an electron acceptor in the case of reactions where the target pollutant is oxidized. The enzyme is therefore regenerated at the end of the reaction and is ready for the next catalytic cycle. The biological origin of enzymes decreases their adverse environmental effects, rendering the treatment of enzymatic wastewater an ecologically viable technique. Microbial technology developments have also been shown to be a cost-effective and eco-friendly alternative to chemical-intensive measures in the pulp and paper industry.

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Microalgal Bioremediation: A Clean and Sustainable Approach for Controlling Environmental Pollution

13

Yuvraj

Abstract

Environmental pollution is a major global threat today, with widespread consequences. Industrial effluents, flue gases, automobile emissions, solid waste, agricultural runoff, amongst others, have loaded air, water, and soil with a plethora of undesirable substances harmful for humans and their surroundings. Common pollutants, such as exhaust gases, heavy metals, pesticides, pharmaceuticals, and many emerging organic and inorganic chemicals, are causing multitude of chronic illnesses. With growing population and rapid industrialization, it is becoming increasingly important to develop efficient, cheap, sustainable, and scalable processes to mitigate these life-threatening pollutants.

Conventional physiochemical methods used for the treatment of industrial, municipal, and agricultural wastewaters and emissions are effective, but they suffer serious drawbacks, such as sludge generation, membrane fouling, and high energy and reagent requirements. This has attracted the use of biological resources in development of sustainable and eco-friendly remediation processes. Microalgae particularly have emerged as a potential microorganism in bioremediation owing to their ability to adsorb, accumulate, and degrade many common pollutants using different mechanisms. Concomitant sequestration of carbon dioxide, generation of oxygen, and accumulation of lipids and carbohydrates with growth are however the real advantages of using microalgae in bioremediation. Moreover, simple and cheap nutritional and cultivation requirements of microalgae make it most suitable bioresource for mitigating pollution. The development of microalgae-based remediation processes is therefore an ambitious goal in environmental biotechnology. This chapter reviews important

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concepts, developments, challenges, and future prospects of microalgal bioremediation.

Keywords

Bioremediation · Environmental pollution · Microalgae · Wastewater

13.1 Introduction

Environmental pollution is on the alarming rise globally, threatening all life forms and ecosystems. Rapid industrialization, urbanization, mining and exploration, with growing population are leading causes of the increasing levels of pollution. Air, water, and soil, which are vital for life, are contaminated with a chemically diverse group of undesirable substances released mainly from anthropogenic activities. These pollutants have severely deteriorated the quality of ambient environment which is fundamental to the healthy living of humans. Incidences of cardiovascular and respiratory disorders, lung, skin and colorectal cancers, chronic kidney disease, and other fatal illnesses linked to pollution are becoming common. Air pollution alone claimed 4.2 million lives worldwide in 2016, when more than 90% of the world's population was living in places where air quality was below standard (WHO 2018). Similarly, contaminated drinking water has been estimated to cause millions of premature deaths each year, with at least two billion people consuming unsafe water (WHO 2019). Another grave consequence of pollution is global warming, resulting from increasing levels of CO_2 and other air pollutants. Soaring temperatures are responsible for rising sea levels and climate change all around the world. Animals are also not untouched by this threat; pollution is making their lives miserable. Millions of seabirds and aquatic animals are killed by marine pollution every year (UNEP 2001). It is believed that climate change has put half of the species of plants and animals at risk in some of the world's most biodiverse regions (WWF 2018). While these disturbing facts represent only a small fraction of the statistics available on menaces and mortalities due to pollution, they are sufficient to understand the serious and widespread impacts of pollution on human health and environment.

Pollution management is a multi-facet programme, with at least two basic components: regulation and control. Pollution prevention laws and policies have been adopted by several nations to limit the adverse effects of pollution. United Nations (UN) and its agencies have enacted international treaties to address common issues in environmental pollution. Practicing such regulations to reduce or eliminate the release of pollutants is called pollution control. Controlling emissions and effluents relies mainly on the use of pollution controlling devices (PCDs) and adopting certain processes and practices, such as recycling, reusing, and reducing the amount of waste. PCDs are based on different physical and chemical techniques to remove pollutants from industrial, municipal, and agricultural wastewaters and emissions. Separation of pollutants from contaminated fluid streams is generally based on their physical size (course filtration, sedimentation, membrane separation), electrical charges (electrostatic attraction, ion-exchange, adsorption), and chemical properties (precipitation, redox, neutralization, complexation, absorption). Although these conventional techniques are efficient in removal of pollutants from their sources, their applications in treatment of wastewater generally have certain limitations and drawbacks. Membrane fouling, reagent requirements, sludge treatment, high energy requirement are some of the disadvantages of physical and chemical methods for treatment of effluents (Crini and Lichtfouse 2019). Moreover, PCDs based on these separation principles are suitable for point sources of pollution or when the pollutant is not widespread. For instance, treatment of oil spills, contaminated groundwater, and polluted land cannot be accomplished with such methods. An alternative approach, which is more eco-friendly, economic, and sustainable, is to use living organisms to mitigate pollution. Known as bioremediation, the technique encompasses all processes employing living organisms (bacteria, fungi, and plants generally) to remediate environment. It takes advantage of biochemical reactions and natural biological processes which enables living organisms to ingest and breakdown nutrients and other compounds. Bioremediation can be performed at the site of contamination (in situ), as in case of oil spills, or away from the site (ex situ) depending on the requirements. Bioremediation is not a new technique; humans have always relied on many natural biological processes to reduce waste, such as the action of soil microbes to decompose human and animal waste. Perhaps the most well-know and important example of bioremediation which has been in practice since decades on a large scale is the decomposition of sewage using bacteria in wastewater treatment plants. Similarly, the use of certain plants for restoration of land and water contaminated with heavy metals and other toxic organic and inorganic substances, known as phyto-remediation, is believed to have evolved over the past several hundred years. Nevertheless, a variety of new and innovative applications and processes in bioremediation to remove wide range of pollutants in many different environmental settings have been increasingly investigated and put in practice (Shah et al. 2020).

13.2 Microalgae and Its Biotechnological Importance

Microalgae are diverse group of unicellular eukaryotic organisms capable utilizing light as a source of energy for fixing carbon dioxide by the process of photosynthesis. They have terrestrial, freshwater, and marine habitats. Microalgae were amongst the first life forms on earth and played a big role in developing our atmosphere by enriching it with oxygen produced as a by-product of photosynthesis. They are still vital for life on our planet as they contribute to approximately half of oxygen in atmosphere by capturing large amounts of carbon dioxide, maintain the flow of matter and energy to aquatic heterotrophs by forming the base of marine food chain, and are a great source of many valuable compounds.

Microalgae are an enormous biological resource, representing one of the most promising sources for new products and applications. They have several important biotechnological applications. Due to their well-balanced chemical composition, they are used as dietary supplements and also to enhance the nutritional value of food and animal feed. They are cultivated as a source of highly valuable bio-molecules such as polyunsaturated fatty acids (PUFA), pigments, antioxidants, and pharmaceuticals (Koller et al. 2014). Their ability to grow on inexpensive sources of carbon (CO_2) and energy (light) makes them a potential feedstock for the production of low-priced biofuels. Worldwide research is in progress to develop cost-effective technologies for production of biofuels from microalgae, especially in the form of biodiesel as an alternative to fossil fuels which are getting depleted and are also responsible for polluting our environment with greenhouse gases contributing to global warming (Brennan and Owende 2010).

13.3 Microalgal Bioremediation

Microalgal bioremediation, known as phyco-remediation, is another potential application of microalgae in biotechnology. Microalgae-based bioremediation has several advantages over other common bioremediation techniques such as phyto-remediation (plant based), myco-remediation (fungi based), and microbial biodegradation of pollutants. The fact that microalgae have simple and inexpensive growth requirements, fix carbon dioxide and produce oxygen, accumulate high amounts of storage molecules (carbohydrates and lipids), act on a wide variety of pollutants, and grow equally well in water and on land have made them an extremely promising candidate in bioremediation. Adsorption and absorption of toxic heavy metals by different strains of microalgae, a phenomenon known as biosorption, assimilation of nitrogen and phosphorus containing organic and inorganic compounds as nutrient source for growth, and removal of several toxic compounds makes microalgae an attractive choice for the bioremediation of wastewater. Similarly, their ability to fix CO_2 and tolerate its extremely high levels has made them an attractive scrubber for bio-mitigation of CO_2 in flue gases. Moreover, their ability to simultaneously perform these different remediation tasks, colloquially known as coupling of processes, is highly beneficial and masks the cost of cultivation.

13.3.1 Heavy Metals

Amongst different classes of water pollutants, heavy metals are most hazardous as they are toxic, persistent, and non-biodegradable. Consequently, they accumulate in nature, causing many serious health problems and environmental issues (Tchounwou et al. 2012). Heavy metal contamination of water and land results mainly from industrial activities as metallurgical, mining, paint, plastic, textile, tannery, agrochemicals, electronics, and other industries continue to use heavy metals in production processes. Arsenic (As), Cadmium (Cd), chromium (Cr), mercury (Hg), and lead (Pb) are some of the common toxic heavy metals present in industrial effluents (Tchounwou et al. 2012). Removal and recovery of heavy metals from wastewater is therefore extremely important. Common physiochemical techniques (chemical precipitation, ion exchange, membrane filtration, electrochemical treatment, chemical oxidation, or reduction) for treatment of heavy-metal contaminated wastewater are efficient but suffer many drawbacks which were discussed earlier. Bio-adsorption (colloquially known as biosorption) and bioaccumulation of heavy metals, particularly using microalgal biomass, offers a comparatively economic, eco-friendly, and efficient solution for heavy metal separation from wastewater and polluted soil (Singh et al. 2019).

The term ‘biosorption’ refers to the phenomenon whereby any biological material, living or dead, adsorbs heavy metal ions. Adsorption of metals, being a surface event, is a metabolism-independent phenomenon and is therefore observed in living as well as in dead biomass. Ion exchange, chelation/complexation, and surface precipitation (microprecipitation) are different mechanisms reportedly involved in the attachment of metals ions on to the surface of biosorbent (Davis et al. 2003). The passive extracellular binding of metal ions is largely dependent on the ion-exchange between metal ions and functional groups of polysaccharides, lipids, and proteins present on the surface of biomass. The other mechanism involved in the removal of heavy metals from contaminated water is the absorption/uptake of heavy metals by active transport of the metal ions across cell membrane. Observed only in living biomass, the absorption of metal ions is thus considered as a metabolism-dependent process. However, as metal ions cannot undergo any change by biochemical reactions operating inside cells, the ions remain (or accumulate) inside the biomass. The bio-absorption is hence termed as bioaccumulation, to distinguish it from biosorption which is entirely a surface phenomenon. Many different biological materials, dead and alive, have been shown to exhibit potential biosorption properties. These include bacteria, fungi, algae, agricultural waste, amongst others (Carolin et al. 2017). Amongst these, microalgal biomass is found to be the most promising biomass for heavy metal removal as it does not require any pre-treatment, low cost, also effective at low metal concentrations, and most importantly has high metal-binding capacity (Anastopoulos and Kyzas 2015). Many different species of microalgae have been demonstrated to remove heavy metals from aqueous solutions (Bilal et al. 2018).

13.3.2 Fertilizers, Detergents, and Other Polluting Nutrients

Like any other microorganism, microalgae need a source of nitrogen, phosphorus, and other macro-elements for growth. However, tolerance of microalgae to high levels of these nutrient minerals and ability to grow on their different sources is what makes them different from other microorganisms in this regard. Nitrogen and phosphorus, being main macronutrients for plant also, are however one of the major water pollutants. These plant nutrients provided as fertilizers for enhancing agricultural productivity contaminate water and land when washed away by agricultural runoff. Similarly, detergents containing nitrates and phosphates, untreated sewage, surface runoff, and wastewaters from other sources (industrial, poultry, and aquaculture) when discharged into water bodies contaminate water with excess nitrogen and phosphorus. This results in a chain of events, known as eutrophication, which deteriorates water quality and disturbs aquatic life. Eutrophication starts when excessive nutrient makes algae and aquatic plants to flourish; however, these plants eventually die as algal blooms blocks sunlight from reaching the bottom of water body. Bacterial degradation of the dead organic matter depletes oxygen, resulting in the death of aquatic animals.

Microalgae, which are the primary cause of eutrophication along with plants, can itself be used to reclaim wastewaters polluted with nutrients. Many species of microalgae have been demonstrated to grow effectively well and efficiently remove nitrogen and phosphorus from wastewaters. Yuvraj et al. (2016) shown that *Chlorella* can completely assimilate nitrate and phosphate from growth medium. There are many advantages of using microalgae over other microorganisms in treatment of wastewater polluted with nutrients. Since microalgae can tolerate high concentration of polluting nutrients, grow under mixotrophic conditions, simultaneously adsorb and accumulate heavy metals, and does not produce any secondary by-products, it is regarded as the most promising organism for bioremediation of wastewaters. Phtyoremediation of nutrient polluted water is also effective and practised widely, but it requires more time, space, and efforts.

13.3.3 Gaseous Air Pollutants

While contributing to half of the oxygen on earth, microalgae sequester billion of tons of CO_2. The ability of microalgae to fix CO_2 by the process of photosynthesis makes it extremely promising amongst strategies to mitigate rising levels of CO_2 in the atmosphere. Burning of fossil fuels to generate energy in power plants, industries, automobiles, and other anthropogenic activities with increasing deforestation has resulted in the global rise of atmospheric CO_2 levels. Over the past two centuries, there has been a phenomenal increase in CO_2 levels by more than 45% due to rapid industrialization (https://www.co2levels.org/). Carbon dioxide, methane, nitrous oxide, and fluorinated gases released mainly from different industrial, transportation, and agricultural activities, traps energy from sun by absorbing and gradually radiating it back as heat, a phenomenon known as greenhouse effect. Increasing levels of greenhouse gases in the atmosphere has caused global warming which is responsible for extreme weather disturbances, climate change, rising sea levels, and altering ecosystems all around the world. CO_2, along with other gaseous air pollutants (CO, SO_2, NO, N_2O, H_2S, benzene, ethylene) released mainly from automobiles, burning of solid and liquid fuels, power plants, and refineries causes serious respiratory conditions. These air pollutants not only affect biotic environment but also abiotic environment. Emissions of nitrogen oxides (NO_X) and sulphur dioxide (SO_2) get dissolved in atmospheric water to form nitric and sulphuric acid, respectively. These acids then precipitate on ground as acid rain, corroding stones and metals, acidifying soil, surface, and groundwater.

Reducing carbon footprint has therefore become a global priority. Lowering the reliance on fossil fuels by promoting and using renewable sources of energy which are cleaner or carbon-neutral, adapting environmental-friendly industrial practices and lifestyle, reducing deforestation and planting more trees can help mitigate air pollution. While numbers of alternatives, schemes, and mandatory measures have been put in practice to reduce the footprint of greenhouse gases globally, it appears that our dependence on fossil fuels can never end completely. Development of technologies to sequester CO_2 and other gaseous air pollutants is therefore extremely necessary. Many efficient physiochemical techniques are available to sequester CO_2 at different scales, but they are costly in operation and are useful for point sources with high CO_2 concentrations (Singh and Dhar 2019). Biosequestration of CO_2 using microalgae is a promising technique for carbon capture. Since microalgae are single-celled photoautotrophs, their carbon fixation rate, and hence rate of growth is higher than plants. Microalgae can grow over a wide range of CO_2 levels (Singh and Singh 2014; Yuvraj and Padmanabhan 2017), and the carbon-capturing process can be coupled with wastewater treatment, biofuel, and feed production to offset the cost of cultivation. Several species of microalgae have been demonstrated to tolerate high levels of CO_2 (Salih 2011). On the other side, microalgae can also grow well in low CO_2 levels due to their carbondioxide-concentrating mechanism (CCM) which enables them to perform photosynthesis when CO_2 levels are limiting (Wang et al. 2015). This characteristic of microalgae is particularly important in case of emissions with low CO_2 levels and offers advantage over physiochemical CO_2-sequestration techniques which are not suitable under such conditions. Another significant advantage of using microalgae for this purpose is its ability to utilize other air pollutants, such as NO_X and SO_X, which are generally found along with CO_2 in emissions from different sources. As these gases (except NO) are fairly soluble in water, they get dissolved in aqueous microalgal cultures to form nitric acid (HNO_3), nitrous acid (HNO_2), sulphurous acid (H_2SO_3), and sulphuric acid (H_2SO_4), which act as sources of nitrogen and sulphur for microalgae.

13.3.4 Pesticides

Pesticides represent a diverse group of chemicals and biological agents which are generally used to protect crops from pests, thereby enhancing agricultural productivity. Pesticides hence occupy an indispensable position in modern agriculture. However, the indiscriminate use of pesticides in agriculture has now become a serious threat to environment and human health. These agrochemicals contaminate soil, ground, and surface water during irrigation and agricultural runoff. As many pesticides, such as organochlorine, are persistent in nature, they gradually accumulate in environment and in higher organisms, including humans (Hassaan and Nemr 2020). Organochlorine pesticides (OCPs), which are classified as persistent organic pollutants (POPs), are also toxic as they stimulate the central nervous system. Most of the OCPs are therefore classified as environmental hazards and are restricted for use. Dichlorodiphenyltrichloroethane (DDT), the most common OCP used in agriculture as an insecticide since 1950s, which is also useful in controlling mosquito-borne diseases, is now banned in many countries due to its potential impact on the environment (Lai 2017). Nevertheless, prohibited OCPs are still used illegally in many countries owing to their different applications in agriculture and chemical industry.

Despite their potential health and environment risks, pesticides cannot be abandoned completely. Therefore, effective treatment of soil and water contaminated with pesticides is extremely crucial in protecting environment and human health from these hazardous chemicals. As in case of other pollutants, physicochemical methods for extraction of pesticides from contaminated resources already exist, but with similar drawbacks and limitations. Biological treatment, which is always eco-friendly, sustainable, and cost-effective, is promising in case of pesticide pollution also as many organisms have been demonstrated to possess potential of restoring pesticide-polluted environment. Like in case of other pollutants, plants, bacteria, fungi, and algae are known to be effective against pesticides also. Although bioremediation of pesticides using microalgae is less studied and only lab-scale studies are available, it is quite promising because of the advantages of using microalgae over other microorganisms in remediation processes are always very attractive. Adsorption, accumulation, and degradation of several pesticides have been reported in different microalgae. The green microalga *Chlorella vulgaris* has been shown to be effective in removal of several pesticides from their aqueous solutions mainly by the mechanism of biosorption and biodegradation (Hussein et al. 2017; Kumar et al. 2018; Baglieri et al. 2016; Hultberg et al. 2016).

13.3.5 Pharmaceuticals

Pharmaceutical compounds (PCs) are yet another class of chemicals which have been widely investigated for microbial degradation. Their increasing residues in wastewater, soil, surface, and groundwater has made PCs an emerging class of environmental pollutants. Eco-toxicity and health risks associated with PCs are a serious environmental concern now (Ebele et al. 2017). Moreover, the increasing levels of antibiotics in environment have accelerated a natural process whereby bacteria develop resistance against antibiotics. This phenomenon, known as antibiotic resistance, is making antibiotics less effective in treatment of bacterial infections. Antibiotic resistance has become a major threat to global health calling for an urgent need to check the indiscriminate use of antibiotics (Morehead and Scarbrough 2018).

The presence of PCs in aquatic environment has shown that conventional wastewater treatment is incapable of removing these substances completely (Snyder 2008). Although PCs do get degraded by natural processes (bio/photodegradation) and can be either low, moderate, or highly persistent depending on the degradation time required, they still possess risk due to their excessive use and constant release into the environment (Snyder 2008). Since PCs are designed to target specific molecular targets and display therapeutic effects at low concentrations, the evolutionary conservation of molecular targets increases the possibility of their pharmacological activity in non-target organisms also. Moreover, pollution of a certain class of PCs possesses a great threat to aquatic and animal life due to their ability to interfere with the endocrine system. These substances, known as endocrine disruptors, are present in several consumer products and pharmaceutical formulations. Although PCs are generally detected at very low concentration in freshwater, bioaccumulation of certain PCs in fishes and algae is yet another reason why these compounds can be life-threatening even at trace levels (Zenker et al. 2014).

Drawbacks of physical and chemical techniques for remediation of PCs (advance oxidation processes, adsorption on activated carbon, membrane separation) and the ability of living organisms to act upon diverse set of chemicals has made bioremediation most suitable in many respect for treatment of PCs also (Shah and Shah 2020). Microbial remediation of PCs and other emerging pollutants—such as personal care products, industrial additives and agents, surfactants, steroids and hormones, food additives, flame retardants, amongst others—is greatly investigated using bacteria, fungi, and microalgae (Girijan and Kumar 2020; Matamoros et al. 2016). Resistance against antibiotics has however raised concern over the use of bacteria for bioremediation of PCs. However, morphology and slow kinetics of fungal growth restrains the use of fungi for large-scale remediation of PCs. Microalgae thus emerged as a promising candidate for bioremediation of PCs and other emerging pollutants. Potential of microalgae in remediation of several emerging pollutants, mainly PCs, have been reported in number of recent publications (Sutherland and Ralph 2019). While biodegradation has been found to be the predominant mechanism involved in microalgal bioremediation of PCs, several studies have also reported biosorption of PCs with varying efficiencies. In addition to common biodegradation which takes place inside cells (intracellular), degradation of PCs outside microalgal cells (extracellular) also occurs with the help of excreted enzymes, and the breakdown products are further degraded inside cells (Xiong et al. 2018).

13.3.6 Radioactive Substances

Radioactive pollution is another form of environmental pollution which is extremely hazardous. Mining and transportation of radioactive ores, production and testing of radioactive weapons, disposal of radioactive waste from research laboratories and biomedical and industrial sites, nuclear explosions, activities and accidents at nuclear power plants, and other operations involving radioactive materials are common sources of radioactive pollution. Radioactive water from nuclear plants and industry contaminates soil, surface, and ground water with radionuclides. Radioactive (or radiological) contamination of environment is life threatening as any exposure to emitted ionizing radiations can lead to serious health conditions, ranging from acute to chronic, depending on the level and exposure to radiation. Conventional treatment process such as solvent extraction, ion-exchange, chemical precipitation, evaporation, and ultrafiltration/reverse osmosis are generally used for the separation of radionuclides from wastewaters. However, along with other general drawbacks of conventional physicochemical methods, these treatment procedures are feasible for point sources of pollutions.

Bioremediation of radioactive contaminants by bacteria has been extensively studied. Biosorption, accumulation, and enzymatic reduction of common radionuclides, such as uranium, thorium, and plutonium using bacteria have been reported in most of the studies (Prakash et al. 2013). Plants, algae, and cyanobacteria are also investigated for their potential towards the remediation of radioactive substances (Fukuda et al. 2014). Amongst these photosynthetic organisms, microalgae are promising microorganisms for the purpose. Potential of different microalgae to remediate wastewaters polluted with radionuclides of elements like uranium, thorium, cesium, radon, iodine, and strontium has been investigated (Pradhan and Sukla 2019; Iwamoto and Shiraiwa 2017; Fukuda et al. 2018, 2014; Lee et al. 2019). Although not much work is reported, microalgal bioremediation is considered as a promising approach for treatment of wastewater and aquatic environment contaminated with radionuclides.

13.4 Microalgae-Based Treatment of Industrial Wastewaters

Industrial wastewaters contain a variety of chemicals, most of which can be separated using microalgae. Effluents from aquaculture, poultry, edible oil refineries, textile industry, distillery, amongst others have been subjected to phycoremediation. Aquaculture effluents, which are rich in mineral nutrients and generally discharged untreated into water bodies, have many negative impacts on the environment. Since microalgae can effectively utilize polluting nutrients of wastewaters, its application in treatment of aquaculture effluents is quite useful. Tossavainen et al. (2019) reported high nutrient removal rates in mixed microalgal cultures with aquaculture wastewater. Similarly, wastewater from poultry and its processing plants is laden with organic waste, proteins, carbohydrate, fats, and other nutrients which can be utilized by microalgae. Viegas et al. (2016) reported high biomass productivities of *C. vulgaris* and *C. protothecoides* cultures with poultry effluent and pig manure. High removal efficiencies for ammonium and phosphate were obtained in treatment of poultry slaughterhouse wastewater using *Scenedesmus obliquus* (Oliveira et al. 2019). Removal efficiencies for ammonium and phosphate were also high when *S. obliquus* was used for the treatment of cattle waste (Mendonça et al. 2018). Wastewater from tannery also contains similar organic pollutants (proteins, fats, and carbohydrates) produced from washing of hides, along with inorganic pollutants (solvents, additives, chromium) which are used in different stages of treatment. Suitability of microalgae for bioremediation of tannery effluents has also been examined in many studies with promising results (Nagi et al. 2020; Pena et al. 2019; Saranya and Shanthakumar 2019).

Like poultry processing, textile industries discharge large volumes of effluents, but with a different set of pollutants. Wastewaters from textile and dyeing industries generally have a strong colour due to the presence of synthetic dyes which are cost-effective and highly stable. However, because of the low fixation efficiencies of these dyes during the dyeing process, most of the material is lost in effluent (El-Kassas and Mohamed 2014). The coloured wastewater not only affects aesthetics, transparency, and gas solubility of water in which it is discharged but may also be toxic and carcinogenic, as is the case with most of the synthetic azo dyes (Lim et al. 2010). These effluents can be potentially hazardous to human health and aquatic life. Mostly bacteria and fungi have been investigated for their potential towards the removal of synthetic dyes from textile effluents. Although there are few reports on microalgae-based remediation of textile wastewaters, the results of these studies have demonstrated the potential of microalgae in biological treatment of coloured wastewaters from textile industries. Lim et al. (2010) reported 50% removal of a dye and concomitant reduction of ammonium and phosphate from textile wastewater using *C. vulgaris*. In a similar study by El-Kassas and Mohamed (2014), *C. vulgaris* has been found to grow well and remove colour from textile waste effluent.

13.5 Conclusion

Potential of microalgae to act upon a variety of synthetic and natural, organic and inorganic, environmental pollutants (gaseous air pollutants, heavy metals, pesticides, fertilizers, detergents, radioactive substances, pharmaceuticals, and other emerging contaminants) and the associated biorefinery concept has emerged microalgae as a promising microorganism in bioremediation of wastewaters and emissions from different sources of pollution. However, more efficient strains which have greater bioremediation potential than the conventional ones need to be identified and developed. Also, as microalgae alone cannot separate all pollutants, phyco-remediation needs to be integrated with bacterial and fungal processes to extend the scope and efficiency of remediation. Lastly, the greater challenge lies in designing efficient cultivation systems which can support high cell densities of microalgae for improving rate of bioremediation and enabling full-scale operation of wastewater treatment coupled with the value addition of waste stream.

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14 Toxicological Impact of Azo Dyes and Their Microbial Degraded Byproducts on Flora and Fauna

Ambika Saxena and Sarika Gupta

Abstract

Azo dyes are the most versatile and immensely used class of dyes. They play an extensive role in industries such as leather, food, paper printing, pharmaceutical, textile dyeing and printing, plastics, cosmetics, etc. The estimated production of azo dyes is about 0.7 million tons annually. They can pass through municipal wastewater plants nearly unchanged due to their resistance to aerobic treatment, which potentially exposes humans and local biota to adverse effects. Approximately, 10–15% of synthetic dyes are dissipated throughout various processes in the textile dyeing and printing industry. Azo dyes cause adverse environmental impacts because of their color, bio-recalcitrance, and potential toxicity to the flora and fauna. The contaminated water takes the solvate (contaminants) to the fields in the vicinity and its consumers, thereby adversely affecting the quality of the agricultural produce, animal and human health causing chemosis, contact dermatitis, exophthalmos, lacrimation, permanent blindness, skin irritation, etc. These azo dyes are also the ones raising the biggest concern due to their mutagenic, genotoxic, and carcinogenic nature. These azo dyes have a distinctive one or more azo bonds (-N=N-) that link different aromatic structures, and the cleavage of this bond biologically or chemically often releases more mutagenic and toxic end products. Under anaerobic conditions, some azo dyes are cleaved by microorganisms forming potentially carcinogenic aromatic amines. The toxicity of an azo dye may be due to the dye itself or aryl amine derivatives generated during the reductive biotransformation of an azo linkage. Aerobic microorganism-based bioremediation is gaining importance as it is proved to have high efficiency in mitigating, detoxifying, and degrading these

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contaminants. It is thus important to explore the possibilities of isolating efficient microbial aerobic degraders for use in the bioremediation of textile effluents.

Keywords

Animal impact · Azo dyes · Bioremediation · Plant impact · Toxicity

14.1 Introduction

A tremendous decrease in the quality of air, soil, and water has been observed due to the industrial revolution that incurred as a result of increasing demands of the population (Sudha et al. 2014). Improving the quality of water and thereby the soil has been a major thrust for scientific research. The effluent/pollutant discharge from various industries poses a severe threat to the ecosystem (Bhainsa and D'Souza 2006). In India alone, 70% of the natural water sources like lakes, rivers, and streams are polluted due to the discharge of industrial effluents into them (Sriram and Reetha 2015). Textile dyeing and printing industries are one of the major sectors that release industrial effluents containing a wide variety of toxicants, including biodegradable organic matter, suspended solids, toxic organic compounds (phenol), synthetic dyes (such as azo, anthraquinone, phthalocyanine, and triarylmethane), heavy metal, and their conjugates that may cause alteration of the physical, chemical, and biological properties of water bodies (Satija and Bhatnagar 2017; Sharma et al. 2007). Azo dyes (acid dyes, basic dyes, direct dyes, disperse dyes, mordant dyes, reactive dyes, and solvent dyes) constitute a major portion of the dyes used in textile industries (Tang et al. 2019; Adebajo et al. 2017; Desai 2017; Yang et al. 2016; Lade et al. 2015; Karunya et al. 2014; Rani et al. 2014; Sudha et al. 2014). Over 1 lakh dyes have been generated worldwide with an annual production of over 7×10^5 metric tons, and approximately 28,000 tons of dyes are being discharged into the public drains without proper treatment that eventually go into the river (Sarker et al. 2019; Singh et al. 2015). Azo dyes are characterized by the existence of nitrogen-nitrogen (-N=N-) double bond, high solubility, and their vibrant color (Santos-Pereira et al. 2019). These may amass to lethal levels causing a vast variety of ecological damage under various environmental conditions.

Various methods are used for the decolorization and treatment of textile wastewater. Physiochemical methods (specific coagulation, filtration, chemical flocculation, use of activated carbon) and chemical methods (ozonation, irradiation oxidation, electrochemical oxidation, Fenton oxidation and ultrasonic chemical oxidation) are used for the treatment (Sarker et al. 2019; Singh et al. 2015; Esteves and Silva 2004; Pearce et al. 2003). These methods have limited usability due to excessive use of chemicals, large amount of sludge generation, production of secondary pollutants, low efficacies, high operational cost, high cost of the electricity, ozone, and radiation (Lade et al. 2015; Leelakriangsak 2013). Biotreatment offers a cost-effective and environmental friendlier alternative for decolorization of textile effluent. In biological treatment processes, various parameters, namely pH, temperature, the degree of agitation, dye concentration and structure, oxygen, and supplementation of different carbon and nitrogen sources have a direct impact on the decolorization of the effluents (Modi et al. 2015). Thus, prior determination of these factors helps make the process more efficient, faster, and practically applicable.

Alternatively, bioremediation technology that employs the ability of microbes like bacteria, fungi, and/or their combination has emerged as an effective method for the treatment of textile dye effluent (Islam et al. 2017; Lade et al. 2015). Bacterial decolonization of azo dyes is found to be used under both aerobic and anaerobic conditions. However, the anaerobic degradation yields certain aromatic amines which are toxic and mutagenic; these byproducts cannot be further broken down under the conditions which generated those (Olukanni et al. 2006). Many bacteria that decolorize azo dye reportedly do not use the dye as the sole carbon source in aerobic conditions (Syed et al. 2009).

The mechanism of fungal decolorization mainly involves two aspects, biodegradation and biosorption. The biodegradation capability of fungi is due to their extracellular, nonspecific, and nonselective enzyme system (Yang et al. 2016). Fungal enzyme production depends on nutrient limitations, and their subsequent dye decolorization ability is achieved depending on the growth conditions.

In particular, the discharge of dye-containing effluents into the water environment is undesirable, not only because of their color, but also because many of the dyes released and their breakdown products are toxic, carcinogenic, or mutagenic to life forms mainly because of carcinogens, such as benzidine, naphthalene, and other aromatic compounds. The dye effluent may contain toxic substances that could be mutagenic, teratogenic, and carcinogenic to aquatic organisms and fish species. These are generally undesirable and toxic for animal and human at extreme minute quantities. The chemicals present in textile wastewater evaporate into the air, which cause adverse effect if inhaled or are absorbed through our skin may cause allergic reaction (Yadav et al. 2014; Kant 2012).

The color causes hindrance in light penetration, which subsequently inhibits the process of photosynthesis (Singh et al. 2015). Moreover, various research studies reveal that the toxic effects of dyes have a major influence over the germination rates and biomass of several plant species (Ghodake et al. 2009).

14.2 Impact of Azo Dye on Flora and Fauna

Azo dyes, except for their deteriorating effect on esthetic beauty and adverse impact in terms of chemical oxygen demand (COD) and biological oxygen demand (BOD), are also reported to adversely affect water resources, soil fertility, aquatic organisms, and ecosystem integrity. They pose lethal effect, mutagenicity, genotoxicity, and carcinogenicity to plants as well as animals.

14.2.1 Azo Dye Synthesis and Types

Azo dyes are one of the most important class of dyes and dominate 70% of the dye market. The elementary structure of azo dyes contains double-bonded nitrogen atoms (-N=N-), combining two identical and/or asymmetrical alkyl or aromatic radical. Mostly aromatic azo compounds are used as commercial colorants in textile industries. These dyes come in a wide range of colors, have better fastening capacity, cost effective in terms of their synthesis and availability of their starting materials, and therefore are preferred worldwide.

The most common procedure for azo dye synthesis is by diazotization of an aromatic primary amine, followed by coupling with one or more electron-rich nucleophiles such as amino and hydroxy. When hydrochloric acid is added to sodium nitrite, it produces nitrous acid. Nitrous acid under acidic conditions produces the nitrosonium ion and releases a water molecule (Fig. 14.1—Eq. (1)). The electrophilic nitrosonium ion attacks an aromatic amine to give a nitrosoamine, which undergoes tautomerism. Under acidic conditions, the OH group can be protonated to release water molecule. The diazonium salt is then resonance stabilized (Fig. 14.1—Eq. (2)) (Benkhaya et al. 2020). The final step for the synthesis of azo dye requires a diazonium salt and a coupling reagent (Fig. 14.1—Eq. (3)). The general synthesis of azo dyes is as follows (Table 14.1):

Others methods used for the synthesis of azo dye include: reduction of nitroso compounds by lithium aluminum hydride, reduction of nitro aromatic derivatives in alkaline medium, condensation of hydrazines and quinones, oxidation of primary amines by potassium permanganate or lead tetra acetate, condensation of primary amines with nitroso derivatives, etc.

Azo Dye Intake into the Food Chain Textile industries play a significant role in the economy of a nation; however, it is also one of the most polluting industrial sectors. The textile industry is responsible for a wide-ranging environmental pollution (Muthu 2017). This effluent prevents light from penetrating into the water bodies and also reduces its oxygen level, thereby reducing the rate of aquatic flora and subsequently its fauna (Imran et al. 2015; Hassan and Carr 2018).

A relatively small section of the chemical industry is represented by the dye industries, which globally produces approximately 800,000 tons of dyes in a year. About 10–15% of synthetic dyes are dissipated during various processes in the textile industry. Dyes are usually categorized into acid, basic, direct, disperse, mordant, metal complex, reactive, sulfur, and vat dyes. Out of the total dyes (approximately 10,000 dyes) exploited in the textile manufacturing unit, azo dyes that possess complex structure and are synthetic in origin contributes nearly 70% to overall produce. Primarily the main cause for the dye release in the environment is the fraction of residual dye in the textile effluent; this is one of the biggest concerns over the last few decades due to their esthetic damage and non-biodegradability (Hassaan and Nemr 2017; Hassan and Carr 2018).

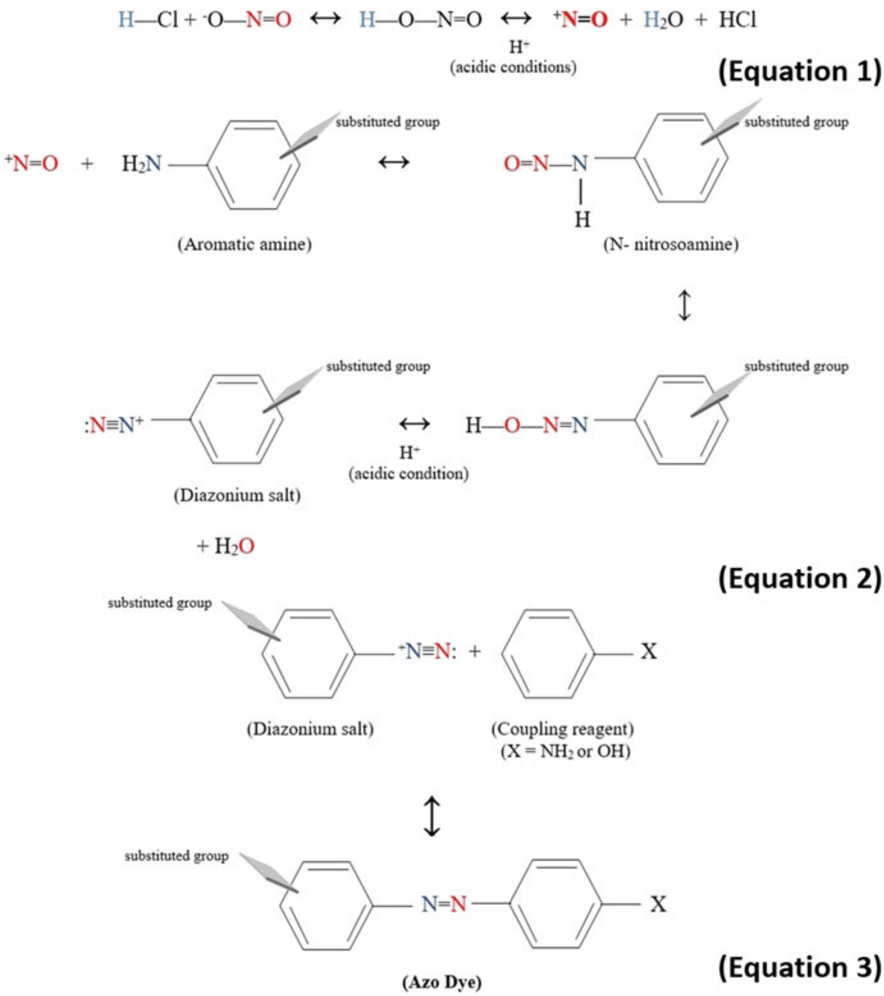

Fig. 14.1 Reactions involved in the synthesis of azo dyes

The solid waste that results from the fabric and yarn scrapings and packaging waste constitute textile sludge. Air pollution is caused by particulate matter, volatile organic compounds, oxides of sulfur and nitrogen. However, water pollution constitutes 80% of the total pollution caused by this sector that results due to discharge of untreated textile effluent into the nearby water bodies is the most harmful. Textile industries utilize large amount of dyes and chemicals. These dyes and chemicals are dissolved in water which is extensively used in various processes like dyeing, printing, bleaching, washing, etc. (Wang 2016). This untreated textile effluent contains dyes, heavy metals, other organic and inorganic dissolved solids,

Table 14.1 Types of azo dyes on the basis of color index

Type	Color index	Chemical formula	Example	Reference
Monoazo	11000–19999	Single (-N=N-) bond	Remazol Golden Yellow	Nigam et al. (1996)
Diazo	20000–29999	Double (-N=N-) bond	Reactive Black 5	Libra et al. (2004)
Triazo	30000–34999	Triple (-N=N-) bond	Acid Black 210	Agarwal et al. (2014)
Polyazo	35000–36999	Three or more (-N=N-) bond	Direct Red 80	Ogugbue et al. (2012)
Azoic	37000–39999	Insoluble ingrain dye, produced during dye process inside the fabric	–	Benkhaya et al. (2020)

which elevates the biological oxygen demand (BOD) and chemical oxygen demand (COD) of the adjoining river/surface water bodies.

These azo dyes are mutagenic and carcinogenic in nature. Since these dyes do not degrade easily, they enter the food chain and show biomagnification as organisms at higher trophic levels show higher levels of contamination compared to that on the initial levels (Newman 2015). When this effluent is used for irrigation purposes, these azo dyes are taken in by the plants where they affect its germination rate and plant growth. The use of these azo compounds also destroys the soil microbial biota (Imran et al. 2015).

14.2.2 Impact of Azo Dye on Flora

The most common methods employed to study phytotoxicity are monitoring of seed germination and plant growth. The study reported the toxicity of Procion Red MX-5B on the root growth in the seeds of *Lactuca sativa*. During the experiment, filter papers with 3 mL dye solution and 20 seeds were lined on petri plates at 21 ± 1 °C for 72 h in dark conditions with 0.05N zinc sulfate solution as positive control and water as negative control. This acute toxicity test showed inhibition of root growth rate in the seeds (Almeida and Corso 2014).

Lobiuc et al. (2018) used Lemna minor to test the toxicity of Congo Red from 5 to 5000 ppm concentration. The paper reported complete inhibition of plant growth, decrease in chlorophyll a content, and increase in carotenoid content at 2500 ppm dye; dye accumulation and necrosis formation. PS II efficiency decreased above 1000 ppm dye while chromosomal aberrations significantly increased at 5 and 1000 ppm dye.

Asses et al. (2018) performed phytotoxicity assays of Congo Red on *Zea mays* and *Solanum lycopersicum* seeds. Three milliliter of dye solution (200 mg/L) each day was used in petri dishes containing ten seeds with distilled water as control for 7 days. Significant reduction in the germination rate, shoot and root length of both the plants was observed. Methyl Red dye at 750 ppm concentration was reported to exhibit 30% germination inhibition in *Sorghum bicolor*, whereas it was 55% in *Triticum aestivum* (Ayed et al. 2011).

Phytotoxicity of Direct Red 2B was tested on *Phaseolus mungo* (mung) plants, ten seeds were placed on a plastic petri dish and 5 mL of 300 ppm dye solution was daily poured on them. It was reported that germination and length of shoot and root were affected in the presence of pure dye (Desai 2017).

Jadhav et al. (2016) conducted a study using synthetic textile dye Blue GL, Blue 2RNL, Direct Red 5B, Golden Yellow, and Scarlet RR on five plant species, namely *Cicer arietinum*, *Vigna radiata*, *Triticum aestivum*, *Vigna sinensis*, and *Vigna aconitifolia*, concluded that increasing concentration of dyes had toxic effect on the development of shoot and root, and showed inhibition of seed germination and inhibition of α-amylase activity.

Kalyani et al. (2009) reported that the percent germination and *Sorghum vulgare* and *Phaseolus mungo* seeds decreased with Reactive Red 2 treatment. Similar inhibitory effects in length of plumule and radicle of *Sorghum vulgare* and *Phaseolus mungo* by Direct Red 5B dye (Khandare et al. 2013) and by Reactive Blue 172 were reported (Lade et al. 2015).

Seed germination test indicated toxicity of textile effluent and contaminated soil on mung bean by reduction in percent seed germination (86.67% and 95.28%, respectively) and drastic decrease in radicle-plumule growth (Khan and Malik 2017).

Another study showed phytotoxicity of R Red M8B, R Navy Blue M3R, R Orange M2R, R Green HE4B, Dt Black BT, Dt Orange RS, R RedM5B, Dt Sky Blue FF, and Dt Blue GLL azo dyes using seed germination and plant growth bioassays in seeds *Guizotia abyssinica* Cass. The percent seed germination, shoot and root length as well as the protein and total carbohydrate content drastically reduced in the presence of pure dyes, indicating their toxic nature (Laxmi and Nikam 2015).

14.2.3 Impact of Azo Dye on Fauna

The dyes in the industrial effluent have been reported to cause several physiological and biochemical changes in the aquatic animals. Once they reach humans after entering the food chain, they cause various physiological abnormalities like hypertension, sporadic fever, renal damage, cramps, etc. (Puvaneswari et al. 2006) and were reported to be mutagenic (Umbuzeiro et al. 2005).

Sani et al. (2018) confirmed the toxicity of dyes through test animals, namely brine shrimps, *Artemia salina*. The study suggested that such compounds may pose serious risks to humans, plants, and other organisms (both soil and aquatic) through ecological interaction in the ecosystem. Asses et al. (2018) used *Bacillus cereus*, and *Escherichia coli* strains were used for microtoxicity evaluation of Congo Red. These strains were grown in nutrient broth (NB) with 200 mg/L CR at 30 °C and 37 °C, respectively, and their growth was assessed by OD at 600 nm recorded at 1 h interval during 8 h, significant decrease in the density was observed in comparison to the control.

Histopathological effects of silk dye waste water in the intestine and stomach of Swiss albino mice showed atrophy of musculature, disintegration of mucosal epithelial cells characterized by cytoplasmic vacuolization, nuclear pycnosis and nuclear fragmentation (Khatun 2017).

Lade et al. (2015) used *Daphnia magna* for acute tests to evaluate lethal toxicity of Reactive Blue 172, which showed 100% mortality indicating the toxic nature of the dye to mammals and humans. The acute toxicity test of Procion Red MX-5B was performed on *Artemia salina* as it acts as a bioindicator of toxicity of industrial effluent since it is sensitive to the salinity and conductivity of textile effluent. Test tubes containing ten larvae in 3 mL of dye solution plus 2 mL artificial sea water and 5 mL artificial sea water as control were used in the experiment and the mortality rate was observed. After 48 h, the experiment concluded that the dye was non-toxic (Almeida and Corso 2014).

This acute toxicity was reported to be a result of dye accumulation up to a critical concentration. Disperse Orange 1 was reported to be genotoxic on the HepG2 cells until it reached the maximum concentration tested where the damage response had saturated despite cell viability being around 90%, which might be due to cytotoxic effects. This cytotoxic effect was confirmed as the dye-induced apoptosis in the cells (Ferraz et al. 2011).

Methyl Red biotoxicity test on *Artemia salina* at 24 h and 25 °C revealed an average lethal concentration (LC50) of 3.5% at 34 g/L (Ayed et al. 2011). Another study performed on *Daphnia magna* with Methyl Red reported water flea to be most sensitive to the dye (EC_{50} 6 ppm). The study also reported the effect of Methyl Red on two freshwater fish, *Poecilia reticulata* and *Gambusia affinis*. The EC_{50} value for erythrocyte counts was reported to be lower than the LC_{50} value for mortality; hence, the dye was concluded to be more toxic on animal physiology than the wholesome effect on the test organism. Since both these fishes belong to the same family *Poecilidae*, species-specific response to the dye was observed as *Poecilia* was found to be more sensitive to dye in comparison to *Gambusia*. This effect to *Daphnia*, *Poecilia*, and *Gambusia* was concluded to be the result of the order of their evolutionary level; hence, the invertebrate is more sensitive than the vertebrate (Sharma et al. 2009).

de Lima et al. (2007) studied mutagenicity of industrial effluent in *Salmonella*/ microsome assay which tested positive. Carcinogenicity of industrial effluent was tested for in the aberrant crypt foci medium-term assay in colon of Wistar rats and confirmed as there was an increase in preneoplastic lesions in the colon of rats (Table 14.2).

Sharma et al. (2007) conducted serum biochemical and hematological studies on Swiss albino rats and stated that the values of white blood cells (WBC), red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hb), and mean corpuscular hemoglobin concentration (MCHC) significantly decreased in wastewater exposed animals (12–46%) with respect to control animals (potable water). Further, reduction in RBC size (13–27%) and the shape modification (poikilocytosis) was observed.

Table 14.2 Effect of untreated dyes on plants and animals

Dye	Application	Environmental impact	Hazardous effect	Reference
Procion Red MX-5B	Dyeing cellulose, nylon, silk, and wool	Plant and animals	Inhibition of root growth rate in the seeds	Almeida and Corso (2014)
Congo Red	Dyeing cotton	Plants	Inhibition of plant growth, decrease in chlorophyll a content, increase in carotenoid content, dye accumulation, and necrosis formation. PS II efficiency decreased; chromosomal aberrations increased and reduction in the germination rate	Lobiuc et al. (2018), Asses et al. (2018)
Methyl Red	Textile dyeing	Plants and animals	Inhibition in germination rate	Ayed et al. (2011)
			Lethal to saline water animals	
Direct Red 2B	Dyeing of cotton, rayon, jute, coir, and linen	Plants	Negative impact on germination and length of shoot and root	Desai (2017)
Blue GL, Blue 2RNL, Direct Red 5B, Golden Yellow, and Scarlet RR	Textile dyeing	Plants	Adverse effect on shoot and root length, inhibition of seed germination and α-amylase activity	Jadhav et al. (2016)
Reactive Red 2	Dyeing of cellulosic fibers	Plants	Inhibition in seed germination	Kalyani et al. (2009)
Direct Red 5B	Dyeing of cotton, rayon, and linen	Plants	Reduction in length of plumule and radicle	Khandare et al. (2013)
Reactive Blue 172	Textile dyeing and printing	Plants and animals	Reduction in length of plumule and radicle	Lade et al. (2015)
			Toxic to freshwater animals, mammals and humans	
Textile effluent	–	Plants and animals	Inhibition in germination rate and length of root and shoot	Khan and Malik (2017)

(continued)

Table 14.2 (continued)

Dye	Application	Environmental impact	Hazardous effect	Reference
R Red M8B, R Navy Blue M3R, R Orange M2R, R Green HE4B, Dt Black BT, Dt Orange RS, R RedM5B, Dt Sky Blue FF, and Dt Blue GLL	Textile dyeing and printing	Plants	Reduction in percent seed germination, shoot and root length, protein and total carbohydrate content	Laxmi and Nikam (2015)
Red, yellow, blue, and orange dye	Dyeing and re-dyeing in textile dyeing and printing industry	Plants and animals	Toxic to humans, plants, and other organisms	Sani et al. (2018)
Silk dye waste water	–	Animals	Atrophy of musculature, disintegration of mucosal epithelial cells, nuclear pycnosis, and nuclear fragmentation in stomach and intestine of Swiss albino mice	Khatun (2017)
Disperse Orange 1	Dyeing of polyester fiber, yarn, nylon, acrylic, and fabric	Animals	Genotoxic and cytotoxic	Ferraz et al. (2011)
Methyl Red	Textile dyeing	Plants and animals	Toxic on animal physiology	Sharma et al. (2009)
Industrial effluent	–	Plants and animals	Mutagenic and carcinogenic	de Lima et al. (2007)
Industrial wastewater	–	Plants and animals	Toxicity of untreated textile dye wastewater to the hemopoietic system of male albino rats	Sharma et al. (2007)

The serum biochemical parameters alanine transaminase (ALT), aspartate aminotransferase (AST), creatinine, urea, and bilirubin significantly increased (5–97%), while cholesterol, glucose, total protein, albumin, and globulin contents decreased (8–53%).

14.3 Pathway of Azo Dye Degradation

14.3.1 Physiochemical Pathway

Azo dyes cause major pollution in the environment; therefore, considerable research has been carried out on the removal of color from textile effluents. Azo dye treatment initially comprises physio-chemical treatment methods including coagulation, adsorption, precipitation, flocculation, oxidation, electrical destruction, ion exchange, ozonation, photocatalysis, etc. (Lade et al. 2015; Singh et al. 2015).

Inorganic coagulants such as polyaluminum chloride (PAC) or polyaluminum sulfate (PAS) have trivalent aluminum ions which react with hydroxyl and other alkaline ionic species. The hydroxides of aluminum are almost insoluble at neutral pH, which precipitate and settle down. In flocculation, coagulants are usually inorganic compounds that produce smaller and lighter flocs and require larger time to settle. It also reflects into the fact that the sludge volume is always greater with inorganic chemicals/coagulants (Ashtekar et al. 2014).

These physiochemical methods have been utilized for removing color especially for wastewaters, containing dissolvable solids. Textile industrial waste water contains large amounts of sludge volume which must be disposed of before further addition of chemicals through sedimentation, precipitation, filtration, etc. However, removal of dye requires high chemical dosage in other processes. In coagulation, trivalent cations have stronger coagulating tendency than divalent cations as cations in coagulants are responsible for neutralization of the negative charge in dye. Certain coagulants mainly used are ferric chloride/sulfate, aluminum sulfate, aluminum chloride, calcium/magnesium oxide, etc. Alum (aluminum sulfate) is one of the most widely used coagulant, and it produces insoluble precipitate of aluminum hydroxide and works better in the narrow pH range close to 5. Ferric compounds such as ferric chloride and sulfate are also some of the popular inorganic coagulants (Merzouk et al. 2011; Aziz et al. 2007).

Each of these methods has their merits and demerits. The chemicals used in these processes include alum, ferric or ferrous sulfate, lime and polyaluminum chloride (PAC), etc. (Saxena and Gupta 2020; Gogate and Pandit 2004).

Ozonation includes ozone bubbling through the effluent which degrades azo dyes. The degradation rate of azo dyes is first order with respect to both azo dye and ozone concentrations (Shu and Huang 1995). In photocatalysis, TiO_2 and ZnO semiconductors are majorly employed photocatalysts for the degradation of several environmental contaminants because of their stability, high photosensitivity, and large band gap. When illuminated with an appropriate light source, the photocatalyst generates electron/hole pairs with free electrons produced in the empty conduction band leaving positive holes in the valence band. These electron/hole pairs are capable of initiating a series of chemical reactions that eventually mineralize the pollutants in this case dyes (Sakthivel et al. 2003).

14.3.2 Microbial Pathway

These physio-chemical methods are non-ecofriendly as they tend to pollute the land and water sources. Microorganisms, however, completely degrade dyes and are environment friendly (Al-Tohamy et al. 2020; Ajaz et al. 2019; Hossen et al. 2019; Asses et al. 2018; Islam et al. 2017; Allam 2017; Lalnunhlimi and Krishnaswamy 2016; Cheng et al. 2016; Laxmi and Nikam 2015). These azo dyes are used as an energy source and a sole source of carbon and nitrogen by these microorganisms by mineralizing them into smaller compounds (Sudha et al. 2014). Bacteria, fungi, and algae are widely reported in literature to have azo dye degrading capabilities (Islam et al. 2017; Yang et al. 2016; El-Sheekh et al. 2009; Olukanni et al. 2006).

Fungal species like *Aspergillus niger*, *A. terreus*, *Alternaria alternate*, *Acrogenospora sphaerocephala*, *Ceriporia lacerate*, *Colletotrichum dematium*, *Corynespora cassiicola*, *Dictyosporium zhejiangensis*, *Fusarium thapsinum*, *F. oxysporium*, *Myrothecium verrucaria*, *Plectosporium tabacinum*, *Penicillium notatum*, etc. (Asses et al. 2018; Yang et al. 2016; Laxmi and Nikam 2015; Almeida and Corso 2014).

A wide range of bacterial species like *Bacillus cereus*, *Alcaligenes faecalis*, *Staphylococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Micrococcus* sp., *Klebsiella* sp., *Sphingomonas paucimobilis*, *Rhizobium radiobacter*, *Arthrobacter soli*, *Citrobacter* sp., *Escherichia coli*, *Micrococcus* sp., etc. have been reported to biodegrade azo dyes (Hossen et al. 2019; Islam et al. 2017; Allam 2017; Khan and Malik 2017; Vimala et al. 2015).

Bacteria mineralize azo dyes through reductive cleavage, which results in the formation of amines. Anaerobic degradation results in the formation of aromatic amines which are nonbiodegradable. However, treatment of these aromatic amines under aerobic condition results in their effective biodegradation. It has been stated in literature that aerobic degradation post anaerobic treatment can be used to degrade recalcitrant and toxic dyes (Puvaneswari et al. 2006).

Other microorganisms like fungi, yeast, algae, and certain bacteria remove azo dyes through biosorption. The biosorption capacity of a microorganism is attributed to the heteropolysaccharide and lipid components of the cell wall, which contain different functional groups, including amino, carboxyl, hydroxyl, phosphate, and other charged groups, causing strong attractive forces between the azo dye and the cell wall. As biosorption is mainly a surface phenomenon, certain pretreatments like autoclaving, treatment with acids like sodium hydroxide, formaldehyde, and calcium chloride change the surface/surface area of the microorganism, therefore changing the capacity of binding sites and affecting the biosorption. The effectiveness of biosorption depends on the following conditions: pH, temperature, ionic strength, time of contact, adsorbent and dye concentration, dye structure, and type of microorganism (Solis et al. 2012).

14.3.3 Enzymatic Pathway

Enzymatic degradation of azo dyes can be done through bacteria, fungi, algae, plant, or other sources (Sarkar et al. 2017). However, enzymes secreted by microorganisms have several advantages compared to other sources due to low maintenance cost, cheaper culture, downstream processing, etc. Microbes reduce the azo dyes by secreting various enzymes such as azo reductase, laccase, oxidase, hydrogenase, and peroxidase. Enzyme systems for the decolorization and mineralization of azo dyes under certain environmental conditions are substrate specific, highly efficient, can be immobilized, and biodegradable and therefore have no adverse impact on the environment (Pandey et al. 2007).

Azoreductase, a reducing enzyme, degrades azo dye into colorless amines through reductive cleavage process with reducing equivalent like FADH or NADH as the electron donor. Azoreductase can be either membrane bound or cytoplasmic. The enzyme cleaves azo bond (-N=N-) and transfers four electrons as reducing equivalent. In each stage, two electrons are transferred to the azo dye (electron acceptor), which results in decolorization of the dye (Singh et al. 2015). Toxic aromatic amines are often formed as intermediates which is later degraded through aerobic processes or microaerophilically. Cell membrane-bound azoreductases utilize redox mediator as an electron shuttle under anaerobic condition. This mechanism that uses redox mediator-dependent reactions are different for both membrane-bound and cytoplasmic azoreductase. Non-sulfonated azo dyes can enter through the cell membrane; therefore, they are degraded by cytoplasmic azoreductase. Degradation in anaerobic condition is more efficient than the aerobic one, as azoreductase is an oxygen-sensitive enzyme. Thus, in aerobic condition, enzyme comes in contact with oxygen, and redox mediator is reduced instead of the azo dye (Sarkar et al. 2017).

Laccase, multicopper oxidase enzyme, oxidizes the aromatic amine using Cu^{2+} as the mediator. Several low molecular weight compounds act as the efficient redox mediator in electron transfer steps. It degrades azo dyes through a free radical-mediated mechanism which is highly nonspecific. The intermediates of this reaction are phenolic compounds rather than toxic aromatic compounds. The enzyme oxidizes the phenolic ring utilizing one electron to produce a phenoxy radical which is furthermore oxidized by the enzyme to generate carbonium ion. Nucleophilic attack of water produces 4-sulfophenyldiazene and benzoquinone; these are unstable in the presence of oxygen. Under aerobic condition, 4-sulfophenyldiazene gets oxidized to phenyldiazene radical, followed by loss of molecular nitrogen, producing sulfonyl radical and ultimately producing sulfophenyl hydroperoxide, scavenged by oxygen (Chacko and Subramaniam 2011).

14.4 Impact of Bacterial Degraded Azo Dye Byproducts on Flora and Fauna

14.4.1 Bacterial Degradation of Azo Dye

Desai (2017) concluded that *Klebsiella* spp. and *Staphylococcus* spp. degrade 98.83% and 98.72% of 200 ppm of Direct Red 2B in Bushnell Hass minimal salt medium at pH 7, 37 °C, and 1% glucose, respectively. *Arthrobacter soli* BS5 showed dye degrading capacity by degrading Reactive Black 5 textile dye with 98% degradation at pH range 5–9, 37 °C, and 120 h of incubation (Khan and Malik 2017). The bacterial strains *Enterobacter asburiae* and *Enterobacter cloacae* were identified to biodegrade (not absorb) up to 98% of 100 mL of textile effluent with 0.1 g biomass at 32 °C and pH 1.67 in 10 min under aerobic conditions (Singh et al. 2017).

Lade et al. (2015) used GC-MS analysis to identify the degraded product of Reactive Blue 172 obtained posttreatment with *P. rettgeri* which were 4-(ethenyl sulfonyl) aniline and 1-amino-1-(4-aminophenyl) propan-2-one based on fragmentation pattern and *m*/*z* values. FTIR and HPLC analyses confirmed the degradation of the dye. Khandare et al. (2013) showed FTIR spectral analysis between Direct Red 5B and its products post decolorization by *Pseudomonas putida*. FTIR spectrum of the dye had different peaks at 1754.7 cm^{-1} for C=O stretch, at 1619.3 cm^{-1} for N=N stretch for azo bond structure; other peaks at 1546.7 and 1486 cm^{-1} show N–O stretch and peaks at 1286.8, 1134.7, 1045.1 cm^{-1} show S=O stretch. FTIR analysis of the degraded dye products by *P. putida* showed few peaks of C–H deformation, indicating cleavage of the dye molecule. Peak at 2318.2 cm^{-1} represented NH_3, indicating the formation of amines, at 1703.4 cm^{-1} C=O for stretch. The decrease in peaks at 1229.5 and 1023.2 cm^{-1} indicated loss of sulfo groups. Further reduction in azo bond structure was also reported. Similarly, Lade et al. (2015) reported complete degradation of 50 mg/L textile azo dye Reactive Blue 172 using *Providencia rettgeri* strain at 30 ± 0.2 °C in 20 h under microaerophilic conditions with activities of azo reductase (159%) and NADH-DCIP reductase (88%). The decolorization was confirmed using HPLC, FTIR, and GC-MS analyses.

The strains *Lysinibacillus sphaericus* and *Stenotrophomonas maltophilia* were reported to degrade consortia of four reactive dyes (Black B, Blue RR, Red RR, and Yellow RR) up to 50–60% in 48–72 h at 2700 mg L and 2100 mg L, respectively (Rajeswari et al. 2014). *Sphingomonas paucimobilis* was reported to decolorize 99.63% toxic azo dye Methyl Red (750 ppm) in Mineral Salt Medium at 30 °C, pH 9 in 10 h confirmed using FT-IR analysis. The degradation of MR was possible through a broad pH (3–11) and temperatures (5–40 °C) range (Ayed et al. 2011). Kalyani et al. (2009) reported that *Pseudomonas* sp. SUK1 decolorizes sulfonated azo dye Reactive Red 2 at concentration ranging up to 5 g/L at 30 °C, pH ranging from 6.2 to 7.5 at static condition with 52% reduction in the COD within 24 h with respect to the activity of lignin peroxidase and azo reductase.

14.4.2 Impact of Bacterial Azo Dye-Degraded Byproducts on Flora

Phytotoxicity of Direct Red 2B conducted by Desai (2017) on *Phaseolus mungo* (mung) plants by petri dish method showed detoxification of Direct Red 2B by *Klebsiella* sp. hence better germination and length of shoot and root while *Staphylococcus* sp. isolate did not show any promising results for detoxification. Textile effluent biodegraded using *Enterobacter asburiae* and *Enterobacter cloacae* was reported to be less toxic for *V. radiata*, *T. aestivum*, and *P. mungo* as revealed after the phytotoxicity studies of percent germination and length of plumule and radicle (Singh et al. 2017).

The phytotoxicity of four mixed reactive dyes (Black B, Blue RR, Red RR, and Yellow RR) and their degraded byproducts by *Lysinibacillus sphaericus* and *Stenotrophomonas maltophilia* was carried out in *Triticum aestivum*. The degraded byproducts were extracted in ethyl acetate, dried and redissolved in water up to 1000 ppm concentration. The plant seeds were watered with 5 mL of dye mix and degraded metabolites, respectively, at 30 ± 2 °C; percent germination was more in degraded byproducts (80%) as compared to dye mix (30%). The phytotoxicity study showed that the length of plumule (3–4 cm) and radical (2–3 cm) was affected in case of the dyes, whereas with degraded metabolites, it showed significant growth (12–13 cm and 8–9 cm, respectively) (Rajeswari et al. 2014).

Sorghum vulgare and *Phaseolus mungo* seeds irrigated with 10 mL of degraded metabolites of Reactive Blue 172 (50 mg/L) using *P. rettgeri* showed 90% seed germination in comparison to 60–70% germination by original dye and better root and shoot elongation (Lade et al. 2015).

The degraded products of Direct Red 5B reported earlier were applied for toxicity analysis on seeds of *Sorghum vulgare* and *Phaseolus mungo* at room temperature. The seeds were soaked in 5 mL solution degraded metabolites at room temperature. The degraded metabolites had lower inhibitory effect on percent germination, shoot and root length of plants as compared to the original dye molecule (Khandare et al. 2013).

The metabolites of Methyl Red dye obtained post degradation using *Sphingomonas paucimobilis* at 750 ppm concentration was reported to exhibit no inhibition in germination of *Sorghum bicolor* and *Triticum aestivum* (Ayed et al. 2011).

The biodegraded products of Reactive Red 2 after degradation with *Pseudomonas* sp. SUK1 was monitored using UV–vis, IR spectroscopy, and HPLC. The final product was 2-naphthol characterized by GC-mass spectroscopy. The study conducted on *Sorghum vulgare* and *Phaseolus mungo* seeds revealed that the degraded metabolites generated after the biodegradation of Reactive Red 2 were less toxic as compared to original dye (Kalyani et al. 2009).

14.4.3 Impact of Bacterial Azo Dye-Degraded Byproducts on Fauna

Acute tests with *D. magna* used for the evaluation of lethal toxicity of Reactive Blue 172 to mammals and humans showed 100% mortality of *D. magna*; however, *P. rettgeri* treated dye completely detoxified the dye as there was no mortality of *D. magna*. The sample of Reactive Blue 172 dye treated with *P. rettgeri* strain was centrifuged and the supernatant was sterilized using 0.45-μm cellulose acetate syringe filter. The filtrate in Erlenmeyer flask was inoculated with five 24-h-old neonates of *D. magna* at 20 ± 0.2 °C for 48 h in dark. The mortality of *D. magna* was observed post exposure to light for 20 s, and no mortality was observed in the *P. rettgeri*-treated dye solution (Lade et al. 2015). Eczema, contact dermatitis, asthma, chronic bronchitis, tuberculosis, hematoma, bladder cancer, and irritation to eyes have been reported among the workers of textile industries (Rajeswari et al. 2014).

Rajeswari et al. (2014) conducted cytotoxicity study of degraded byproducts of four mixed reactive dyes (Black B, Blue RR, Red RR, and Yellow RR) by *Lysinibacillus sphaericus* and *Stenotrophomonas maltophilia* on human embryonic kidney cell line (HEK 293) using MTT assay to evaluate their toxicity. The assay at concentration of 31.25–500 μg/mL degraded metabolites showed percentage cell viability in the range of 79.41–98.42. The study concluded that degraded metabolites at high concentration had a mild interference in cell viability, therefore are nontoxic. *Erwinia* sp. bacterial strains decolorized brilliant green efficiently than Evans Blue. The decrease in the toxicity of brilliant green was interlinked to decolorization of brilliant green was connected with decrease of zootoxicity in *D. magna*. However, Evans Blue decolorization had no impact on its zootoxicity (Przystaś et al. 2012). Methyl Red biotoxicity test on *Artemia salina* revealed no significant change in the acute toxicity in relation to the test organism reported by Ayed et al. (2011) (Table 14.3).

14.5 Impact of Fungal Degraded Azo Dye Byproducts on Flora and Fauna

14.5.1 Fungal Degradation of Azo Dye

A. niger degradation of CR was correlated with lignin peroxidase and manganese peroxidase production, showing 97% degradation with 2 g mycelia incubated with 200 mg/L dye at 28 °C, pH 5 for 6 days under 120–150 rpm confirmed using LC-MS/MS spectral analyses (Asses et al. 2018).

Laxmi and Nikam (2015) reported that *Aspergillus flavus* degraded various azo dyes like R Red M8B, R Navy Blue M3R, R Orange M2R, R Green HE4B, Dt Black BT, Dt Orange RS, R RedM5B, Dt Sky Blue FF, and Dt Blue GLL in 3–7 days at pH 5, 30 °C, initial dye concentration of 40 mg/L as a result of biotransformating enzymes like lignin peroxidase, laccase, manganese peroxidase, and tyrosinase.

Table 14.3 Effect of bacterial degraded dyes on plants and animals

Bacteria	Isolated from	Dye	Impact of degraded byproduct	Reference
Klebsiella sp., *Staphylococcus* sp.	Chemically contaminated sites	Direct Red 2B	Better germination and length of shoot and root in *Klebsiella*-treated dye	Desai (2017)
Enterobacter asburiae, *Enterobacter cloacae*	Textile effluent	Textile effluent	Less toxic for *V. radiata*, *T. aestivum*, and *P. mungo*. Increase in percent germination and length of plumule and radicle	Singh et al. (2017)
Lysinibacillus sphaericus, *Stenotrophomonas maltophilia*	–	Black B, Blue RR, Red RR, and Yellow RR	Increase in length of root and shoot of *Triticum aestivum*	Rajeswari et al. (2014)
			Degraded metabolites at high concentration had a mild interference inhuman embryonic kidney cell line (HEK 293) cell viability, hence non-toxic	
Providencia rettgeri	–	Reactive Blue 172	20% better germination and increase in length of root and shoot in *Sorghum vulgare* and *Phaseolus mungo*	Lade et al. (2015)
			100% mortality in original dye while no mortality posttreatment in *D. magna*	
Portulaca grandiflora, *Pseudomonas putida*	Root-associated bacteria	Direct Red 5B	Lower inhibitory effect of degraded metabolites on percent germination, shoot and root length of *Sorghum vulgare* and *Phaseolus mungo*	Khandare et al. (2013)
Sphingomona spaucimobilis	Textile wastewater	Methyl Red	Degraded metabolites had no inhibition in germination of *Sorghum bicolor* and *Triticum aestivum*	Ayed et al. (2011)
			No significant change *Artemia salina*	

(continued)

Table 14.3 (continued)

Bacteria	Isolated from	Dye	Impact of degraded byproduct	Reference
Pseudomonas sp. SUK1	Soil sample collected from the waste disposal site of textile processing and dye manufacturing units	Reactive Red 2	Degraded metabolites are less toxic as compared to original dye *Sorghum vulgare* and *Phaseolus mungo*	Kalyani et al. (2009)
Erwinia sp.	Wastewater treatment plant	Brilliant green and Evans Blue	Decrease of zootoxicity in *D. magna*	Przystaś et al. (2012)

Almeida and Corso (2014) reported in a study indicating *Aspergillus terreus* and *A. niger* to test the biodegradation and biosorption of Procion Red MX-5B. In the biodegradation study with the fungus *A. terreus*, in 336 almost 100% decolorization was achieved, and UV–Vis and FTIR spectroscopy revealed molecular degradation and the formation of secondary metabolites, such as primary and secondary amines, while biosorption was effective in both decolorization and reducing the toxicity of the solutions.

Another study used two fungi to conclude that fungi exhibit different tendencies in liquid medium and tube overlay method. *Aspergillus niger* recorded maximum decolorization of the dye in liquid medium Basic fuchsin (81.85%), Nigrosin (77.47%), Malachite green (72.77%), and dye mixture (33.08%) under shaking condition while maximum dye decolorization in tube overlay method was recorded in Malachite green and Nigrosin (92.85% and 93.33%, respectively), Basic fuchsin (90.05%) and then dye mix (10.4%). In *P. chrysosporium*, maximum decolorization occurred in Nigrosin (90.15%), Basic fuchsin (89.8%), Malachite green (83.25%) followed by dye mix (78.4%) in liquid medium while Malachite green (71.42%), Basic fuchsin (70%), dye mix (9.6%), and least in Nigrocin (8.33%) in tube overlay method (Rani et al. 2014).

14.5.2 Impact of Fungal Azo Dye-Degraded Byproducts on Flora

Asses et al. (2018) concluded that Congo Red degraded with *A. niger* showed less toxicity to *Zea mays* and *Solanum lycopersicum* seeds with almost no effect on their shoot and root length posttreatment. Azo dye degradation using *A. flavus* dye-decolorized samples, the percent seed germination, shoot and root length, and the protein and total carbohydrate content were reported similar to that of the control (Laxmi and Nikam 2015).

Almeida and Corso's (2014) experiment that used *A. terreus* for biodegradation of Procion Red MX-5B showed a significant increase in toxicity, inhibiting the growth of *L. sativa* seeds by 43%, and the acute toxicity tests confirmed lack of molecular degradation following biosorption with *A. niger*, as toxicity to *L. sativa* seed reduced from 5% to 0%.

Another study reported the effect of untreated dye solution and biodegraded dye on wheat seed germination. Sterilized wheat seeds kept soaked in untreated dye solution and biodegraded dye showed 90% higher seed germination percent in treated dye while original dye inhibited the seed germination after 4 days at 25 ± 1 °C (Rani et al. 2014).

Similar effects were reported in the phytotoxicity studies on *Triticum aestivum* and *Ervum lens* Linn. as high percent seed germination along with significant growth in plumule and radicle of both the plants occurred in malachite green decolorized sample using *Penicillium ochrochloron* (Shedbalkar and Jadhav 2011).

Kalyani et al. (2008) reported better germination rate and plumule and radicle growth in *Sorghum vulgare* and *Phaseolus mungo* when treated with Red BLI metabolites post decolorization as compared to the original dye.

14.5.3 Impact of Fungal Azo Dye Degraded Byproducts on Fauna

Asses et al. (2018) concluded that Congo Red posttreatment with *A. niger* was less toxic on *Bacillus cereus* and *Escherichia coli* strains. The experiment confirmed that Congo Red dye is detoxified by *A. niger*, indicating the degradation of the amines in the solution. The experiment concluded that the improper disposal of the dyes can exert a negative impact on aquatic ecosystems (Table 14.4).

Almeida and Corso's (2014) experiment that used *A. terreus* for biodegradation of Procion Red MX-5B showed a significant increase in toxicity, leading to a 100% mortality rate among the *A. salina* larvae. *A. niger* did not have any effect on the toxicity of Procion Red MX-5B.

14.6 Future Perspective

Azo dyes are the most widely used dyes and contribute to about 50% of the dyes produced annually. Approximately 70% of them are discharged of as residual dye in textile wastewater. As they are highly toxic to the ecosystem, their degradation from textile wastewater has become a significant challenge over the past few decades. As the norms are getting stricter with respect to the industries following all the waste disposal rules and regulation, there is a need for more appropriate methods that are not only effective, cheap, but also do not harm the environment in the long run. As we learn the three R's, we must also try to reuse and recycle the water and reduce the waste produced. Microbial degradation of azo dyes has been very promising as it is accessible, effective, environmentally friendly, and cheap. Microbial treatment not only decolorizes the dye but also results in its detoxification. Chemical treatment, on

Table 14.4 Effect of fungal degraded dyes on plants and animals

Fungi	Source	Dye	Impact of degraded byproduct	Reference
Aspergillus niger	Processing wastewater	Congo Red	Less toxicity to *Zea mays* and *Solanum lycopersicum* seeds with no effect on shoot and root length of degraded metabolites	Asses et al. (2018)
			Decreased toxicity on *Bacillus* cereus and *Escherichia coli* strains	
Aspergillus flavus	Textile dye-contaminated site	R Navy Blue M3R, R Red M8B, R Green HE4B, R Orange M2R, R RedM5B, Dt Orange RS, Dt Black BT, Dt Blue GLL, and Dt Sky Blue FF	Percent seed germination, shoot and root length, protein and total carbohydrate content were similar in both control and posttreatment metabolites	Laxmi and Nikam (2015)
Aspergillus terreus, *Aspergillus niger*	–	Procion Red MX-5B	Significant increase in toxicity, inhibiting the growth of L. sativa seeds by *A. terreus*	Almeida and Corso (2014)
			A. niger reduced toxicity to *L. sativa* seed from 5% to 0%	
			A. terreus increased toxicity post treatment (100% mortality rate in *A. salina* larvae). *A. niger* had no effect on toxicity	
Aspergillus niger, *Phanerochaete chrysosporium*	Dye effluent soil	Malachite green, Nigrosin, and Basic fuchsin	90% higher seed germination of wheat seed	Rani et al. (2014)

(continued)

Table 14.4 (continued)

Fungi	Source	Dye	Impact of degraded byproduct	Reference
Penicillium ochrochloron	–	Malachite green	High percent seed germination and significant growth in plumule and radicle *Triticum aestivum* and *Ervum lens* Linn.	Shedbalkar and Jadhav (2011)
Pseudomonas sp. SUK1	Soil sample collected from the waste disposal site of textile processing and dye manufacturing units	Red BLI	Increased germination rate, plumule and radicle growth in *Sorghum vulgare* and *Phaseolus mungo*	Kalyani et al. (2008)

the other hand, increases the chemical load in the environment worsening the pollution of water bodies and associated land masses. Since microbial degradation treats the wastewater in terms of both color and toxicity without any significant chemical load, it will increase the reusability of the water and reduce the water need of each industry and minimize toxic chemical discharge into the environment.

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Industrial Wastewater Treatment in Bio-electrochemical Systems

15

Rishi Gurjar and Manaswini Behera

Abstract

The age of industrialization has brought a new dawn of global order and has led to advancement of mankind. The dynamics of industrial growth accompanied with rapid population increase has created stresses on the natural resources. These industries requires tremendous source of energy and freshwater for generating various consumable products. The aftermath of these industrial activities leads to production of huge amount of wastewater that requires treatment prior to its disposal. The wastewater generated has a wide range of pollutants, which are produced uniquely in different type of industries implementing an array of processes. Furthermore, the ever-increasing complex nature of industrial wastewater (IWW) makes the conventional treatment fall short of achieving the respective discharge standards of individual industries. Furthermore, to improve their treatment efficiency, efforts by combining physico-chemical (filtration, coagulation etc.) and biological (activated sludge process, up flow anaerobic sludge blanket, sequencing batch reactor, moving bed biofilm reactor) have been devised, thereby making it highly energy-intensive. However, use of sustainable solutions, that is, bioelectrochemical systems (BESs), will carry out efficient treatment and also help recover energy in the form of electricity, hydrogen, and other valuable products. The BESs convert chemical energy to electrical energy with the help of microbes operating under ambient conditions unlike the extreme operational condition of conventional polymer electrolyte membrane fuel cells. These systems have been used to treat different types of wastewater from various industries, viz. dairy, electroplating, chocolate, brewery, paper, textile, pharmaceutical, rice mill wastewater, etc. In this chapter, we will

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discuss the characteristics and challenges posed in the treatment of dairy, brewery, textile, pharmaceutical, paper industry, and rice mill wastewater in BES, such as microbial fuel cell, microbial electrolysis cell, microbial desalination cell, and microbial remediation cell.

Keywords

Industrial wastewater · Bioelectrochemical systems · Rice mill wastewater · Pharmaceutical wastewater · Textile wastewater

15.1 Introduction

The industrial revolution in the nineteenth century was a significant turning point in the history of humanity. It brought hopes of a better future and innovation to the world. This progress overlapped with the population explosion, thereby creating enormous stresses on the resources to cater to swelling global demands. Water is a vital resource that is required for all industries in different proportions. Several industries use various methods to obtain the desired end-product and contribute by-products to the environment. These by-products comprise organic, inorganic, emerging pollutants, and heavy metal compounds. Several industries produce toxic and complex effluents. However, in this chapter, effluents from some industries, i.e., rice mill (RWW), textile (TWW), and pharmaceutical wastewater (PWW), have many compounds generated during the manufacturing processes.

Continuous development of synthetic chemical compounds as an alternative to natural resources for use leads to the production of highly polluted effluent. The wastewater generated from textile and pharmaceutical industries (manufacturing units, hospitals, and residential areas) contributes chemical compounds to the wastewater. These chemical compounds are highly inhibiting or refractory to biological processes and interfere in achieving efficacy of other physico-chemical processes. In TWW, chemicals such as acid, alkalis, and dyes are present. The dyes are the most complex component of textile wastewater, as many factors affect its removal. The different types of dye used are acid, azoic, basic, direct, disperse, mordant, reactive, solvent, sulfur, and vat, depending upon the fabrics. Similarly, PWW is a set of chemical compounds containing disinfectants, antibiotics, surfactants, etc. The prevalent pharma compounds are penicillin, diclofenac, carbamazepine, bisphenol, and ethinyl estradiol, etc. Their treatment is far more complex due to the presence of several compounds in varying concentrations ranging from milligrams to nanograms in metabolized and un-metabolized forms in the effluent. Therefore, complete knowledge of the inventory is necessary before selection of treatment method and sequence. In contrast, the RWW is mainly organic and contains few objectionable compounds for treatment, e.g., phenols and lignin. However, the wastewater temperature is another concern as the most employed method is parboiling for rendering rice consumable.

Conventionally, the treatment of wastewater generated from the industries mentioned above has been open-ended and no generalized sequence or guidelines can be created for individual sectors. Furthermore, no single type of treatment technique can be implemented to render the effluent to discharge safely. As a result, the use of multiple methods and development of hybrid treatment methods, e.g., membrane bioreactor, advanced oxidation processes, etc., has been on the rise. But these methods increase initial cost and energy expenditure to meet stringent norms set by the regulatory body. Moreover, bioelectrochemical systems (BESs) can provide a techno-economic and sustainable solution for removing pollutants and energy recovery, thereby making them the treatment technologies for the future.

The chapter discusses in detail the characteristics of industrial effluent generated from three industries, viz., textile, rice mill, and pharmaceutical industries and their feasibility of treatment in BESs.

15.2 Industrial Wastewater Characteristics and Potential

15.2.1 Pharmaceutical Wastewater (PWW)

The twentieth century saw medical research improve leaps and bounds to provide medicine for countering diseases. The achievement of medical sciences in improving the average lifespan of humans and animals is commendable. The pharma industry market share is estimated to be 15 billion dollars (National Pharmaceutical Policy 2012) and is bound to increase manifolds in the future. The production and constant improvement of drugs make active pharmaceutical ingredients (API) an inalienable part of the ecology.

The PWW comprises waste and effluent generated during the manufacturing of pharma products and their use in hospitals. Both humans and animals consume antibiotics for treating microbial infection and other compounds, such as steroids, hormones, etc., as a growth promoter. But the discharge of medicines from residential areas and animal farms has been rising (Tong et al. 2011). They enter the ecosystem either in metabolized or unmetabolized form or by dumping expired products. These compounds are at inhibitory levels when generated from the hospital, but dilution reduces it to low levels. These low levels led to resistance development in the target entity; thus, more potent or higher concentration compounds are required to tackle resistant species of the disease (Fluit et al. 2000).

After the treatment of PWW, some compounds persist either in biosolids or effluent. This treated effluent is used for irrigation or gardening or discharged into water bodies. As a result, the compounds become part of soil, ground, and surface water. Their ability to bioaccumulate ensures their entry into the food chain (Kümmerer 2003). The wastewater components exert high BOD and COD with APIs, such as antibiotics, surfactants, hormones, and volatile organic compounds (VOCs) (Pal 2018). It is challenging to generalize pharmaceutical compounds and relationships between terms like COD/BOD or TOC/COD ratio in the effluent. The concentration of these compounds is present in nanograms in domestic wastewater; whereas, they exist in milligrams in hospital wastewater (Lindberg et al. 2005).

These challenges make their treatment highly difficult and require rigorous characterization for the selection of treatment methods, their design and operation. Surveying the use of pharma products by people or sold by pharmacy outlets can give an idea about the presence of any particular compound in the wastewater (Tjandraatmadja et al. 2010). Generally, it includes quinolones such as ciprofloxacin, sulfonamides, roxithromycin, dehydrated erythromycin, and others (Lindberg et al. 2005). Penicillin, a strong antioxidant, anti-degradation, and inhibitory to microbial growth, was entirely removed by employing a treatment sequence of anaerobic, hydrolysis, and aerobic units for an overall HRT of 30 h (Li et al. 2008). Antibiotics used for treatment of animals, such as tetracyclines, quinolones, metronidazole, virginiamycin, cyclosporin, sarafloxacin, etc., have been found in the soil, sewage sludge, and sediments (Marengo et al. 1997; Hamscher et al. 2002). Their persistence enables them to be present in sludge in mg per kg of dry weight (Lindberg et al. 2005).

The broad classification of treatment includes physico-chemical, biological, and filtration techniques. The physico-chemical includes coagulation and flocculation, ion exchange, adsorption, evaporation, advanced oxidation process (AOPs), and solvent extraction. A preliminary method, autoclaving, is also known as supercritical fluid carbon dioxide sterilization. It is used to degrade noxious chemicals and microbes that are present in the wastewater. Despite high removal efficiency ($>98\%$), the economy of the process restricts its use to small volumes (Hossain et al. 2011).

In general, the PWW has low biodegradability and demands the removal of specific components resistant to biological treatment processes by particular preliminary or primary treatment. The type of treatment provided mainly depends upon the target compound. The precipitation air flotation method shows a higher COD removal efficiency over the coagulation–precipitation process, but the operating costs of chemical precipitation is 25% lower. Phosphorous removal is mainly performed by these methods (El-Gohary et al. 2010). The coagulant selection is critical and requires careful study of the target compound under observations. But the high cost of chemicals makes it unpopular for use.

Furthermore, the use of natural coagulants can provide an economical solution (Joshi and Nanoti 1999). Electrocoagulation can be implemented to overcome the cost barrier. Deshpande et al. (2005) managed to improve the biodegradability of real pharmaceutical wastewater from 0.18 to 0.3. Types of electrocoagulation, such as peroxi-photoelectro-coagulation and photo-electrocoagulation, can also improve biodegradability of the wastewater. Moreover, integrating AOPs and electrocoagulation can drastically enhance the removal of compounds (Boroski et al. 2009). The advanced oxidation process (AOPs) allows mineralization of refractory compounds while electrocoagulation majorly removes colloidal organic substances and suspended materials. Boroski et al. (2009) used a system of Fe/Fe electrodes under a current density (763 A m^{-2}), reaction time (90 min) and pH (6) to obtain a removal rate of 91% and 86% for turbidity and COD, respectively. But the study found the presence of recalcitrant material, which was then removed by photo-oxidation (TiO_2/UV/H_2O_2) at pH (3) and irradiation time (4 h). It enhanced the overall efficiency to 97%. Therefore, a combination of techniques that help overcome the drawbacks of other processes can help achieve higher efficiency to treat PWW. Fenton's method has managed to increase the biodegradability of wastewater four times (Tekin et al. 2006). Similarly, the AOPs can function as a pre- or post-treatment unit depending upon the characteristics of the wastewater. In the same context, AOPs as a pretreatment prior to a sequential batch reactor was used. This arrangement helped in increasing the biodegradability of the wastewater from 0.06 to 0.23. It broke complex compounds into biodegradable compounds using a molar ratio of H_2O_2/Fe^{2+} between 150 and 250.

In another method, removal of specific compounds by adsorption can be efficient. The type of components must be known to perform adsorption efficiently. In adsorption, the batch mode is preferred for small quantities, while continuous mode is required for a large volume. But demands exhausted adsorbents to be regenerated and replaced frequently. Zeolites have been used to remove nitrosamine (De Ridder et al. 2012). Furthermore, use of nanoparticles, nanoclays, nanotubes, and nanocomposite material has shown promising adsorption of pharma compounds (Ghauch et al. 2009). However, their use as an adsorbent still requires considerable research before becoming a mainstream treatment. These materials have shown the capability to adsorb compounds from both aqueous and gaseous systems.

The fate and transport of pharmaceutical compounds are unknown and require extensive study. The compounds present in pharmaceutical wastewater cannot be removed with a single treatment, as they tend to reappear. Therefore, a combination of different methods is key to their removal. However, the processes are not uneconomical and unsustainable due to high energy demand. Most of the time, the antibiotics are not completely processed by living beings and enter the ecosystem as active compounds. Here the doctors prescribing medicine as well as pharmacists can play a vital role to curtail the concentration of these compounds.

15.2.2 Rice Mill Wastewater (RWW)

Rice is one of the most consumed forms of food. Globally, approximately 759.6 million tons (503.9 million tons, milled) are produced (FAO 2018). Around 95% of total rice production is consumed in countries like Bangladesh, China, India, Indonesia, Japan, Korea, Malaysia, Sri Lanka, Thailand, etc. (Pradhan and Sahu 2004). In India, the majority (65%) of the population consumes rice as a staple food. The primary process used to render the rice consumable is parboiling. It is a hydrothermal process employing soaking paddy, steaming, and drying before milling. The process is preferred as it ensures taste, nutritional value, and nonsticky nature with higher head rice yield (Kato et al. 1983). This, in turn, assists in avoiding malnutrition, food shortages, and food losses (Tomlins et al. 2005). The process followed in rice mill is shown in Fig. 15.1.

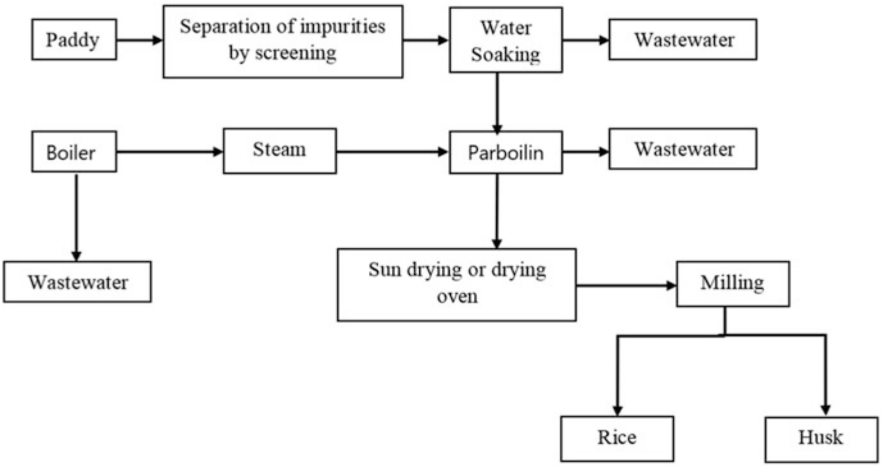

Fig. 15.1 Process followed in rice mills for paraboiling

In parboiling, the harvested paddy is first cleaned for soil or dust and then soaked in water for hours. The moisture content of about 30–40% is desired through sufficient soaking and then transferred to the steam-induced boiling tank. On sufficient boiling, the paddy is subjected to drying either naturally or mechanically before milling. During milling, the husks are removed from the paddy, and the rice is polished by separating bran. Furthermore, separation of broken rice takes place before supply. The separated husks are employed as a fuel source in the boiler, while bran is used to extract oil (Pradhan and Sahu 2004). The rice mill generates waste in solid, liquid, and gaseous forms. Gaseous pollutants, such as SO_x, NO_x, CO_2, etc., are caused by burning husk, while residual ash forms solid waste. Parboiling is a water-intensive process that has a multitude of parameter variation, thereby producing effluent with broad characteristics.

The main steps of parboiling are fixed; however, the parameter values vary geographically and seasonally to produce different quality of rice, such as water absorption capacity, grain quality vitamin (B1 Thiamine), content, etc. (Kumar et al. 2016). During summer, the temperature is maintained in the range of 70–80 °C, while in winters, between 90 and 100 °C. For soaking in summer, a period of 4 h is sufficient to 4–6 h in the rainy and winter season (Asati 2013). For soaking, the water required is about 1.25 times the weight of rice, which amounts to 1–1.2 L kg^{-1} (Rajesh et al. 1999). The pollution caused by rice mill effluent from small industries in its vicinity has been on the rise as they do not have proper treatment facilities as well as noncontinuous operation in large industries. The effluent is primarily yellowish, having pungent odor with organic materials such as cellulose, phenol, lignin, humic substances, and impurities (Kumar et al. 2016). The high organic strength and nutrients in the effluent will result in eutrophication of water bodies, whereas when discharged on land, will affect the vegetation and create odor problems.

The effluent, along with organics, also consists of lignin and phenol. Lignin, a plant-based material, is highly recalcitrant. On the other hand, phenol is a noxious organic toxin. The toxicity test of rice mill wastewater on seeds of *Vigna radiate* (plant) restricted shoot and root length (Kumar et al. 2016). For *Lebistes reticulatus*, it was acutely toxic, resulting in death (Giri et al. 2016) and affected reproductive rates for zebrafish (Galus et al. 2013).

As a result, the disposal of rice mill wastewater demands treatment before its reuse or discharge. Nowadays, with land shortages, the conventional treatment, that is, oxidation ponds, facultative ponds, etc., have been replaced by more compact treatment processes, such as activated sludge process (ASP), up-flow anaerobic sludge blanket (UASB), sequencing batch reactor (SBR), etc., with a focus on nutrient removal. The principal treatment methods that have been used for the treatment of rice mill wastewater are biological treatment and adsorption. In biological treatment, Rajesh et al. (1999) studied the suitability of a two-stage UASB reactor to treat rice mill wastewater. The UASB at an OLR of 3 kg COD m^3 day^{-1} obtained an overall BOD and COD reductions of 89% and 78%, respectively, with a methane production of 0.34 L kg^{-1} COD. Giri and Satyanarayan (2015) conducted a comparison study employing two media, Biopac and Fugino spiral, in a modified fixed-film UASB to treat rice mill wastewater at various OLR. The Biopac was found to be efficient than Fugino spiral, producing a better COD and BOD removal efficiency of 80–89% and 83–93%, respectively. Furthermore, Queiroz et al. (2007) used cyanobacteria, *Aphanothece microscopica Nägeli*, to remove organic as well as nitrogen from the parboiled rice effluent at 30 °C in a stirred batch reactor for a 12 h dark/light photoperiod. The study obtained the removal of organic (COD) and nitrogen (TKN-N) of 83% and 73%, respectively. Also, the phytoremediation techniques are promising eco-friendly treatment methods prevalent in countries that are still economically developing. Mukherjee et al. (2015) employed water lettuce (*Pistiastratiotes*), a floating plant, to treat rice mill wastewater. The treatment ensured soluble COD, nitrate, ammonical nitrogen, and soluble phosphorous removal to be 65%, 70%, 98%, and 65%, respectively. It also suggested bi-weekly harvesting of plants. The treatment used dilute wastewater in a ratio of 1:1 with tap water and pond effluent, as the acclimatization of the plant was inhibited by raw wastewater.

On the other hand, the treatment of rice mill wastewater through adsorption has been employed to remove complex organic matter (lignin) and color. Most commonly activated carbon has been used as an adsorbent; however, new adsorbent materials such as chitosan, rice husk ash are being used. Chitosan was used to treat rice mill wastewater (COD: 2200 mg L^{-1}) under batch mode (Thirugnanasambandham et al. 2013). An efficient removal of 98% was obtained under optimum condition (agitation time = 4 min; pH = 4.5; chitosan dose = 600 mg L^{-1}; settling time = 20 min). Kumar et al. (2015) investigated the removal of organic, color, lignin, and phenol from the rice mill wastewater with the help of rice husk ash followed by coagulation by $MgCl_2$. Under optimum conditions (agitation time = 4 min; pH = 4.5; adsorbent dose = 10 g L^{-1}; reaction time = 120 min; pH = 5), the removal of COD, color, phenol, and lignin were, respectively, 68%, 69%, 73%, and 66%. On addition of $MgCl_2$ (pH = 12; dose = 10 g L^{-1}), the removal was further enhanced by 40%, 75%, 40%, and 75% for COD, color, phenol, and lignin, respectively. The silica recovery (from rice husk ash) and $MgCl_2$ from sludge rendered the whole treatment highly economical. The focus of these conventional methods has been removing organic matter and color; however, other components such as phenols and lignin have not been studied in-depth for these treatments. However, treatment processes, such as chemical precipitation and electrocoagulation, have been used with the latter being new and effective.

Karichappan et al. (2013) studied the effect of continuous electrocoagulation of rice mill wastewater using stainless steel electrodes. The optimum efficiency in treating rice mill wastewater (COD: 2200 mg L^{-1}, TSS: 768 mg L^{-1}) for COD and TSS removal was 97% and 89%, respectively. The optimized parameters were pH (7), current density (15 mA cm^{-2}), electrode distance (5 cm), and flow rate (70 mL min^{-1}) from a response surface methodology technique. In another study, Choudhary et al. (2015) implemented aluminum and iron as sacrificial electrodes to observe the treatment of COD, BOD, and TDS removal in rice mill effluent (COD: 2886 mg L^{-1}, BOD: 2401 mg L^{-1} TDS: 1773 mg L^{-1}). The Fe-Fe electrode combination gave the maximum removal of COD, BOD, and TDS with 70%, 76%, and 59%, respectively, at a current density (40 mA cm^{-2}) with a reaction time of 60 min.

In contrast to other parameters under observation, viz. electrode spacing, pH, and current density, the current density was a primary governing parameter in the treatment of rice mill wastewater. The electrocoagulation, despite using no chemicals as compared to chemical precipitation, will pose a problem for disposal of sludge on a large scale. The high initial cost of these methods has resulted in delaying their commercial applications. The tighter norms and goal of sustainable treatment demands a combination of different types of units having a specific purpose for removing certain compounds with energy recovery.

15.2.3 Textile Wastewater (TWW)

Textiles comprise 8% of the total goods manufactured in the world. The industry is broadly classified into two categories: dry and wet textile processing units. The dry processing mills mostly generate solid wastes in terms of rejects of raw materials, while the primary cause of pollution is the wet processing industries. The wet industries house chemical-intensive processes such as sizing, desizing, scouring, bleaching, mercerizing, dyeing, printing, and finishing. The raw material is first screened for the presence of unwanted material. After screening, it is subjected to sizing, as it imparts strength and smoothness to the fabrics for weaving with the help of starch, polyvinyl alcohol, carboxymethyl cellulose, and acrylates. Once weaved, the sizing material is removed, that is, desizing by 0.5% H_2SO_4 or enzymes.

Furthermore, the fabric is boiled with NaOH, Na_2CO_3, Na_2O_2, and sodium silicate with a small amount of surfactants to separate impurities such as greases,

Table 15.1 Process in textile industries

S. No	Methods	Purpose	Pollutant contribution
1	Sizing/ Slashing (pal book)	To impart strength and smoothness for weaving by starch and starch derivatives.	Organic
2	Desizing	After weaving the material used for sizing is removed by 0.5% H_2SO_4 or enzymes (for starch).	pH, solids and organic.
3	Scouring	To separate out impurities like greases, waxes, oil, fats, dirt's, dusts, etc. by boiling with NaOH, Na_2CO_3, Na_2O_2, and sodium silicate.	pH, solids and organic.
4	Bleaching	To remove natural colors of fabrics by Na_2O_2, H_2O_2, and NaOCl.	pH and organic
5	Mercerizing	To provide luster, strength, absorbency and dye affinity to fabrics by boiling cloth with NaOH (20%).	pH
6	Dyeing	For coloration of textile materials with the help of different dyes.	Organic dyes.

waxes, oil, fats, dirt, dust, etc. Besides, the natural colors imparted by impurities on the fabrics are removed by Na_2O_2, H_2O_2, and NaOCl. Finally, the fabric is prepared to develop an affinity toward dye by boiling with NaOH (20%). It also imparts luster, absorbency, and strength to fabrics. The main and complex components of the textile wastewater are generated from the dyeing processes. After dyeing, the fabrics are moved to printing and finishing sections to render the products usable. The printing process in the industry has recently become popular with a change in the lifestyle of the urban population. The various processes used in textile and their purposes are explained in Table 15.1.

All the processes mentioned above demand the use of freshwater to carry out their designated function to make clothes resistant to physical, chemical, and biological agents. It adds chemicals, such as acids, alkalis, starch, surfactants, H_2O_2, dispersing agents, soaps of metals, and dyes in the effluent generated (Paul et al. 2012). These processes confer the effluent with high organic loading, nitrogen, phosphorus, color, solids and heavy metals with high pH, and temperature. This complex mix of chemicals, if allowed to discharge, will pose a great threat to soil, surface, and groundwater.

The presence of heavy metals and chlorine in the wastewater can be toxic to aquatic animals. The dyes affect the photosynthetic ability of aquatic plants due to low light penetration in the water bodies. This phenomenon reduces the self-cleaning ability of water bodies. Also, their presence can hamper the effectiveness of some treatment processes, such as UV disinfection. In addition, the by-products derived from decomposing dyes have been proved toxic to aquatic flora and fauna (Georgiou et al. 2002). These color pigments have a tendency to bioaccumulate, which may promote respiratory failure, neurological disorder along with infections, allergies, and nausea (Foo and Hameed 2010).

The dyes are organic compounds, which are water-soluble. During the dyeing process, the unused dyes (about 10–15%) find their way to wastewater in low concentration (Al-Degs et al. 2000). The major dyes used are acid, azoic, basic, direct, disperse, mordant, reactive, solvent, sulfur, and vat, depending upon the fabrics. Nowadays, using aromatic and heterocyclic dyes has produced complicated and stable structures of chemical compounds, posing great difficulties in degradation (Ding et al. 2010). Among these dyes, azo dyes have been popularly used as they span over an entire range of colors, thereby making up 95% of reactive dyes. They are highly stable to oxidants, washing, and light. Furthermore, the various color shades are obtained by subjecting heat to the dyed fabric. The dyes on the basis of color are classified into 25 structural classes, that is, color index.

The textile wastewater generated has different characteristics from different processes performed in the industries. The main compounds present are suspended solids, nitrates, chlorides, heavy metals, e.g., lead, manganese, chromium, lead, iron, copper, along with high organic loading. Since the process of every industry is unique with respect to their objectives, the plan and design of treatment will revolve around the techniques incorporated in the manufacturing of the textile and regulatory standards and economy. The treatment methods broadly include pretreatment, secondary, and tertiary treatment methods. First, the equalization of the wastewater is essential to average the multiple strength of wastewater generated from various processes at different times during the day. It enables systematic treatment without overloading a particular unit.

The textile wastewater consists of many components that are nonbiodegradable, thereby demanding the use of physico-chemical methods. These methods enhance the biodegradability index (BOD5/COD). The first method includes coagulation and flocculation. It adds coagulants, such as aluminum sulfate, aluminum chloride, and sodium aluminate, and iron-based, such as ferric sulfate, ferrous sulfate, ferric chloride, and ferric chloride sulfate, magnesium carbonate, hydrated lime, etc. The addition is followed by rapid mixing and slow mixing to allow the formation of flocs to sweep compounds to the bottom. Polyelectrolytes in flocculation can assist in the formation of larger and stable flocs. The flash mixing occurs for 30–60 s at 80–100 rpm; whereas, the slow mixing occurs for 20–30 min at 20–40 rpm, followed by a clarification in conventional settling in a primary sediment tank or tube settler. Once a desirable biodegradability index (>0.30) is achieved, the wastewater can be subjected to biological treatment. The biological methods are mostly classified as anaerobic, anoxic, and aerobic. Dye is considered the most difficult compound for removal and perceived by the community as an indicator of pollution. It has been the yardstick against checking the efficacy or selection of the treatment processes. The high chemical cost and a large amount of chemical sludge limit their use (Liang and Wang 2015). Individual applications have shown only 50% color removal by using alum/ferrous sulfate for azo dye. The efficiency is further hampered when water-soluble dyes are present (Hao et al. 2000). Coagulants having higher stability and coagulating effects, such as polyaluminum ferric chloride (PAFC), have been compared with polyaluminium chloride and polyferric sulphate to remove the active and dispersed dye. The decolorization efficiency for purplish red P-R (dispersed), light yellow K-4G (active), bright red K-2BP (active) were, respectively, 100%, 94%, and 74% (Gao et al. 2003). The PAFC is an inorganic and positive-charged coagulant. But overdosage of PAFC can also degrade the color removal efficiency for light yellow K-4G dye. These methods not only improve the biodegradability of effluent but also help in decolorization.

Once the biodegradability reaches sufficient levels, the effluent can be subjected to biological treatment. Furthermore, the anaerobic and aerobic methods work better together. The former helps in removing organic loading but have low color-eliminating capacity. A SBR treatment method was used to see the effect of high salinity in textile wastewater on color and COD removal. When administered a saline shock by increasing TDS from 1 to 5 g L^{-1}, the removal efficiency decreased from 81% to 59%; whereas, the removal of Remazol Brilliant Blue R (reactive dye) was consistent at 60% (Mirbolooki et al. 2017). In another method, a sequence of anaerobic fluidized-bed reactor and activated sludge process was used to remove four types of dyes, i.e., Acid-Orange 7, Acid-Red 14, Acid-Orange 8, and Acid-Orange 10 for a complete removal with the exception of aromatic amine by-products (Seshadri et al. 1994). The biological processes are capable of removing only organic matter present in the TWW. In most situations, the anaerobic unit is followed by the aerobic unit. The anaerobic treatment can be used to produce methane, which in turn can be used for the energy-intensive aerobic processes for the sustainable treatment of TWW. The mechanism involved for the degradation of dyes is based on the stroke of the enzymes such as laccase, lignin peroxidase, NADH-DCIP reductase, tyrosinase, hexane oxidase, and aminopyrine *N*-demethylase (Solís et al. 2012). The biological treatment efficiency along with controlling various parameters can also be enhanced by using an acclimatized strain of microbes and enzymes. Furthermore, the use of fungi and algae to degrade a wide variety of dyes is becoming popular.

The fungal cultures have enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase that are suitable for the degradation of dyes. Especially, white rot fungi, such as *Coriolopsissp*, *Penicillium simplicissimum*, and *Pleurotus eryngii* (Holkar et al. 2016). However, sustaining a fungal culture over a long period is difficult (Anastasi et al. 2011). The algae use both the mechanism of biodegradation and biosorption. The type of algae such as *Green macroalgae Cladophora*, *Shewanella algae*, and *green macroalgae Enteromorpha species* are employed to remove azo dye, because they possess azoreductase enzyme. The biological degradation mechanism is not fully understood for most of the dyes except for azo dyes. The azoreductase enzymes under anaerobic conditions break azobonds and may lead to the formation of colorless toxic intermediates, which can be treated either in anaerobic or aerobic conditions. Certain bacterial strains, such as *Pseudomonas* sp. *SUK1*, *Alcaligenes faecalis PMS-1*, and *Enterobacter* sp. *EC3*, *Enterobacter* sp. *F NCIM 5545*, and isolated *Pseudomonas* sp. *SUK1* is used (Holkar et al. 2016).

Another method popular in color removal is adsorption. The main parameters governing the selection of adsorbent are affinity, regeneration, and economy. However, it is advisable to use an adsorption process whenever the concentration of the pollutants is less or low-cost adsorbents are available. The most popular adsorbent is activated carbon, but their high cost and difficult regeneration have driven research to obtain low-cost adsorbents. Several adsorbents are being used, such as peat, fly ash, polymeric resins, zeolite, clay, chitosan, fly ash, wheat residue, treated ginger waster, groundnut shell charcoal, date stones and potato plant waste, etc.

In an investigation carried out by Patel and Vashi (2010), activated carbon for the removal of BOD, COD, and color was undertaken with an adsorbent dose of 11 g L^{-1}. It resulted in 81%, 88%, and 90% removal of BOD, COD, and color, respectively. In the same study, a comparison with different coagulant, viz., alum, ferric sulfate, and ferrous sulfate, were conducted at different doses. The reduction of BOD, COD, and color was 74%, 80%, and 85%, respectively. Wheat residue as a low-cost alternative produced adsorption rates similar to commercial activated carbon for removal of Reactive Red-24 (Zhong et al. 2011). The wheat residue was prepared by treating the residue with NaOH and epichlorohydrin. The study showed pseudo-second-order kinetics with 95% absorption for a dye concentration of 50–200 mg L^{-1} for an adsorbent dose of 2 g L^{-1}.

The filtration technique houses a great potential for the treatment of wastewater for recycling and reuse but requires thorough characterization of the content and temperature of TWW. The main objective of these treatment processes is the removal of colloidal, suspended particles, heavy metals, etc. However, the presence of organic compounds and soluble compounds passing through the membrane is a cause of great concern. They can move through the membrane and lower the treatment efficiency. This method has also been used for the recycling of dyes (Chollom et al. 2015). The membrane filtration method despite achieving high removal efficiency suffers from the problem of concentrated reject, frequent fouling, and high initial cost.

Furthermore, integration of membrane filtration techniques with other treatment methods, e.g., physico-chemical, biological, and AOPs, will enhance quality of water. Nowadays, hybrid treatment like MBR has become popular for the treatment of textile wastewater. The MBR is of two types: aerobic and anaerobic. The aerobic MBR has been frequently implemented in contrast to anaerobic MBR for TWW treatment. The aerobic MBR has obtained efficiency for color removal in a range of 50–98% (Schoeberl et al. 2005). The color removal was observed mainly by biomass adsorption than degradation. When sequential anaerobic and aerobic MBRs were used to treat textile wastewater, 90% removal of COD and 99% removal of dye were obtained (Pal 2017). The limitation of the anaerobic method for treating nutrients is one of the reasons for using anaerobic and aerobic sequence. There are different types of membranes used for removing various kinds of dyes. A polyamide membrane for methylene blue (80%) and brilliant blue (87%) (Pal 2017), ceramic membranes for azo dyes (96%) (Barredo-Damas et al. 2012), and a combination of RO and NF for methyl orange rejection (99%) (Nataraj et al. 2009) have been studied.

On the other hand, the AOPs under ambient conditions can be used to mineralize dyes, toxic, and complex organic compounds, which are either partially or not removed by other treatment methods (Asghar et al. 2015). The hydroxyl radicals generated have the potential to degrade most dyes used in the textile industries. Among various AOPs, Fenton's reagent is potent for TWW, as it manages to degrade both insoluble and soluble dyes. However, the color-laden iron sludge generated is a limitation. These AOPs, when combined with other treatment techniques, result in enhanced efficiency (Babuponnusami and Muthukumar 2014). Feng et al. (2010) used Fenton's method at optimum conditions (H_2O_2 dosage = 17 mmol L^{-1}, pH = 5, Fe^{2+} = 1.7 mmol L^{-1}, reaction time = 35 min) for removal of TOC and Reactive Blue 4 color by 39% and 69%, respectively. The dye (Reactive Blue 4) is considered to be the most recalcitrant compound in TWW. The study was then integrated with Fenton's process with an MBR to obtain an overall TOC removal of 86%.

Another way to oxidize the components other than AOPs is to use chemical oxidation, which has a low rate of degradation compared to AOPs. The methods mainly use ozone and hydrogen peroxide as an oxidizing agent. This method forms a nonselective hydroxyl under alkaline conditions and is effective in degrading aromatic rings and conjugated double bonds of dye chromophores (Tehrani-Bagha et al. 2010). Ozone is an oxidizing agent preferred because of on-site preparation and no sludge. However, the method is costly and may produce toxic by-products with a short half-life at pH 7. The main factors affecting ozone oxidation are pH, salts, and temperature. The ozone decomposition is faster under alkaline conditions (pH $>$8.5) (Tian et al. 2014) The ozone when used for a removal of Reactive Black 5 (RB5) took 60 min and a high dose of 1.7 g L^{-1} for color removal; however, the time was drastically reduced by combining electrocoagulation with ozone. This combination reduced both time and dose to 18 min and 0.3 g L^{-1} to achieve 95% efficiency (Bilińska et al. 2019). Another way to produce free radicals from ozone or hydrogen peroxide is with the help of energy-dissipating components, e.g., ultrasound, UV, and sunlight (Saharan et al. 2014).

Several studies treating synthetic and real TWW have been conducted to render the wastewater suitable for discharge or reuse, or recycle. The combination of treatment units increases with the presence of multiple compounds and their complexity. Therefore, the selection of treatment units should be in such a manner that it can accommodate various functions and is compatible with other techniques.

15.3 Bio-electrochemical Systems

Bioelectrochemical systems (BESs) are biologically assisted systems capable of converting the stored chemical energy in organic compounds to energy in electricity or hydrogen. These systems can be operated under ambient conditions and have been used to treat different waste, for example, leachate, landfill leachate, domestic, rice mill, pharmaceutical, petroleum and textile industry wastewater, etc. In the past decade, these systems have taken various forms, such as microbial fuel cell, microbial electrolysis systems, microbial desalination systems, microbial remediation cell, photo-electrochemical system, constructed wetlands-MFCs, soil-MFC, carbon capture fuel cell, etc., to serve the user-specific requirement. The development has not only to develop new types of BESs systems but has majorly switched to utilizing low-cost materials to replace costly materials used currently. The electrode material, such as platinum, is being replaced with carbon materials; whereas, the expensive polymer membrane is being actively replaced with ceramic membranes along with improving the architecture of BESs. These BESs systems have been used as a post- or pre-treatment for various conventional technologies to achieve sustainability and meet regulatory demands, such as the Fenton process, forward osmosis, electrocoagulation, and sustain robotic activity and to develop biosensors.

The microbial fuel cell (MFC) is the simplest form of BESs comprising anode and cathode chambers with electrodes. These electrodes are connected by an external circuit to transfer electrons, while the cation exchange membrane is used to transfer protons to the cathode. Both the electrons and protons are generated in an anode chamber by bio-oxidation of organic matter. Furthermore, modifications are carried out by supplying electric current to cathode to obtain hydrogen in an electrolysis systems or adding a compartments having anion exchange membrane in desalination cell for water recovery. The BESs have mostly focused on the treatment of readily biodegradable waste with a focus to harness maximum energy. However, in the past decades, the BESs has been treating complex industrial wastewater such as a brewery, dairy, rice mill, textile, pharmaceutical, petrochemical industries, etc. The versatile nature of BESs allows it to be tailor-made to overcome a particular problem by changing membranes, electrode material and adding compartments to remove harmful compounds. Generally, the treatment of refractory pollutants produces toxic metabolic intermediates either from other treatment methods or from BESs anode chamber (anaerobic conditions), which requires further treatment before discharge. The compatibility of BESs allows it to be combined with any treatment that can be deemed suitable for the treatment of harmful compounds. It also provides an alternative for the treatment as well as recover energy. However, the treatment of complex compounds is still in its early stages and requires a lot of research to understand the mechanism of treatment of these compounds.

15.3.1 Phenolic Compounds

Phenol is a harmful compound generated from various industries, such as coal, rice, pharma products, fertilizer, dyes, pesticides, leather, ceramic, etc. Although phenol is biodegradable, it can inhibit the growth of microbes, which reduces treatment efficiency at higher concentrations levels (>400 mg L^{-1}) (Uygur and Kargi 2004). The removal of phenol by solvent extraction, adsorption, chemical oxidation, ion exchange, and biological treatment has been undertaken. Tay et al. (2001) used a UASB reactor for phenol degradation with glucose concentration having a HRT of 12 h. The study observed quicker sludge acclimatization and phenol degradation at a glucose concentration of 1000 mg L^{-1} to degrade incremental phenol concentration from 105 to 1260 mg L^{-1}. The UASB utilizing glucose expedited the acclimatization period to 4 months with a large granular size (2.76 mm) and phenol removal of 98% as compared to UASB without glucose taking 7 months having a granular size of 1.77 with 88% removal efficiency. These treatments are associated with high cost, production of toxic by-products, and low removal efficiency.

Therefore, a net positive energy alternative in BES that can achieve simultaneous removal of pollutants must be explored. All biological treatment methods for removing organic chemicals suffer from the fact that their efficacy is limited by electron acceptors. However, the BESs provide an in-situ inexhaustible electron acceptor in the form of electrodes.

The degradation of phenol under anaerobic conditions is carried out by iron, sulfate-reducing, and denitrifying bacteria, while low energy is obtained from methanogens (Veeresh et al. 2005). For instance, the exhaustion of these electron acceptors will inhibit phenol degradation. Luo et al. (2009) used a mixture of phenol and glucose as a substrate in MFC to study the degradation of phenol. When a mixture of both compounds was used, a twin peak of voltage was obtained, at the first peak (>650 mV) and the second peak (approx. 600 mV). The first peak was due to the glucose degradation with 20% phenol degradation, whereas the second peak was majorly due to phenol up to 90%. Also, the maximum power densities were 9.1 W m^{-3} and 28.3 W m^{-3} for MFCs using phenol and glucose–phenol mixture as the fuel, respectively. During the first peak, glucose provided abundant electrons accompanied by electrons from phenol degradation. The study does not establish any relationship of the first peak with glucose due to intermediate products generated but relates the second peak with phenol degradation. This conclusion was based on a similar time (approx. 60 h) required for phenol degradation in both cases. The performance was further enhanced by using an external resistance instead of an open circuit. The open-circuit restricted electrons flow within an anode, thereby making it an anaerobic mechanism. This gave a free run for other metabolic pathways to prosper and hamper the electrical performance of MFCs. The higher electrochemical activity shown by MFC is due to the fermentable glucose producing nonfermentable (ethanol and acetate) for consumption.

In another study, Song et al. (2014) studied degradation of phenol by acclimatizing anode microbial community with different substrates, viz. glucose, sodium acetate, and sodium propionate. The anodic biofilm enriched with glucose produced a higher power density of 31 mW m^{-2} while treating phenol as a sole substrate. The voltage drop was clear when phenol concentration increased from 100 to 600 mg L^{-1} with a constant substrate dose of 1000 mg L^{-1}. The MFC produced a nearly similar potential (>590 mV) for different proportions till phenol was used as a substrate. The voltage dropped drastically with a maximum potential of 414 mV using glucose with respect to sodium acetate (193 mV) and sodium propionate (105 mV). The study also investigated the performance of MFC acclimatized with phenol and anaerobic sludge, which produced a stable potential of 242 $\pm$ 6 mV after 30 days of operation. A phenol degradation kinetic parameter having a value of 0.035 h^{-1} was obtained for MFC with glucose. The use of different sources of acclimatization is done to find the effect it had on the anodic biofilm community. The degradation of phenol in MFC acclimatized with glucose was higher by 16% compared to the open circuit MFCs. Similarly, Huang et al. (2011) obtained a 100% improvement in removal rates. In addition, the coulombic efficiency (CE) can be more as the phenol theoretically contain thrice the amount of coulombs present in glucose. Hedbavna et al. (2016) carried out treatment of simulated phenol contaminated groundwater using dual-chamber MFC to produce nearly 1.8 mW m^{-2} of power density.

Both the study shows electroactive bacteria responsible for enhancing the removal of phenol, but do not generate current immediately. A simple reason for this behavior would be the exoelectrogens preference for simpler compounds to generate electricity (Kiely et al. 2011). This delays degradation of phenols only after other preferable compounds are removed, thereby ensuring a syntrophic relationship for phenol degradation. This relationship primarily depends upon the microbial community, which is a function of the acclimatization procedure implemented. Both studies did not discuss the degradation pathway of phenol. However, there are other pieces of literature that can provide the most probable pathway carried out by microbes present in anode. The phenols are degraded into 4-hydroxy-3-methylbenzoic acid (4H3MB acid) and 4-hydroxybenzoic acid (4HB acid) by oxidation or para-carboxylation. The anaerobic oxidation of *p*-cresol (Bossert and Young 1986) and 2,4-xylenol and para-carboxylation of phenol (Boll and Fuchs 2005) and *o*-cresol (Bisaillon et al. 1991) produce 4HB and 4H3MB, respectively. The fermentation of phenol includes hydroxybenzoic acids degraded to acetyl-SCoA and finally to acetate (Madigan 2000). A higher concentration of hydroxybenzoic acid than phenols in the anode indicates degradation of phenols.

Furthermore, the above studies also explain the main factors responsible for affecting MFCs performance are: (1) The presence of an external and open circuit MFC affects the respiratory method occurring in the anode as nonelectrode electron acceptors are facilitated. The use of open circuit MFCs allows the growth of nonexoelectrogens (iron, sulfate, and denitrifying bacteria), which may oxidize phenolic compounds. (2) The acclimatization method implemented for MFCs with a substrate or target pollutant forms the basis for phenol degradation efficiency, as it determines whether the exoelectrogens become a part of anodic biofilm or not. (3) The inter chamber mass transfer of oxygen or substrates along with electromigration of dissolved ions (Luo et al. 2009; Song et al. 2014; Hedbavna et al. 2016).

15.3.2 Dyes

The azo dyes under anaerobic conditions produce amines and sulfanilic acids as intermediate products by enzymes, redox mediators, and other electron acceptors (sulfide, iron, and denitrifying bacteria). These amines are toxic and can be removed by providing an aerobic process as a post-treatment unit (van der Zee et al. 2001). A dual MFC system offers a unique architecture of having sequential anaerobic and aerobic conditions in anode and cathode, respectively. This trait of sequential treatment embedded with energy recovery can provide a sustainable alternative for the treatment of textile wastewater. The biocathode in the presence of oxygen can remove the amines to assure improved electrical performance (Freguia et al. 2008; Li et al. 2010). Fundamentally, the BESs are anaerobic treatment processes with high degradation efficiency, less sludge generation, and energy recovery. The cathodic electrons can be used to reduce the azo dye's chromophoric linkage, thereby producing aromatic amines having high biodegradability (Frijters et al. 2006). Mu et al. (2009) carried out the degradation of dyes (AO7) at the cathode while using an anode for substrate oxidation. The degradation is carried out at the cathode by transferring electrons and protons through an external circuit and cation exchange membrane, respectively. At the cathode, the intermediates are formed, which are toxic. Li et al. (2010) used an anaerobic and aerobic sequence to treat Congo red dye with glucose to eliminate the intermediate toxins generated by the anaerobic processes.

BESs performance factors for dye degradation are substrate, nutrients, pH, constructional material, structure, and concentration of dyes. The decolorization rate (DR) will increase with dye concentration, whereas the decolorization efficiency (DE) may decrease with dye concentration (Mu et al. 2009; Sun et al. 2009). But the effect of concentration on the rate is ambiguous (Solanki et al. 2013).

The power output of MFC depends on catholyte pH. Studies by Liu et al. (2009) and Mu et al. (2009) suggested acidic catholyte to be essential for higher dye removal. The maximum power density reached was 34.77 mW m^{-2} at pH 3, whereas the power density was only 1.51 mW m^{-2} at pH 9 for methyl orange (Liu et al. 2009). Mu et al. (2009) reported AO7 decolorization efficiency decreased from 91% to 20% when cathode pH was increased from 6 to 10. The chemical structure of dyes plays an important role in their removal. The monoazo dyes are comparatively easy to decolorize than polyazo dyes (Hsueh et al. 2009). Furthermore, the position of electron-withdrawing groups near the azo bond assists in degradation. Depending on their decolorization rate, the position is ranked as high to low, i.e., para > ortho > meta.

In most of the studies observed, a low external resistance has produced high decolorization, as high resistance restricts electron flow. In an anode, the electro-oxidation of dyes is affected. Mu et al. (2009) obtained a 50% decrease in dye removal when resistance was increased from 3 to 100 Ω. Similarly, a 15% dip in efficiency was obtained when resistance increased from 50 to 5000 Ω. The removal obtained at 5000 Ω also resulted in a double time required to achieve a 90% efficiency at 50 Ω. The low resistance allows high power generation, which improves color removal. Under high power density, the microbes consume more substrate to keep their metabolism rate intact. This paves the way for using higher substrate or organic matter. Higher COD removal from 45% to 67% was delivered when power density increased from 25 to 387 mW m^{-2} with simultaneous improvement in coulombic efficiency from 0.9% to 5.2% (Li et al. 2010). It is a well-known fact that under sustainable growth of biofilm, the increase in HRT will provide higher contact to the biofilm with the substrates or other pollutants under consideration and will result in better removal. A high HRT will give a higher power density, whereas a low HRT will reduce the power density and affect color removal proportionally (Mu et al. 2009).

Cao et al. (2010) showed electrons generated from substrate degradation to be used for dyes removal. Therefore, establishing color degradation to be a co-metabolism process. Li et al. (2010) observed stepping up glucose concentration from 100 to 4000 mg L^{-1}, increased the CR from 43% to 77% in the anode with an overall CR from 69% to 93%. However, an optimum dose of the substrate must be determined as a higher concentration will lead to a high residue of the substrate, increasing the oxygen requirement in the subsequent cathode or aerobic process. The effect of substrate on the decolorization rate depends on the electron-donating ability of a substrate. Sun et al. (2009) and Cao et al. (2010) studied the effect of MFC power density with three different co-substrates, i.e., glucose, sucrose, and acetate. They found highest effectiveness for color removal by glucose followed by sucrose and acetate for active brilliant red (ABRX3) and Congo red dyes. However, the suitability of substrate consumption also depends upon the microbial community that has grown on the anodic biofilm as high power density has been obtained when acetate is used as a substrate (Nimje et al. 2012). Furthermore, the addition of co-substrate may increase the cost of treatment. Still, it may not be needed as textile wastewater will have organic matter and the anodic biofilm will be acclimatized according to the substrate it is fed with. Kalathil et al. (2012) used real textile wastewater in a single chamber MFC with a granular activated carbon biocathode to obtain a power density of 8 W m^{-3}. The power density decreased from 234 to 110 mW m^{-2} when ABRX3 concentration was increased from 300 to 1500 mg L^{-1} (Sun et al. 2009). In this study, it was also noted that when the medium with only glucose was added into the anode chamber, it resulted in recovering original performance with a power density of 274 mW m^{-2}.

The power density was highest for glucose (103 mW m^{-2}) followed by sodium acetate (85.9 mW m^{-2}) and ethanol (63.2 mW m^{-2}). It is also because the internal resistance for the MFC using glucose was 97 Ω followed by sodium acetate (163 Ω) and ethanol (253 Ω). The glucose and ethanol gave nearly similar dye removal rates in the initial phase up to 24 h; however, the degradation at the end of 30 h was >96% for all types of substrates. Electroactive bacteria consume these products ethanol and acetate, thereby keeping the pH values under a check in the anode. This would have been difficult in an anaerobic system for fermentable products, such as ethanol and acetate. These products would have inhibited microbes by decreasing the pH of the anode and led to lower methane recovery (Asad et al. 2007) compared to electricity in BESs. The consumption of a substrate also depends upon the redox potential associated with the substrates and anode to obtain.

The azo dyes were observed to split to form phenyl derivatives. As a result, their concentration reduced in the anode and the remaining dyes with the intermediate products generated were further removed in the aerobic compartment. The color removal was primarily incurred due to biodegradation rather than biosorption by living cells (Sun et al. 2009). The biosorption has amounted to 11.7% (Cao et al. 2010) and confirmed in a photo electrochemical degradation for methyl orange (Ding et al. 2010). Till now, it can be concluded that both the electrons and protons are required for breaking azo bonds; however, in BESs both the objectives, i.e., electricity generation and dye removal, must be satisfied in an optimized manner rather than compromising one for the sake of the other (Işik and Sponza 2005). This use of electrons for dye removal will suppress electricity generation but will increase the removal of dyes when supplied in abundant quantities either by varying the

Table 15.2 The performance of BESs treating different types of dyes

S. No	Dye	Anode	Cathode	Power density (mW m^{-2})	Reference
1	Active brilliant red X-3B (300 mg L^{-1})	Carbon paper	Platinum	234	Sun et al. (2009)
2	Methyl Orange (0.016 mg L^{-1})	Carbon felt	Carbon felt	35	Liu et al. (2009)
3	Acid orange 7 (0.06 mg L^{-1})	Granular graphite	Granular graphite	0.31	Mu et al. (2009)
4	Congo Red	Carbon paper	Platinum	103	Cao et al. (2010)
5	Methyl Orange (10–20 mg L^{-1})	Unpolished graphite	Rutile-coated graphite	–	Ding et al. (2010)

external resistance or limiting the anode and cathode surface. Therefore, it can be concluded that the microbial community apart from electroactive microbes might be having strains that are using dyes as a mediator compound by reducing them and using electrons for electricity generation (Cao et al. 2010). The performance of BESs for treating dyes is shown in Table 15.2.

15.3.3 Pharmaceutical Compounds

Pharmaceutical wastewater is a set of chemical compounds containing disinfectants, antibiotics, surfactants, etc. The prevalent pharma compounds are penicillin, diclofenac, carbamazepine, bisphenol, and ethinylestradiol, etc. Their treatment is far more complicated due to the presence of several compounds in varying concentrations ranging from milligrams to nanograms in metabolized and un-metabolized forms in the effluent. Ismail and Habeeb (2017) compared a unique dual-chambered MFC having biofilm bearers, such as granular activated carbon and high-density polyethylene in anode treating real PWW. The study demonstrated similar performance having a nearly identical COD removal and power density of about 83% and 205 mW m^{-2}. The coulombic efficiency obtained was very low, 1.6 and 1.4% for GAC and HDPE, respectively. The high COD removal with low CE values may be due to the dominant metabolic pathway of nonexoelectrogens (iron-sulfate reducing, fermentative, and methanogens) over exoelectrogens, low concentration of microbes on anodic biofilm, high organic loading, diffusion of oxygen, and system internal resistance (Strycharz et al. 2011). These conditions, supplemented with the heterogeneity of real wastewater treatment, aided the reduction of CE. The study developed a mathematical model for mass and charge balances for biomass and substrates in 2D-anode biofilm. Despite the model's high correlation (0.991), a significant difference between the experimental and modeled data during the initial phase was obtained. However, once the MFC's biofilm stabilized, after 16 days of operation, the model predictions complimented experimental data. Later it was concluded that the model did not account for the initial acclimatization or lag phase or underdevelopment of anode biofilm.

Liu et al. (2012) used the steroidal drug industry effluent in an air cathode single-chamber MFC. The wastewater is estimated to contain several components, such as ethanol, methanol, acetate, chlorobenzene, benzene, toluene, acetone, pyridine, ethyl acetate, cyclohexanone, dichloroethane, chloroform, reaction intermediate, fermentation medium, and residual products. The wastewater also comprises nitrogen and sulfate along with organic material. The maximum COD removal was obtained when the wastewater strength was 50% strength, whereas the maximum nitrogen and sulfate removal were, respectively, 63% and 26% at 20% strength of real PWW. On increasing concentration, the COD removal was more or less the same. However, the nitrogen and sulfate removal was affected significantly and reduced to only 39% and 16% when subjected to the full strength of real wastewater. The coulombic efficiency also decreased to 10% at full strength from 30% when the wastewater was at 10% strength. The study also analyzes the presence of various microbes, which would have been following a different metabolic pathway than the exoelectrogens. The study reiterates that the acclimatization method of the biofilm holds a crucial key in removing complex compounds successfully. The study observed the strength of wastewater to be insignificant on electrical performance. The MFC operated at 20% strength produced a maximum potential and power density of 0.52 V and 22.3 W m^{-3} as compared to full strength wastewater producing only 0.46 V. This behavior can be explained by the effect of osmotic pressure on the microbes due to an increase in the substrate concentration, thereby inhibiting the metabolism rate and power production efficiency. Furthermore, the complex nature of wastewater facilitated the growth of other microbes. It used electrons generated from substrate degradation to reduce other compounds, for example, sulfate, whose concentration increased with the wastewater strength. As a result, high consumption of electrons led to lower CE and power production (Huang and Logan 2008; Liu et al. 2012).

Velvizhi and Venkata Mohan (2012) also carried out the degradation of real-time recalcitrant PWW under different organic loading ranging between 1.98 and 7.98 kg COD m^{-3}. The increase in the organic loading increased the potential and power density of the MFCs and achieved a maximum potential of 346 mV and 206 mW m^{-2} at an OLR of 7.98 kg COD m^{-3}. In an MFC, the presence of various microbial strains causes different metabolic pathways to flourish by substrate degradation. This led to a phenomenon known as electron quenching, which demands electrons. The aftermath of increasing substrate concentration satisfies this demand and results in less electron loss at high substrate loading. The conductivity of the wastewater increased with OLR, resulting in reducing the resistance and increasing power.

Wen et al. (2011a) studied the effect of different penicillin concentrations in a glucose medium with a single chamber air cathode MFC. In the initial stage, the addition of penicillin resulted in low potential; however, when the microbial community acclimatized, the potential increased. The voltage was 322 mV for glucose (1 g L^{-1}), which kept on decreasing and reached 190 mV for glucose and penicillin (1 g L^{-1} and 30 mg L^{-1}). But after a period of 90 h on increasing the penicillin concentration, the voltage reached 325 mV with penicillin (50 mg L^{-1}). Furthermore, in another setup, glucose (1 g L^{-1}) and penicillin (50 mg L^{-1}) was added, which after lagging for a period of 150 h, stabilized and reached a maximum voltage of 440 mV. It shows the adaptation of exoelectrogens to the substrates and is in suitable condition to generate electricity, as they develop deterrence to penicillin. However, a separate study in which only penicillin was added in MFC resulted in a maximum voltage of only 190 mV. This has been proved from many studies that the treatment of the targeted pollutant phenol (Luo et al. 2009); pyridine (Zhang et al. 2009) cannot be done alone as the desired energy recovery can only be made when they are subjected to co-metabolism.

The electrical performance of the MFC when using glucose (1 g L^{-1}) with an incremental concentration of penicillin (0–50 mg L^{-1}) showed that the OCV, power density, and current density increased from 372 to 517 mV, 14.7–101.2 W m^{-3}, and 1.61–7.33 A m^{-2}, respectively. This improvement in the electrical performance of the MFC is due to the effect of penicillin on the membrane and cell wall of microbes. This will allow an easy and faster exchange of substrates and enzymes for enhanced electron transfer (Ramanavicius and Ramanaviciene 2009). Since the current density indicates enhanced electron transfer, the internal resistance was found to decrease from 19 to 7 Ω for penicillin concentration of 0–50 mg L^{-1}. Similarly, the total impedance for whole cell (anode) dropped from 20.6 (9.3) to 9.3 (3.8) for penicillin from 0 to 50 mg L^{-1}. These reductions in the resistance of cells also contributed heavily to increase power density. Penicillin is a β-lactam antibiotic that binds β-lactam functional group with an enzyme (DD-transpeptidase). This enzyme helps in the formation of peptidoglycan cross-links in the bacterial cell wall. Therefore, binding to it sabotages the cell wall formation (Hedberg et al. 1996). The cell membrane consists of peptidoglycan and other nonconductive materials. They restrict electron movement from cell to membrane. The addition of penicillin resulted in compromising the microbe's cell wall, which expedited electron transfer and improved electrical performance of MFC. The study also presents the kinetics for COD, glucose, and penicillin were 0.167 h^{-1}, 0.224 h^{-1}, and 0.282 h^{-1}, respectively. In another study conducted by Wen et al. (2011b), another β-lactam antibiotic, ceftriaxone sodium, in a single chamber air-cathode MFC and obtained a higher power density of 113 W m^{-3} when performing co-metabolism of glucose (1 g L^{-1}) and ceftriaxone sodium (50 mg L^{-1}). The BESs performance for treating PWW is presented in Table 15.3.

15.4 Zero Liquid Discharge

The demand for freshwater sources by industries has been increasing every day to meet the demand of globalization. The industries except for using water are also depleting these sources, thereby reducing safe water resources for humans and creating more stresses on the existing sources. These dire conditions have made the governments and the scientific communities worldwide to implement "Zero Liquid Discharge (ZLD)." This tossed term allows the industries to reuse and recycle

Table 15.3 Performance of BESs for treatment of different pharmaceutical compounds

S. No	Pharmaceutical wastewater	Anode	Cathode	Power density (mW m^{-2})	Reference
1	Real wastewater	Graphite rods	Graphite rods	204.9	Ismail and Habeeb (2017)
2	Real wastewater	Carbon brush	carbon cloth	22.3	Liu et al. (2012)
3	Real wastewater	Graphite plates	Graphite plates	205.6	Velvizhi and Venkata Mohan (2012)
4	Penicillin (10–50 mg L^{-1})	Carbon felt	Pt-Carbon felt	101.2	Wen et al. (2011a)
5	Ampicillin (10–25 mg L^{-1})	Stainless Steel	Graphite Plates	90.3	Bagchi and Behera (2020)

water and remove harmful components before discharging effluent in the nearby vicinity (CPCB 2015). The ZLD has been classified into three categories based on the degree of treatment applied: (1) High-end systems for recovery and reuse of water, (2) Measure to dispose of high TDS waste effluent through high rate transpiration systems, (3) Small quantity of effluent (5 m^3 day^{-1}) with artificially operating evaporation systems.

The various techniques that form the part of the ZLD system are reverse osmosis, mechanical vapor recompression, multi-stage flash distillation, multiple-effect evaporation, agitated thin film drier, electro-dialysis, etc. However, the use of high-end technologies can cause the prices of the commodity to increase, thereby affecting the trade internationally by competition from other countries having low-cost products. The cost incurred by these systems can be reduced by employing mathematical tools such as analytical hierarchy process and grey relation analysis to select a techno-economic option for the industrial wastewater (Shende et al. 2020).

15.5 Challenges and Limitations

The BESs have been limited by their application to the laboratory-scale reactor. Its application on a large-scale results in high internal resistance and long-term operation. The long-term application leads to the deposition of extracellular polymeric substances produced by microorganisms on the membrane surface. This buildup of material on the membrane surface causes hindrance in the proton transfer, hence, reducing the electrical performance of the BESs. Furthermore, there are several low-cost materials for electrodes and membranes, which have already been proven for use. Despite successful implementation, the interaction of these materials with the microbes and complex chemical compounds in the anode and biocathode warrants extensive research. From the literature, it has been observed that the presence of chemical compounds aids the growth of non-exoelectrogens. These causes several metabolic pathways to operate simultaneously within the anode chamber; therefore, demanding optimization of various parameters to control the pathways to maximize energy generation. The degradation kinetics of the target pollutant must also be determined to understand the effect of varying operational parameters. Most of the studies have used MFC as the preferred choice for treating industrial effluent, thereby focusing on other BESs can open new avenues of research.

15.6 Future Perspective and Conclusions

Physico-chemical and biological processes have been conventionally used for the treatment of complex industrial wastewater. These treatments are expensive and energy-intensive. In response, the BESs fairs well and provide a promising techno-economic alternative to other methods. Like other methods, BESs suffers from some advantages and disadvantages. Still, its ability to tailor to the user-specific demand and compatibility with other methods allows it to treat a nexus of chemical compounds. The effectiveness of BESs entirely rests on the anodic biofilm. The development of different microbes capable of dissolution of pollutants is a function of the acclimatization method and period. Besides, the presence of several microbial strains will support unrelated combinations of metabolic pathways. These constraints are the biggest hurdles in executing large-scale and real-time industrial wastewater treatment in BESs. However, mathematical tools for subduing hindrance by factorial design methods, response surface methodology density functional theory, and principal component analysis. These tools will help in the optimized performance of BESs. The BESs processes can perform as a portable treatment solution for small-scale industries to meet regulatory norms limited by space and economy. At last, the BESs system can be used to develop biosensors to detect toxic compounds in the wastewater streams.

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16 Novel Economic Method for Dynamic Noninvasive Optical Monitoring of Turbidity

Frederick Vivian Lubbe and Hendrik Gideon Brink

Abstract

Optical density and turbidity are important parameters used both in the laboratory and a variety of industries. The aim of this study was to develop and demonstrate a low-cost, easily reproducible system for optical analysis of turbidity that can be used in the field, laboratory, or any other environment where budget and simplicity are important. The image-capturing system was manufactured by using off-the-shelf materials, consumer-grade 3D printing and Webcams, and open-source software. The performance of the system was tested during an experiment on algal growth by benchmarking it against UV-Vis absorbance measurements at 680 nm. The results were found to correlate well with the experimental results up to an absorbance of 0.8 ($R^2 = 0.9347$). Additionally, the Webcam was able to detect attached growth in contrast with UV-Vis measurements. The results demonstrate the feasibility of the system, of which the primary limitation is the quality of the imaging device. Despite this, the specific setup used in the study has promising potential for use in low-turbidity applications and can be reproduced for as little as 50 USD.

Keywords

Low-cost absorbance measurement · Optical density · Turbidity

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16.1 Introduction

Turbidity and optical density are important process parameters in many process industries, the water industry, as well as in a biological laboratory environment where the measurement of optical density is frequently used as an indicator for microbial growth (Baruth 2005) or the effectiveness of water treatment processes (Gillett and Marchiori 2019). In industry, various methods, both online and offline, are used (e.g., laser turbidimetry, transmittance turbidimetry, and scatter turbidimetry), which vary in terms of capital outlay and complexity (Baruth 2005). In the laboratory environment, spectrophotometric methods such as UV-visible light spectrophotometry are commonly applied (Rouessac et al. 2014).

For spot turbidity measurements, offline instruments that require a liquid sample remain in common use in both industry and the laboratory environment (Baruth 2005). In such cases, sample handling is manual; therefore, for applications where there is a risk of injury or contamination, there is a requirement to employ expensive workarounds or online measurements for the safety of employees, which may be beyond the budget of smaller organizations.

In literature, there have been several applications documented of using consumer-grade technology to perform turbidity and light measurements for scientific and engineering applications. Ismail et al. (2013) demonstrated the concept of constructing a luxmeter using an off-the-shelf Webcam. Their setup demonstrated that it was possible to accurately measure absolute light intensity using a Webcam under controlled conditions. Liu et al. (2018) demonstrated the concept of image analysis to measure sewage particle concentration, and Yao (2018) applied image analysis technology to predict the chemical composition of tea. Toivanen et al. (2013) developed a device that operates in conjunction with a mobile phone to take images of water and send it to a central image processing database to determine turbidity. The system uses crowdsourcing to build an observation database and thus improve on the accuracy of the readings. Na et al. (2013) demonstrated the use of a Webcam for tank level and algal growth measurement using a Webcam and proprietary software. Mullins et al. (2018) developed a measurement system using a specialized monochrome complementary metal oxide semiconductor (CMOS) camera in conjunction with an image analysis script run on the proprietary mathematical software package MATLAB. Gillett and Marchiori (2019) developed a low-cost sensor for in situ measurement of turbidity; however, this sensor was built from "scratch" using electronic components sourced directly from suppliers, therefore requiring specialized electronic skills for the assembly of the system. It is further uncertain what software was used for data analyses that potentially introduces cost related to software required for data logging and interpretation. At the higher end of the spectrum in terms of development complexity and cost, Jia et al. (2015) developed a multiwavelength optical density sensor for continuous algal monitoring using laser diodes as light sources, photodiodes as detectors, a flow cell, and a temperature controller to accurately measure algal cell concentrations of up to 1050 mg/L.

This chapter demonstrates the use of a Webcam and image analysis using open-source software to semi-continuously measure the optical density/turbidity of an algal growth system in a laboratory environment under general conditions. This application has the potential to be extended to a process environment for on-the-spot field measurements using cheap, off-the-shelf consumer equipment and open-source software for which no costly license is required.

16.2 Methods

16.2.1 Design and Procurement of Instruments for Optical Density Measurement System

16.2.1.1 Design Philosophy

Instead of using an absolute measurement of light intensity, the system uses a relative indication of light intensity by means of an external control solution measured using the same camera setup to zero the measurement. This allows multiple measurements to be compared against the same basis. The system can also use a saved image in addition to the initial image of the measured solution to zero the measurements.

16.2.1.2 Camera Sensors

Four off-the-shelf Web cameras (Logitech C170, 640 $\times$ 480 true optical resolution) were purchased online for R249.00 (approximately 20 USD at the time of purchase) each. The cameras' built-in light-emitting diode (LED) indicators were taped shut with black duct tape to prevent interference.

16.2.1.3 Housing

Each camera was mounted onto a two-part housing. The housing was designed to minimize external light interference; the overall design is shown in Fig. 16.1. The first part is attached to the camera via rubber bands and was termed the inner housing (Fig. 16.1a). The second part—the outer housing—acts as a light shaft that is attached to the source (an Erlenmeyer flask in this case) (Fig. 16.1b) and slides over the first part as seen in Fig. 16.1c. Between the two parts, a light diffuser was installed consisting of a layer of white paper and masking tape (Fig. 16.1d). The diffuser acts to reduce the image into a diffuse light source (Ismail et al. 2013), which is required for the light intensity/turbidity measurement. The Webcam (connected to a USB port of a computer) was controlled with custom-written Python language software utilizing the OpenCV library.

The housing was designed in-house and printed in PLA plastic using an additive deposition modeling 3D-Printer (MBot Cube). The tolerances were such that the housing parts could easily but firmly fit together.

16.2.1.4 Software/Algorithm Setup

The online data acquisition software was developed to enable semi-continuous monitoring of the turbidity; the Python code is provided as supplementary material to this text. The software was designed to capture images at discreet intervals, the spacing of which can be set by the user, and the user is able to individually adjust the

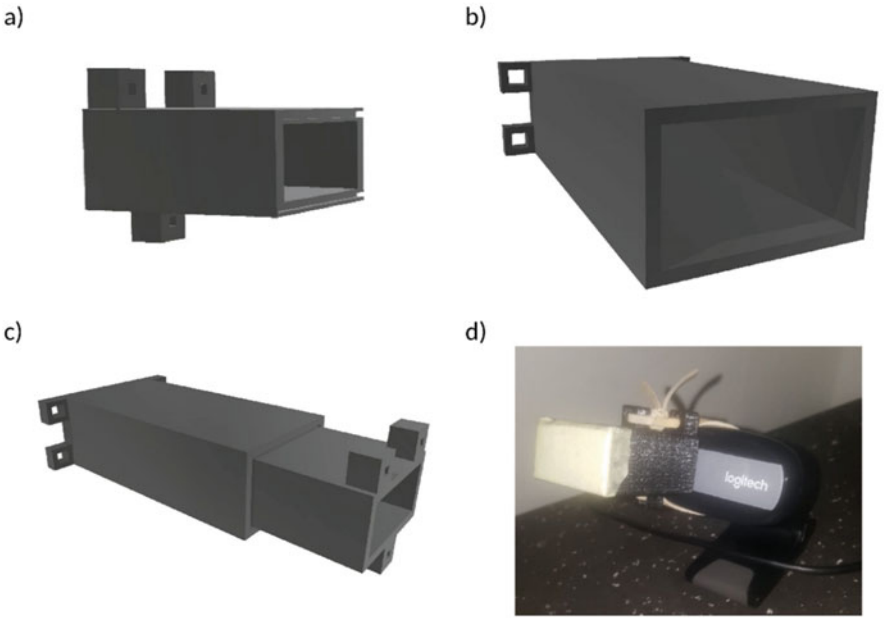

Fig. 16.1 The housing design showing (**a**) the inner housing, (**b**) the outer housing, (**c**) the assembled housing, (**d**) the diffuser attachment

camera parameters. The images are converted from the red–green–blue (RGB) color space to the monochrome color space using the grayscale vector presented by Bradski and Kaehler (2008), and the average light intensity of the image is consequently calculated using the pixel values. The absorbance measured by the Webcam system (ABS_{WC}) for a specific point in time was calculated based on the average light intensity measured from the experimental image and the calibrated image, where ABS_{WC} is given by the formula in Eq. (16.1) (Rouessac et al. 2014):

$$ABS_{WC} = -\log_{10}\frac{(\text{Light Transmitted by Object})}{(\text{Total Light Intensity})} \tag{16.1}$$

The software logs the light intensity and the calculated absorbance, the date and time, and camera number to a log file for later use. Each instance of the software run on the computer can run only one camera at a time, but running multiple instances allows the user to track multiple cameras at the same time, which is only limited by the number of processor cores installed in the computer.

16.2.2 Experimental Setup

The Webcam absorbance measurement method was tested and demonstrated by observing the growth curve of microalgae, grown as part of a different project

(Lubbe and Brink 2019). Three cameras were setup in parallel to individually observe the growth of the algae experiments in triplicate. This was done with a series of slow-growing, low-density experiments, and repeated with a series of fast-growing, high-density experiments. The differences in growth rates were mainly attributed to the supplied inorganic carbon sources. Periodic absorbance measurements for each run obtained using UV-Vis spectrometry (WPA, Light wave II, Labotech, South Africa) at 680 nm (ABS_{680}) were used as control. A fourth camera was attached to an Erlenmeyer flask filled with ultrapurified water for zeroing the measurements. 2× Osram L 36W/77 Floura lights were used to supply the required lighting for measurements and were situated at 90° to the camera angle. The camera control parameters were set as per Fig. 16.2b. The software was set to capture an image every 5 min for each camera. The lighting timer worked on a 14/10 light–dark cycle. To enable data capture at night, the lighting timer switched on for the first 10 min of each hour at night. Figure 16.2c shows a photograph of part of the experimental setup with the Webcam system connected.

Data captured during the dark periods were scrubbed from the logs. Furthermore, the average value of light intensity for the zero measurement was calculated and used throughout, replacing the individual value for each measurement. Data was indexed and sorted according to date.

16.3 Results and Discussion

16.3.1 Transient Growth Curves

The transient measurement results showing the semi-continuous measurements using the ABS_{WC} as well as periodic ABS_{680} are shown in Fig. 16.3. The low growth algal runs are shown in Fig. 16.3a–c, and the high growth algal runs are shown in Fig. 16.3d–g.

As can be seen from Fig. 16.3a–c, the low growth rate ABS_{680} correlates well with the ABS_{WC} values for the entire first run (Fig. 16.3a), from the start to Day 9 for

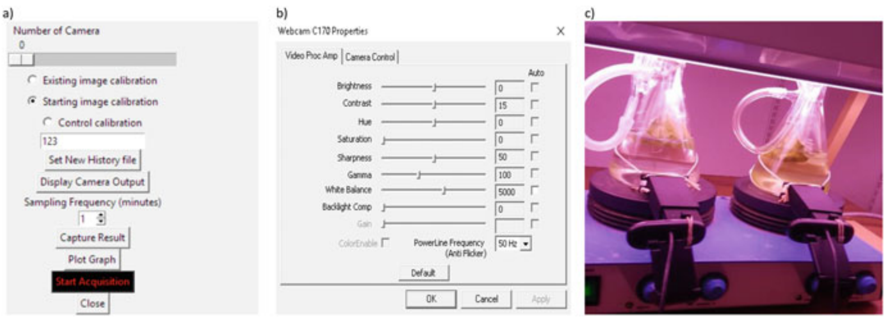

Fig. 16.2 (**a**) Main software interface, (**b**) camera experimental parameters, and (**c**) example of experimental setup

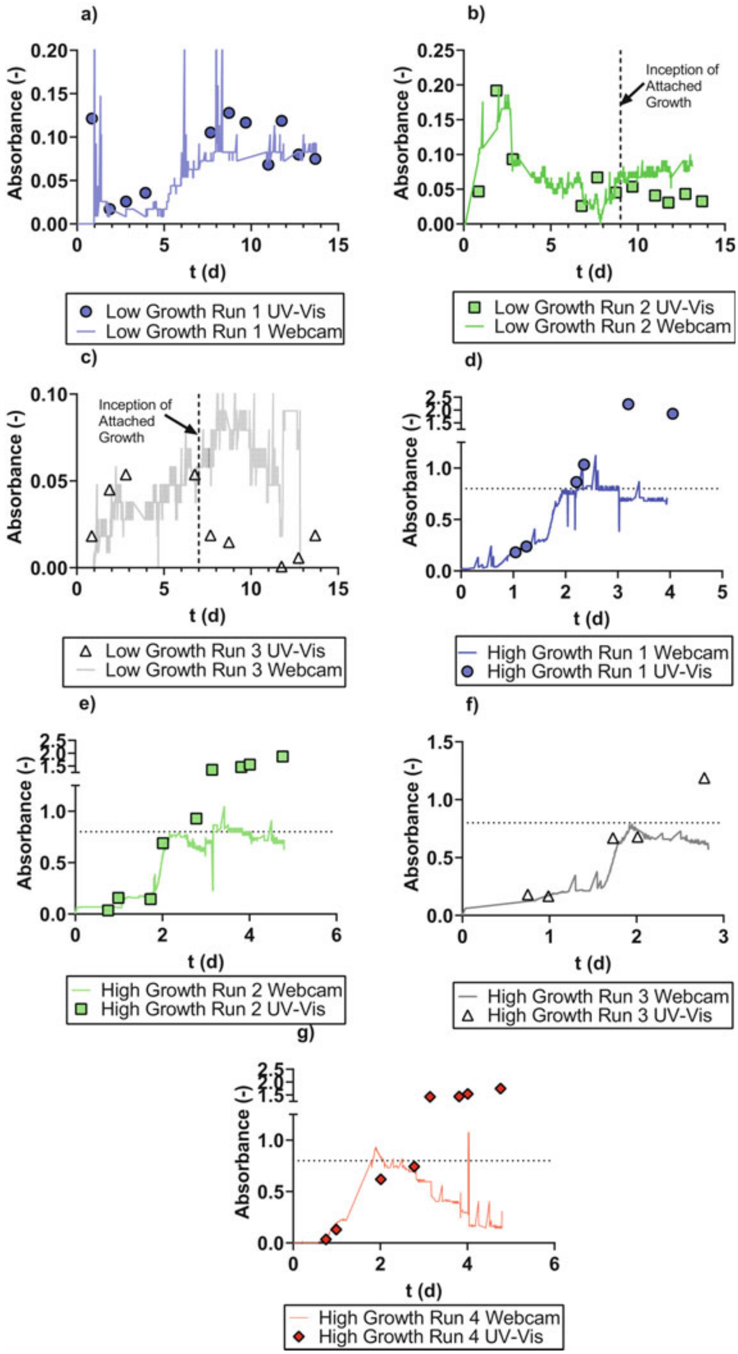

Fig. 16.3 (**a–c**) Results of the low growth rate runs 1–3, respectively, the inception of attached growth in runs 2 and 3, that is, (**b**) and (**c**), are indicated by a dashed lines. (**d–g**) Results of the high growth rate runs 4–7, as well as the "cutoff" absorbance of 0.8 observed for the ABS_{WC} measurement, respectively

run 2 (Fig. 16.3b), and from the start to Day 7 for run 3 (Fig. 16.3a). The decrease in ABS_{680} for runs 2 and 3 at these respective times (as indicated by the dashed vertical lines in Fig. 16.3b, c) is due to the microalgae in these runs preferentially aggregating as attached growth onto a nylon mesh framework (Lubbe and Brink 2019), as compared with the dispersed growth of run 1. Despite the fact that the algae was not dispersed throughout the medium (see Fig. 16.2c, which displays run 1 to the right and run 2 to the left), this method was still able to detect the growth because the green mass of algae in the center of the medium still absorbed an increasing amount of light as it grew (Lubbe and Brink 2019), and hence the light detected by the imaging sensor of the camera still decreased over time. In contrast, the ABS_{680} measurements were unable to detect the attachment event as the measurement was made by sampling and analysis of the growth medium that, as expected for attached growth, saw a significant decline in biomass. This result is significant as the detection and quantification of attached biomass in bioreactors are notoriously difficult (Brink and Nicol 2014a, b).

The high growth rate ABS_{680} and ABS_{WC} correlated well up to an absorbance value of approximately 0.8 (Fig. 16.3d–g), which was observed to be the cutoff optical density for the low-cost model of camera and the lighting setup used. For the species of algae in question, an absorbance of 0.8 correlated with approximately 182 mg cells/L of solution. Typical industrial algal cultivation concentrations range from 2×10^6 to 20×10^6 cells/mL (Lavens and Sorgeloos 1996), which translates to a typical dry cell weight concentration range of 14–140 mg/L and a corresponding ABS_{680} of approximately 0.09–0.9 (Jia et al. 2015). Therefore, the current setup would be usable in all but the highest density algal growth solutions. As absorbance measured is a function of the sensitivity of the sensor as well as the light intensity reaching, it is expected that using a higher quality Webcam and increasing the strength of the light source will yield a higher absorbance threshold. Additionally, the current setup has practical application in industries with liquids on the lower end of the turbidity range, such as monitoring of the final domestic wastewater effluent quality from treatment plants or in the potable water industry.

16.3.2 Quantitative Comparison of ABS_{680} and ABS_{WC} Measurements

The parity plot of the measured ABS_{680} and corresponding ABS_{WC} measurements, as well as the residual error plot as a function of the ABS_{680}, are shown in Fig. 16.4. These results show that for ABS_{680} values significantly less than 0.8, the Webcam predicts the conditions in the reactor well. Once the ABS_{680} in the reactor increased to the threshold value of 0.8, the residual error increases with increasing optical density. It can further be seen from Fig. 16.4a that the ABS_{WC} in the high growth rate runs 3 and 4 (Fig. 16.3f, g) decreased significantly while ABS_{680} maintained a steady increase. This means that to quantitatively evaluate the measurements, it is necessary to consider the history of the curve to assess which data to compare and which to disregard. From Fig. 16.3d–g, it can be seen that the most accurate predictions followed the initial growth curve up to the saturation absorbance of 0.8. Once the saturation absorbance was reached, the ABS_{WC} results compared progressively worse with increasing ABS_{680}.

To quantitatively evaluate the correlation of the ABS_{680} and the ABS_{WC} data, the data obtained from the Webcam system for the low growth rate runs 1 and 2 (Fig. 16.3b, c) after attached growth was observed, that is, 7 days and 9 days for low growth rate runs 2 and 3, respectively, were ignored. For the Webcam results from the high growth rate runs (Fig. 16.3d–g), the data for the growth from the start of the experiment until the first ABS_{680} data point after saturation absorbance (i.e., an absorbance of 0.8) was reached were considered and the remaining data disregarded. This meant that, for example, in the case of the high growth run 1 (Fig. 16.3d) all data after 2.5 days were disregarded (i.e., after ABS_{680} data point 3), while in the high growth rate run 2 (Fig. 16.3e) all data after 2 days were disregarded (after ABS_{680} data point 4).

The results from this quantitative analysis of the correlation using the data shown in Fig. 16.4a are summarized in Table 16.1 and show a strong correlation between the ABS_{WC} and ABS_{680} values (correlation coefficient – $R^2 = 0.9020$) for the 36 sets of values compared. Any potential elevation of the correlation by the number of pairs is accounted for by the adjusted R^2, showing that the comparison is not unduly influenced by the number of *X*–*Y* values (*n*). It is interesting to note that a higher Pearson correlation coefficient (*r*) with an accompanying r^2 of 0.9283 was observed than the current system generates, indicating that a stronger linear correlation exists between the variables. The ABS_{WC} can be calibrated using the calibration curve in Eq. (16.2) with a linear regression coefficient – $R^2 = 0.9347$. It can be noted that Eq. (16.2) demonstrates that a very small zero point shift (+0.007233) and an increase in the ABS_{WC} value is a relatively small adjustment required considering the ABS_{WC} were generated using only data from the Webcam system and Eq. (16.1).

$$ABS_{680} = 1.062(ABS_{WC}) + 0.007233 \tag{16.2}$$

16.3.3 Novelty of Method and Practical Application

The novelty of the method lies in the ease with which it can be implemented by any user, even a home user, the only prerequisite being access to a Webcam, a personal computer/laptop (which can easily be substituted in an educational institution with an interface such as a Raspberry Pi), and some basic programming skills with Python. The software used in the development of the application was open source and free (Open CV 2019; Python Software Foundation 2020). The material cost of manufacturing the 3D printed parts was less than 2 USD at the time of development, but the parts can easily be replaced by ones manufactured through other methods if desired.

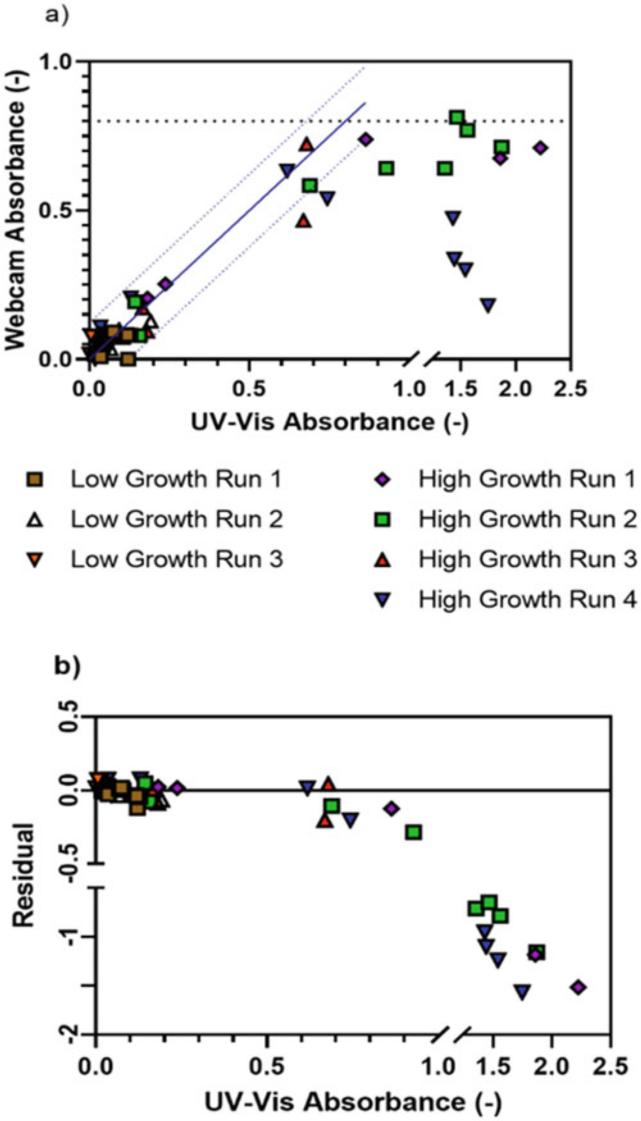

Fig. 16.4 (**a**) The parity plot of the ABS_{680} measured against the corresponding ABS_{WC} measurements—the 95% confidence prediction band (the likely location of additional data points) are indicated by the dashed interval; (**b**) the residual errors of the parity plot (**a**) as a function of the measured ABS_{680}

In comparison with Toivanen et al. (2013), where the data and image analysis was processed at a different location to the user, and Na et al. (2013), who used proprietary licensed software (Labview), no commercial methods or software was

Table 16.1 Correlation results from the comparison of the ABS_{680} and ABS_{WC} measurements

Parameter	Value
Number of *X*–*Y* values (*n*)	36
R^2	0.9099
Adjusted R^2	0.9124
RMSE	0.06112
Pearson correlation coefficient (*r*)	0.9668
95% confidence interval of Pearson *r*	0.9353–0.9831
Pearson's r^2	0.9347

used during the data analysis and the application was written entirely in Python and OpenCV by a programmer with rudimentary programming skills. Additionally, the methodology used in this study can be replicated for as little as 50 USD when the budget is the main consideration, the main expense being the Webcams (two Webcams being the minimum).

16.4 Conclusion

It has been demonstrated that turbidity can both be measured cost effectively and reasonably accurately using an equipment setup that costs less than 50 USD. This system is ideal for field measurements and laboratory setups where solutions with a lower range of turbidity need to be measured relatively accurately. The measurement cutoff point for this method is, however, dependent on both sensor quality and light intensity of the source used to illuminate the solution. The experimental results are promising as they are not only illustrating success at the lower end of the quality continuum, that is, using a low-cost, low-quality Webcam, but also demonstrating the ability to detect and monitor attached growth. In terms of field application, this setup can quickly be replicated and used in a process plant with low turbidity effluents.

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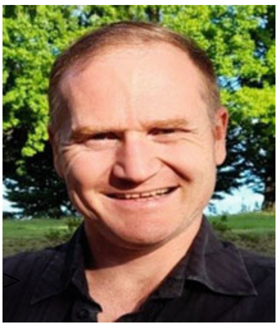

Hendrik Gideon Brink's passion lies in understanding and deciphering how the natural sciences can be implemented for the sustainable managementManagement of the biosphere. Through the study of Chemical Engineering, with specific emphasis on the study of reactor engineering, he has gained an in-depth appreciation for the impact of science on the future of the Anthropocene. Dr Brink has been employed as the resident Environmental Engineer in the Chemical Engineering Department at the University of Pretoria since September 2015 where he was awarded the University's "Exceptional Young Researcher" award in 2019.

Exploring the Less Travelled Path of Ecofriendly Handmade Paper Production

17

Sunita Chauhan, Shri Baleshwer Prasad, Shri Badri Lal Meena, and Pradeep Bhatnagar

Abstract

Conventional papermaking through paper mills is one of the most polluting industries of the world, and it also contributes toward deforestation and global warming concerns, but handmade papermaking is the tree-free and ecofriendly option. Handmade papermaking neither utilizes the forest-based raw materials nor does it use the toxic, harmful chemicals. Rather, the handmade paper industry utilizes the waste of textile industry/tailor cuttings as the principal raw material and the waste lignocellulosic materials (viz., straws/leaf fiber/bast fibers, etc.) as alternative raw materials besides recycling the waste papers. Handmade paper is therefore a green and clean option in the truest sense of its meaning. Handmade paper is actually the sheet of paper produced by hand in contrast to the mechanically produced paper in conventional paper mills. The process of making handmade paper uses minimum machinery and equipment, and it is very simple to understand, learn, and adopt. It is a drive towards mass employment and an important tool of women empowerment. Thus, handmade paper industry is a green and sustainable enterprise.

Although handmade papermaking is very relevant and significant in today's context, yet the industry faces numerous challenges in actually realizing the huge potentials involved. The present chapter investigates various aspects of ecofriendly handmade paper production with coverage of the challenges faced by the industry and the various measures that can be adopted to address those challenges. The huge potential involved with the various biotechnological

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applications for handmade paper industry has also been explained with special mention.

Keywords

Biotechnological applications · Challenges · Cleaner production techniques · Cotton rags · Machine made and handmade paper · Natural fiber and sustainable enterprise

17.1 Introduction

17.1.1 Paper: An Industrial and Commercial Commodity of Great Use Today

During the period of evolution, when man first started recording various events through drawing or writing, the process raised his intellectual powers to a vastly higher plane than could have been achieved through the mere utterance of vocal sound, that is, by speaking. With drawing in sands and upon the walls of caves, he started using materials like wood, metal, stone, ceramics, leaves, barks, cloth, papyrus, and parchment as basic surfaces upon which to incise or inscribe hieroglyphics and characters but none of these materials could be regarded technically as paper. A true paper is actually the thin felted material formed on flat, porous molds from macerated vegetable fibers. Although the popular literature often characterizes CaiLun of China as being the inventor of paper in the year 105 AD (Hubbe and Bowden 2009, p. 1737), there are ample evidences to show that the paper was invented independently in Indian Buddhist times around 250 BC. However, it could not be accepted as a pious product that time (Singh and Chauhan 2000, p. 67), and it was replaced by the more useful palm leaf and birch bark later on. Archeological findings at Gilgit in the valley of Kashmir indicate that paper was already applied in the Himalayas in the sixth-century AD (Teijgeler 2002, p. 185). For centuries, paper has been competing with the human mind and now with the computer as a preferred means of storing knowledge. Memory systems based on writing have proven advantageous; especially when it is important to retain the facts reliably for long periods of time or among large number of people. But as on date, paper has become an indispensable part of our life today. We begin our life with newspaper and end up while writing the diaries. Per capita consumption of paper is considered as a yardstick of literacy and development of any society (Teijgeler 2002; Chauhan and Bhatnagar 2009; Chauhan and Hussain 2009).

Paper is more than an industrial and commercial commodity today. The welfare of a nation cannot be achieved without rise in the consumption of all kinds of cultural and industrial qualities of paper. We cannot safeguard our human rights like the right to education, right to culture, and right to information. Paper is also used as a packaging material. As we know, packing and wrapping is an index of standard of living. It is the fact that for any increase in national income there will be a

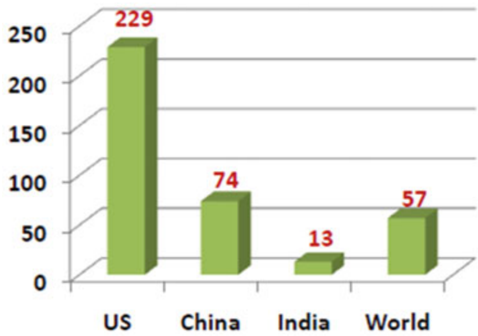

Fig. 17.1 Per capita consumption of paper (CARE Ratings 2018)

proportionate increase in paper consumption (Reddy 2015, p. 71). The per capita consumption of paper of different countries against the world average of 57 kg (Fig. 17.1) clearly signifies the development status of the specific country.

17.1.2 Indian Paper Industry: Present Status and Future Demands

Globally, India is the fastest growing paper market as reflected from the 5-year consumption (FY11–FY16). CAGR of consumption is 8% vs. world at 1%. China and Indonesia have grown at 1% CAGR and developed markets of the USA and the UK have declined at 1% and 3% CAGR in the same period, respectively. Paper demand grows in tandem (*Global paper market-current review* 2019). World production of paper and paperboard is around 390 million tons and is expected to reach 490 million tons by 2020.

The paper industry, which has been dominated by North American, North European, and East Asian companies, it is expected that the two countries India and China will become key players in the industry. As per the CARE Ratings'18, the domestic demand of paper in India grew from 9.3 million tons in FY'08 to 15.3 million tons in FY'16 at a CAGR of 6.4%. For the Indian paper industry, strong economic growth has been accompanied by equally robust demand for paper. The demand drivers and growth triggers have come from a combination of factors such as rising income levels, growing per capita expenditure, rapid urbanization, and a larger proportion of earning population, which is expected to lead to consumption, widening spread of education and increase in literacy rates, rising circulation and number of newspapers and magazines, etc. Packaging paper and board segment is expected to have significant capacity addition across the country. So, there is enormous potential of paper industry in the country (*Paper industry update by CARE ratings* 2018). With the increasing users of new and e-commerce, media will have a big impact on the paper industry too. New market outlets stemming from a rising middle class in emerging markets and increasing demand from hygiene products are going to be the key drivers for the industry growth (*Global paper market-current review* 2019). On having a look into the statistics of paper industry, it can be seen that the paper consumption in India is expected to touch 28 million tons by 2025 from the current level of 12.7 million tons, a growth of more than 120%. Thus, a timely attention to the continuously rising demands of paper may help the industry to cater to the needs in future. Various workers (Khan 2012; Day 2014; Kathuria 2014; Tripathi 2017; Thacker 2018) have reported the status, prospects, and problems of the Indian paper industry from time to time. Paper is actually a simple commodity with complex nature, so it needs to be understood properly to address the problems.

17.1.3 Serious Environmental Concerns of Paper Industry

The striking point to know here is that for 1 million tons of paper, 27 trees of Eucalyptus are required (based on 45% yield, 50% moisture, and 6″ girth 30′ ht.). Accordingly, for an increase in per capita consumption by 1 kg, the estimated extra trees required would be 32 million.

Paper industry thus causes huge deforestation, thereby contributing to the global warming and various pollution problems. Production and use of paper has a number of adverse effects on environment, which are known collectively as *paper pollution*. Paper, being a cheap commodity today, has led to a high level of paper consumption and paper waste. Chlorine and chlorine compounds used for bleaching of pulps generate significant quantities of dioxins. Dioxins are persistent organic pollutants that are generally recognized among the most toxic human-released pollutants in existence. On having a look at the scenario of world's forest area, India has merely 22% of its total land area as the forest area and that too is depleting faster (Table 17.1) (FAO 2015). However, the point to be noted here is that the production and use of conventional mill made paper has a number of adverse effects on the environment that are collectively known as "paper pollution." Besides the deforestation problems, it includes the generation of "dioxins" (persistent organic pollutants recognized as the most toxic human-released pollutants in existence) having

Table 17.1 Top ten countries of the world by forest area (Source: FAO 2015)

S. No.	Country	Forest area (×1000 ha)	FA as % of country area	% of global forest area
1	Russian Federation	814,931	48	20
2	Brazil	493,538	58	12
3	Canada	347,069	35	9
4	USA	310,095	32	8
5	China	208,321	22	5
6	The Democratic Republic of Congo	152,578	65	4
7	Australia	124,751	16	4
8	Indonesia	91,010	50	2
9	Peru	73,973	58	2
10	India	70,682	22	2
Total		2,686,948		67

mutagenic and carcinogenic effects, huge emission of greenhouse gases (NO_2, SO_2, and CO_2), and large amount of color in the effluents. Paper use is an ecological concern that has triggered many paper intervention actions around the world such as the Paper Task Force in the USA, Sustainable Paper Alliance in China, and Paper and Beyond in Europe (Ezeudu et al. 2019).

17.1.4 Handmade Paper: An Ecofriendly Option

In contrast to the conventional papermaking through mills, traditional papermaking generally employs renewable resources and can be carried out in such a way so as to avoid environmental pollution besides helping to bind communities and social groups together (Hubbe and Bowden 2009). Taori (2002) has proposed that the handmade paper manufacturers take further steps to become a more truly ecofriendly industry.

Handmade paper is defined as a sheet of paper or board produced by hand in the sheet-making process. If the sheet is formed by means of a cylinder mould and vat or on a Fourdrinier table, it cannot be called a handmade paper even if the subsequent pressing and drying operations are carried out discontinuously. It should however be noted that Khadi and Village Industries Commission (KVIC) includes paper and boards made in the Cylinder Mould Machine/Vat (CMM or CMV) up to a definite maximum deckle width of 102 cm in the handmade paper category.

Handmade paper is an ecofriendly option of the conventionally produced mill paper. Handmade paper is one of the socially responsible products because it is the tree-free, cleanly produced, environmental-friendly product. The process of handmade paper production is green and clean in the truest sense of its meaning because the handmade papermaking neither utilizes the woody, forest-based raw materials nor it uses the toxic, harmful chemicals (Chauhan and Sharma 2012). It has been reported with full justifications that production of handmade paper can be categorized as a cost-effective, ecofriendly, profitable, energy, and resource-saving technique (Reddy 2015). Keeping in view of the positive role that traditional crafts can play in a society, increasing attention is being paid to them (Biggs and Messerschmidt 2005).

17.1.4.1 Differences Between Handmade and Machine-Made Paper

Although the mill paper has superseded the handmade paper production all over the world, yet the art of handmade papermaking is flourishing in many countries due to the certain distinctive features. The handmade paper has its own specific attributes and qualities to charm anybody for its use. Therefore, in the new millennium, the art of making handmade paper has not only survived but it is moving proudly to heights new and horizons high. Although the basic principle of papermaking is same in both cases, still there are some differences in the papers produced by machines and by hand (Chauhan and Hussain 2009). They are described in Table 17.2.

Table 17.2 Differences between the mill paper and handmade paper (Source: Chauhan and Hussain 2009)

Parameters	Machine/mill made paper	Handmade paper
Raw materials utilized	1. Woody trees (hard woods/soft woods).	1. Traditionally used principle raw material: cotton rags (hosiery waste/textile industry waste/tailor cuttings/denim waste).
	2. Nonwoody plants (straws/bagasse, etc.).	2. Alternate raw materials: locally available, easily pulp able waste materials of ligno-cellulosic nature.
	3. Different types of waste paper.	3. Different types of waste paper.
Pulping procedures utilized	Pressurized digestion at high temperatures is done through kraft pulping/soda pulping/sulfite pulping, which are highly polluting and energy intensive.	Pulping is not required for cotton rags/waste paper.
		For the alternate raw materials, relatively cleaner pulping procedures, viz., open hot digestion/ash lye pulping/lime/urea digestion/alkaline peroxide pulping (APP) process are used.
Freedom to use any fibrous raw material	Due to the giant scale operations in a continuous manner, there are certain limitations to use any new raw material for papermaking in the mills.	Any fibrous raw material whether available in plenty or not can be used because of the utilization of simpler batch procedures at small scales in handmade paper sector.
Pulp yield and brightness levels	Pulp yields lie generally in the range of 40–50% and huge amounts of chemicals are used for achieving the desired brightness levels.	With low lignin content in raw materials and lignin-preserving pulping methods, the pulps produced are the so-called 80/80 class. This means 80% pulp yield and 80% ISO brightness.
Recyclability of the paper	The mill paper can be recycled only for 3–4 times.	Maximum limit of recycling handmade paper is almost double, that is, eight times.
Durability/permanence/endurance	Most of the mill paper made not more than 100 years ago, after less than 50 years is so fragile that it cannot be handled with any degree of safety.	Paper shaken by hand in a mold endures for a much longer period than that formed on the traveling wire of a paper machine.
	The modern paper produced on the machine is far more perfect in formation and in mechanical development than the paper made by hand in the fifteenth century but the endurance and lasting quality of present day papers are often sacrificed for speed of production.	Handmade papers are more durable than the machine-made papers.
		It may be interesting to note that papers made not only 400–5000 years but thousands of years ago are still in excellent conditions.
Strength isotropy	On the traveling wire of the machine, the course of the fibers is limited to the side-to-side shake, which has a	Handmade paper sheets are shaken four ways, causing them to cross and intertwine in formation.

(continued)

Table 17.2 (continued)

Parameters	Machine/mill made paper	Handmade paper
	tendency to throw the fibrous material in one direction only.	
	For this reason, paper formed on machine has a grain and tears more easily one way than the other.	Therefore, handmade paper sheets will tear with almost the same resistance in all the four directions.
		So they show better strength isotropy.
Natural shrinkage	In the case of mill-made papers, artificial drying on heated cylinders produces paper that has not been permitted natural shrinkage.	Handmade papers are dried without any restraint and hence the sheets thus produced have natural shrinkage.
Deckle edges	The edges of mill-made paper are generally very smooth and perfect. However, some machines have also been invented to put mechanical deckle edges on cheap paper by means of a rotating cutting device and thus artificial deckling is done on demand.	Deckle edges are the imperfections in the handmade paper sheets caused by the moist fibrous pulp running against the boundary frame of wooden deckle of the mold.
		Previously, these rough edges were regarded as blemishes and therefore were discarded. But now they are considered to be artistic and desirable.
Difficulty in printing	The sheets of mill-made paper do not find any difficulty in printing and are used very easily and most commonly for the purpose.	There are numerous uncontrollable imperfections in the sheets of handmade paper that cause difficulties in printing, viz., deckle edges, disparity of thickness in separate sheets, as well as within the same sheet, uneven edges, etc.
		Still there is a particular fascination about the product that makes HMP desirable and suitable for the printing work of certain books but such paper should not be used indiscriminately without regard to its traditions.

17.1.5 Indian Handmade Paper Industry: Status

Indian handmade paper industry has travelled a long journey from the *Golden Age* during Mughal Empire to the *Dying Age* in the British period to the Gandhian-led *Revival Stage* during freedom struggle movement. In the Post-Independence era, the umbrellas of KVIC and KNHPI have further supported the industry in one or the other way. Consequently, the industry has been able to earn a very good image in the national and international market. The industry had also shown a mercurial growth in the 1990s due to the continuous, rigorous, and collaborative efforts of KNHPI and KVIC in association with the UNDP. KNHPI has emerged from a UNDP-assisted Government of India project (IND/90/037/A/01/37) titled as "Strengthening the Handmade Paper Industry in India." The institute has now become a center with the capacity to develop and transfer the technology and to provide services to the handmade paper industry to increase the productivity besides improving the quality and marketability of handmade paper products. Various reports are available in literature to explain about the status, prospects, and problems of the Indian handmade paper industry (Chauhan et al. 2016; Saakshy et al. 2015). Due to the global recession and a discontinuity in the coordination of concerted efforts of the concerned agencies, the market of Indian handmade paper and products has witnessed a big blow and the industry is striving hard to earn its original name and fame. Therefore, there is an immediate need to explore various methods so as to address the challenges being faced by the industry.

Teijgeler (2002) has reported the past, present, and future of Indian handmade paper industry. Similarly, history, craft, and science of handmade papermaking have also been reviewed nicely (Hubbe and Bowden 2009).

17.1.6 Challenges Being Faced by Handmade Paper Industry and the Possible Remedies

An exclusive study of the Sanganeri handmade paper industries has been reported with the water and power scarcity as well as market competition with their counterparts as the major challenges being faced by them (Jain et al. 2017). Similarly, challenges faced by the handmade paper industry in Kalimpong region of West Bengal have also been reported (Ray 2016). Although the handmade paper industry can play a very significant role in the present scenario of environmental friendliness, yet it could not have been possible to the expected extents because of the certain challenges being faced by the industry. They are described below herewith along with the possible solutions or remedies:

Lack of Knowledge and Awareness Among the General Public

The general public in India does not have much knowledge of handmade paper and its characteristic features. People are generally not aware of the significance of handmade paper and just use the mill paper conventionally in a knowing or unknowing manner. Because of this lack of knowledge and awareness, people neither use handmade paper or products nor do they try to enter into its business. Most of the handmade paper and paper products produced in the country are exported to different countries of the world. However, in the current scenario of "*Polythene Mukta Bharat*" and "*Swatchh Bharat Campaign*," Indians have started exploring various alternatives and finding handmade paper as a solution so the knowledge is also growing and therefore handmade paper is surely going to play a big role in near future.

Scarcity and Sky Scrapping Prices of Traditionally Used Cotton Rags
With growing concerns about the resource conservations and recycling avenues for closed-loop generations, the textile industries have themselves started exploring ways to reduce the disposal of cotton rags and are trying to recycle them at source. Consequently, the availability of cotton rags from the textile industries is getting limited day by day. At the same time, the demand of cotton rags is increasing with the rising demands of handmade paper and paper products. As a result of such developments, the cost of cotton rags is rising continuously and has reached around Rs. 70/kg in the year 2019 as compared to Rs. 34/kg in the year 2009.

Utilization of Alternative Raw Materials
Keeping in view the shortage and rising prices of the traditionally used cotton rags as principal raw material for handmade papermaking, a strong need of alternative sources of raw materials was realized for the sustainability of the handmade paper industry. With the exhaustive research and development activities at KNHPI, abundantly available waste sources of lignocellulosic raw materials have been evaluated and found to be suitable alternative raw materials for making handmade paper. Various bast fibers (ankra—*Calotropis procera*, paper mulberry—*Broussentia papyrifera*, jute—*Corchorus capsularis/Corchorus olitorius*, silk mulberry—*Moru alba*, etc.), leaf fibers (banana—*Musa sapienthum/Musa paradisiaca*, pine apple—*Ananas comosus*, sisal—*Agave sisalana*, etc.), agro residues (wheat straw, rice straw, lemon grass, Bagasse, Mentha, Bodha grass, etc.), and recycled fibers (elephant dung, cow dung, shredded currency waste, etc.) are now proven alternative raw materials for handmade papermaking as recorded in the monograph published by KNHPI as well. A detailed list of the alternative raw materials is given in Table 17.3. However, challenge is the actual utilization of such alternative raw materials by the industry because the handmade paper manufacturers are more or less inclined towards making handmade paper from traditionally used cotton rags and are reluctant towards adopting newer raw materials.

Development and Utilization of Cleaner Production Technologies
Handmade paper industry is actually a green and clean industry in the truest sense of its meaning because it has been traditionally using cotton rags and tailor cuttings as the principal raw material for making handmade paper. Since they are cellulosic in nature, so there is no need to use any cooking or pulping chemical. Instead, they are directly processed into the beater just after necessary sorting/chopping and dusting. Few chemicals are used only during sizing for making writing printing grades of paper. Thus, the green and clean image of handmade paper has been a strong driving force for greater inclination of consumers towards it. However, while processing natural fibers for making handmade paper, the use of pulping and bleaching chemicals becomes imperative, which may question the ecofriendly credentials of handmade papermaking. Therefore, one of the major challenges before the handmade paper industry is to develop and utilize the cleaner production technologies so as to minimize the actual usage of chemicals in handmade papermaking. In this

Table 17.3 Different categories of alternative raw materials for handmade papermaking (Source: Chauhan and Sharma 2012[17])

Category of the alternative raw material	Common name	Botanical name/specific mention of the material
Bast fibers	Sunn hemp	*Crotolaria juncea*
	Hemp (Bhang)	*Cannabis sativa*
	Jute	*Chorchorus capsularis/ Chorchorus olitorius*
	Ankara	*Calotropis procera*
	Paper mulberry	*Broussonetia papyrifera*
	Silk mulberry	*Morus alba*
	Shogun	*Daphne papyracea*
	Bhimal	*Grewia optival/ Grewia oppositifolia*
	Ramie (China Grass)	*Boehmeria nivea*
	Kenaf	*Hibiscus cannabinus*
	Flax/linen	*Linum usitatissimum*
	Salago	*Wikstroemia species*
Leaf fibers	Banana	*Musa sapiethum/Musa paraqdisiaca*
	Pine apple	*Ananascomosus*
	Sisal	*Agave sisalana*
	Water hyacinth	*Eichornia crassipis*
	Abaca	*Musa textilis*
Grasses/agro-residues	Sabai	*Eulaliopsis binata*
	Bodha	*Chloroxylon coloratus*
	Lemon	*Cymbopogon ciatrus*
	Mentha	*Mentha piperita*
	Wheat straw	*Triticum vulgare*
	Rice straw	*Oryza sativa*
	Bagasse	*Saccharum officinarum*
	Kheep	*Leptadenia pyrotechnica*
	Besharam (Kolumu)	*Ipomea carnea*
Recycled fibers (RCF)	Elephant dung	Pachyderm
	Cow dung	Gobar
	Shredded currency waste of RBI	
	Jute gunny bags	Waste grain bags
	Mixed office waste (MOW)/news print waste (NPW)/magazine waste (MW)	Post-consumer paper waste
	Handmade paper trims/in-house waste of paper industry	Pre-consumer waste of paper

context, lot of technology development and upgradation work has been conducted by KNHPI including biotechnological applications.

Energy Conservation While Using Cotton Rags

The basic process of making handmade paper from the cotton rags is highly intensive in terms of beating energy. Therefore, energy conservation in beating process is a main challenge. Keeping in view of this, KNHPI has evaluated certain identified enzymes for treating the hosiery waste. This kind of enzymatic treatment has shown great promise in saving the energy while beating because the enzyme treatment could result into a saving of about 16–21% of the beating energy as compared to the control.

The treatment of the cellulosic raw materials like cotton hosiery waste (both white and colored hosiery)/denim waste/hosiery mixed with synthetic fiber with certain identified enzymes under the optimized conditions of pH, temperature, pulp consistency, and enzyme dose has resulted into the following advantages:

- Reduction in beating energy to the tune of 15–25% as compared to the respective controls
- Improvement in pulp drainage
- Slightly better strength properties of the handmade paper pulps produced through the enzymatic route

The technology thus developed has also been applied for getting patent.

There are reports showing that the process of enzymatic refining could result into a saving of around 16% beating energy in the case of alkaline-cooked banana pulp (Kumar et al. 2013). Apart from the process of biorefining, it has been suggested that minor steps, viz., rag chopping in proper size, using proper beater consistency and the adoption of pre-soaking of the rags can also result in significant amounts of saving in beating energies (Table 17.4) (Prasad 2006).

Similarly, Prasad (2006) has demonstrated through industrial trials that by increasing the consistency from 2.6% to 3.0% there is almost 20% saving in energy consumption during beating and it also resulted in improved pulp quality as reflected by the strength properties recorded in Tables 17.5 and 17.6. Beating process needs to be properly monitored to get a uniform pulp quality and also lower energy consumption. This monitoring is generally done during the time of beating, whereas monitoring should be done by measuring the freeness at regular intervals by means of the Canadian Standard Freeness (CSF) Tester, which is a simple mechanical instrument, easy to operate, and is readily available in the country. A proper schedule of the workshop shall be prepared and submitted immediately on optimizing the process parameters.

Water Conservation and Recycling

The driving forces in the recycling of water in pulp and paper industry are the high cost of fresh water, inclination of the industry towards environmental friendly process, laid down discharge norms by regulatory authorities, community

Table 17.4 Simple measures evaluated (under laboratory scale) for energy conservation in handmade papermaking (Source: KNHPI)

S. no.	Particulars	Inches	Consistency, %	Beating time	HP	Power consumption (kWh/kg)	% reduction in beating energy
A. Change in size of chopped rags							
1.	Small size	1.5	1.49	20 min	0.75	1.865	40%
2.	Big size	3.0	1.52	35 min	0.75	3.263	
B. Change in consistency of beater							
1	Small size	1.5	1.49	20 min	0.75	1.865	25%
2	Small size	1.5	1.64	15 min	0.75	1.398	
C. Change in energy consumption on soaking							
1	Without soaking	1.5	1.50	35 min	0.75	3.263	23%
2	Soaking 24 h	1.5	1.49	27 min	0.75	2.517	

Table 17.5 Comparison of physical strength properties at different beating consistency (Source: KNHPI)

Consistency (%)	Beating time (h)	CSF (mL)	Tensile index (Nm/g)	Tear index (mN m^2/g)	Burst index (kPa m^2/g)	Double fold (no.)
2.60	4.50	625	14.18	12.15	0.21	62
3.00	4.50	400	26.43	20.26	1.44	165

Table 17.6 Industrial trial of the pulp beating at different consistency (Source: KNHPI)

S. no.	Unit I	Pulp consistency 2.60%	Pulp consistency 3.0%
1.	Size of Hollander beater	30″ × 36″	30″ × 36″
2.	Electric motor installed	30 HP	30 HP
3.	Quantity of raw material	100 kg/charge	120 kg/charge
4.	Beating time	4.5 h	4.5 h
5.	Final freeness of pulp	625 mL	400 mL
6.	Total energy consumption	100 kW h	100 kW h
7.	Energy consumption/kg of pulp	1.00 kW	0.83 kW
8.	Cost of energy/kg of pulp	Rs. 5.00	Rs. 4.16

perception, and high cost of secondary effluent treatment process (*CPPRI* 2008). With the systematic studies carried out for the handmade paper industry, approximately 89% of water can be recycled if collected properly in the case of handmade papermaking from cotton rags and similarly approximately 81% of water can be recycled while making handmade paper from natural fibers like sisal/banana (Chauhan 2011).

Utilization of Azo-Free Dyes in Ecofriendly Manner

Traditionally, direct dyes are used for producing colored handmade paper sheets due to their easy solubility and ready attachment to the fibers. Further, due to the good light fastness characteristics of direct dyes, they are better choice for handmade paper manufacturers. Most of the direct dyes available in the market possess azo group, which on reduction release carcinogenic or harmful amines. Therefore, utilization of azo-free dyes in ecofriendly manner is a challenge for handmade paper industry so as to maintain its ecofriendly credentials for a long run. KNHPI has conducted extensive studies in this area while exploring the extraction and application of different types of natural dyes for coloring handmade paper pulps (Saakshy et al. 2013, 2010). The studies have shown very promising results.

Waste Water Treatment

Although the process of handmade papermaking is cleaner and the handmade paper produced comes under the category of ecofriendly product, still there are concerns involved with the treatment of waste water and effluent generated so as to enable them to be recycled back to the process or to discharge them safely into the fields or water bodies. Color removal is of major concern. Therefore, lot of work has been carried out by KNHPI to address this challenge. Diversified methods, viz., use of low-cost fly ash or activated carbon as color adsorbent (Agarwal et al. 2016a, b), process of ozonation (Agarwal et al. 2014) process of fungal decolorization (Chauhan et al. 2014, 2015c), etc., have been explored for treating effluents from handmade paper industry.

Uniformity in the Production of Handmade Paper Sheets
Although pride is taken in the fact that no two sheets are the same with HMP, uniformity in basis weight, color, whiteness, and general appearance are important parameters of quality that can be achieved through a professional and scientific methods of operation. When chemicals are added to stock, they must be weighed and not simply put into the beater. To maintain stock consistency, the stock water level should be maintained by painting level marks on the beater. The new Japanese method of sheet formation and the European method of stock recirculation in the vat are things that can be adopted for finer varieties of HMP. For improvement in sheet formation of thinner grades of paper, the use of formation aids should be taught to the papermakers. The formation aids could be either synthetic chemicals or preferably vegetative mucilages. The vegetable mucilage controls the fiber dispersion as well as the drainage rate. Mucilage of Cactus (*Opiuntia belloni*) has been tested with good results (Kumar and Maheshwari n.d.). Therefore, maintaining a proper uniformity in the making of different handmade paper sheets is a major challenge for the handmade paper manufacturers. A trained person or a skilled worker can be helpful in addressing these concerns of the handmade paper industry.

Quality Control
Quality control is another very important challenge before the Indian handmade paper industry. Generally, the process of handmade papermaking is considered as an art and their diversified creations are often appreciated as well. However, bits of dirt and specks are invariably found in sheets of handmade paper mainly due to open drying under sun in the lawns and use of recycled fibers. In mottled or decorative papers, this is of course not a serious problem, but in white or semi-white sheets, such as drawing and art paper or stationery papers, it speaks of low quality. The proper selection of raw materials, careful cleaning of work areas, drying of sheets in covered halls, and a sense of cleanliness and good housekeeping could solve the problem to a great extent (Kumar and Maheshwari n.d.).

Cost Competitiveness in the Domestic Market
The handmade paper and its products are relatively costlier than the mill-made paper, and therefore, people show reluctance to use them in the country. Therefore, making them cost competitive is a greater challenge for the handmade paper industry. This can be achieved by substituting costlier cotton rags with the suitable alternatives. In this regard, waste paper/handmade paper trim/gunny bags/waste or rejected stuff of khadi fabric, cow dung, waste polythene materials, etc., have been found to be appropriate options. Recently, KNHPI has developed cost-effective

carry bags by partially substituting rags with some of such alternatives. The carry bags thus developed are being supplied to various sales outlets of Khadi India for consumption in the domestic market. These carry bags are becoming very popular day by day.

Lack of BIS Standards for the Handmade Paper

There are no standards available for the diversified varieties of handmade paper except drawing paper (BIS 3064/1986), paper for permanent and semi-permanent records (BIS 1774/1986), and pulp board (BIS 4664/1986). Due to the lack of BIS Standards, handmade paper manufacturers get exploited by the traders and they also find problems in bulk supplies. So, the development of BIS Standards is one of the major challenges for the handmade paper industry. Efforts are being made by KNHPI in association with the organizations like the Central Pulp and Paper Research Institute (CPPRI), Saharanpur, and the Indian Institute of Packaging (IIP) to formulate certain standards for the specific varieties of handmade paper separately (Sharma 2009).

Competition from Duplicate Handmade Papers

The industry is also having challenge to cope up with the competition from the duplicate handmade paper sheets produced by conventional paper mills, which are also available at a cheaper price. So, people are instigated to purchase those sheets under the impression of being the handmade paper sheets. Therefore, the industry needs to not only popularize the identifying features of true handmade paper sheets but to also make them available at lower/affordable prices.

Competition from the Countries Like China

Indian handmade paper industry is striving fast and facing challenges from the major players like China, Philippines, Thailand, and Indonesia. Therefore, the handmade paper manufacturers should focus upon the cost-effectiveness and quality of the handmade papers produced.

GST Concerns of the Industry

The Indian Government has recently imposed a GST of 12% on the handmade paper and its products. The industry people find it a challenge and have concerns that the imposed GST should be either removed similar to the khadi products or the GST rates should be reduced to the bare minimum. The idea behind the demands of GST reductions/removal is that the handmade paper is an ecofriendly product and its manufacturing process generates a huge employability potential especially in the rural sections of the Indian society with the additional prospects of women empowerment.

17.1.7 Biotechnological Applications for Handmade Paper Industry: Huge Potential

Having realized the great potential associated with the biotechnological applications especially for the handmade paper industry due to the utilization of small-scale batch procedures (Singh and Chauhan 2000; Jain et al. 2009a), KNHPI, Jaipur, developed a vision to work upon this important area. With the set up of a separate Biotechnology laboratory in the year 2001–2002 and its further extension in the year 2008–2009 and 2019–2020 KNHPI, Jaipur, has been doing pioneering research work in the area of biotechnological applications in the handmade paper industry. Having done extensive studies carried out so far at KNHPI, the following applications of biotechnology have been proved to be very useful for the handmade paper industry:

- Bioenzymatic pulping of shredded currency waste of Reserve Bank of India (RBI) for making handmade paper (Chauhan et al. 2009; Khandelwal and Chauhan 2011)
- Biobleaching of handmade paper pulps (Chauhan and Bhatnagar 2009; Chauhan et al. 2006; Choudhury et al. 2006; Chauhan and Pant 2005).
- Biorefining of handmade paper pulps
- Bioretting for the handmade paper industry (Chauhan and Sharma 2014; Pant et al. 2005; Jain et al. 2009b; Chauhan et al. 2013)
- Enzymes in making tissue paper from banana/jute fibers (Chauhan et al. 2015b).

Out of these potential applications, institute has already applied for a patent on "Biorefining." Apart from this, the other two patents applied, viz., "Improved process of making tissue paper from banana fiber" and "Improved process of recycling shredded currency waste of RBI for making handmade paper," also have the basis of enzyme utilization in the newer process. Efforts are being made to motivate the entrepreneurs of the handmade paper industry for adopting newer technologies developed by the institute. With the development of these technologies, newer areas have also been explored continuously from the viewpoint of their potential utility in handmade papermaking. With the studies carried out so far, utilization of microbial cultures (viz., white rot fungi) for pulping/bleaching/decolorization of effluents has also proven very effective (Chauhan et al. 2014, 2015a, c; Aswal et al. 2020). Thus, biotechnological applications have a very huge potential that needs to be harnessed for addressing many of the challenges of the Indian handmade paper industry.

17.2 Solid-State Fermentation of the Bast Fiber of Paper Mulberry: A Case Study

The solid-state fermentation (SSF) that involves the growth and fermentation by microorganisms, especially fungi on moist, water-insoluble and solid substrate in the absence or near absence of free water, was chosen as a pretreatment option to modify the Alakline Peroxide Pulping (APP) process because it offers distinct advantages of low-cost, no need of fermentation control, lesser water volumes required, considered the self-control process, no processing required for dewatering (Krishna 2005), and above all this might prove to be industry-friendly. When SSF involves “cultivation on natural substrates” like starch or lignocellulose based agricultural products or agro-industrial sources, the substrate serves both as support and a nutrient source, while SSF that utilizes “cultivation on an inert support impregnated with a liquid medium,” the substrate serves only as an anchor point for the microorganisms (Krishna 2005).

As a part of the exhaustive studies on biotechnological applications for the handmade paper industry, the bast fiber of paper mulberry (*Broussentia papyrifera*) was subjected to solid-state fermentation (SSF) with the standard culture of the white rot fungi procured from the Institute of Microbial Technology (IMTECH), Chandigarh, that is, *Pleurotus ostreatus* (MTCC code-142) under the conditions shown in Table 17.7. The paper mulberry fiber was inoculated with the fungus in the presence of different nutrients like sucrose (S), corn powder (C), and the additives like EDTA, the chelating agent (Q) and the surfactant like Tween-80 (T). The incubation was done at a temperature of 25–30 °C (27 °C) for a period of 2 weeks (Chauhan and Bhatnagar 2008).

After the completion of the incubation period, all the treated and control fibers were harvested and cooked in the bomb digestor using the alkaline peroxide pulping (APP) process with 7% NaOH and 4% hydrogen peroxide at a temperature of 95 °C for a period of 3 h. Black liquors were collected in every case and pulps obtained were washed thoroughly. The black liquors generated during pulping were characterized for all the parameters of lignin, color, and Residual Active Alkali (RAA). All the pulps thus obtained were evaluated for the optical and strength properties after beating.

17.2.1 Effect of SSF on Various Parameters of Interest for Handmade Papermaking

During SSF of the paper mulberry fiber with *P. ostreatus*, the fungus was found to grow in a flowering manner with varying speed in the presence of different substances being maximum in the presence of additional carbon sources including sucrose and corn powder. The fungal mycelium seemed to be growing like floral petals elongating from the center (i.e., from the mycelial pellet used for incubation). Black liquor of all the treated cases showed more amounts of total solids, lignin, and color than that of the control pulp. While RAA was lower than control (Table 17.8). Pulp yield was lesser in all the treated cases than the pulps obtained from control pulp yield. Brightness was more in all the pulps obtained from fiber pretreated with PO except when SSF was carried out in the presence of water only and maximum brightness was obtained when SSF was carried out in the presence of sucrose. As far as strength properties are concerned, all the parameters including tensile index, burst index, and tear index were found to be higher than the control pulp. Thus, the maximum gain of four points in the pulp brightness was obtained in the case of paper mulberry fiber subjected to SSF with PO in the presence of sucrose (Table 17.9) (Chauhan and Bhatnagar 2008).

Table 17.7 Conditions used during fungal treatment of paper mulberry

	A	B	C	D	E	F	G
Parameters	(F + H_2O)	(F + Q)	(F + T)	(F + Q + T)	(F + Q + T + S)	(F + Q + T + C)	(control)
Mycelium	50 discs	50 discs	50 discs	50 discs	50 discs	50 discs	Nil
C. powder	Nil	Nil	Nil	Nil	Nil	1%	Nil
EDTA	Nil	0.2%	Nil	0.2%	0.2%	0.2%	Nil
T-80	Nil	Nil	0.1%	0.1%	0.1%	0.1%	Nil
Sucrose	Nil	Nil	Nil	Nil	1%	Nil	Nil
Incubation period	2 weeks	2 weeks	2 weeks	2 weeks	2 weeks	2 weeks	2 weeks

Table 17.8 Analysis of black liquors of pulp from PO-treated fiber

	A	B	C	D	E	F	G
Parameters	(F + H_2O)	(F + Q)	(F + T)	(F + Q + T)	(F + Q + T + S)	(F + Q + T + C)	(control)
T.S. (%)	4.10%	4.42%	4.85%	4.20%	4.28%	4.54%	3.95%
RAA (gpl)	1.10	1.18	1.16	1.26	1.20	1.05	1.21
pH	9.63	9.84	9.70	10.18	10.11	9.67	9.80
Lignin (gpl)	7.03	7.58	7.99	7.60	8.90	8.20	6.17
Color (PCU)	2.3×10^4	2.6×10^4	2.8×10^4	2.4×10^4	2.8×10^4	2.9×10^4	1.8×10^4

Table 17.9 Optical and strength properties of pulp from PO-treated fiber

	A	B	C	D	E	F	G
Parameters	(F + H_2O)	(F + Q)	(F + T)	(F + Q + T)	(F + Q + T + S)	(F + Q + T + C)	(control)
Pulp-CSF	360	400	355	390	400	390	365
Pulp yield	65.3%	59%	57%	58%	60%	61.3%	68%
Brightness	25.20%	30.60%	30.50%	29.30%	32.12%	31.67%	28.50%
Tensile index	33.22	30.05	29.62	29.61	31.20	28.63	29.62
Burst index	3.99	2.67	2.60	2.45	2.28	2.67	2.27
Tear index	15.20	13.66	14.13	12.86	16.83	12.95	12.77

The drainability of almost all the pulps obtained from fungal pretreated fiber was found to be better than the control pulp, which is in concurrence with the previous findings This might be because degradation of parenchymatic tissues is reported to be a common characteristic of fungal attack on different herbaceous materials (Akin et al. 1995; Barrasa et al. 1995), which has a positive effect on pulping as these parenchymatic cells contribute to the fine fraction of pulp. This fraction exerts a negative effect on pulp dewatering on the paper machine. So its removal results in improved drainage properties of the pulp (Camarero et al. 1998). Similarly, Sabharwal et al. (1996) reported that the pulps obtained from fungal-treated bast strands at lower CSF levels were easier to drain compared to untreated samples (Sabharwal et al. 1996). While observing the strength properties of the pulps, the best strength properties were found with PO as compared to the control. It has been reportedly explained during fungal decay of wood that *Pleurotus* species shows selective mode of degradation, thereby resulting into separation and preservation of the integrity of cellulose fibers (Sabharwal et al. 1996).

The pulps obtained from the APP of the fiber subjected to SSF with white rot fungal strains were found to be better than the control pulp, which may be because of the fungal softening and lignin modification of the paper mulberry fiber that occurred during SSF. This has been also reported in the literature with wood chips because it was suggested that the fungal pretreatment of wood chips removed some of the lignin and modified other lignin; these changes might make it easier to remove lignin in the subsequent pulping process. Fungal treatment causes softening and swelling of wood cells and that these fungus induced changes and the removal/modification of lignin might result in improved chemical penetration during pulping operations, which could result in better pulp. Similarly, the softening of middle lamella and subsequently controlled fibrillation, which restricts the formation of fines, was reported to be responsible for the enhancement of strength properties obtained in the biomechanical pulps of kenaf as compared to control (Sabharwal et al. 1996) and a better fiber separation and less damage to cell wall during refining of fungal-treated bagasse was considered responsible for the improved strength properties obtained in biologically treated pulps.

Fibrils of bast fibers lie more or less parallel to the fiber axis, unlike wood fibers whose fibrils are spirally wound. Therefore, bast fibers can be split lengthwise by mechanical action to yield fine, relatively long threads (Clark 1978). In woody and nonwoody plants, lignin is typically concentrated toward the outer cell wall and middle lamella, whereas in the bast fibers, lignin is more evenly distributed. Besides, the lignin content of bast fibers is comparatively lower than conventional woody and nonwoody materials used for papermaking and their lumen size is generally larger. Therefore, low lignin content and large lumen size have beneficial effect during refining. This might be the reason behind the time saved during beating of pulps obtained from fiber pretreated with fungal strains as compared to the time taken by the beating of the pulp obtained from untreated fibers. Besides, Sabharwal et al.

(1995) have also reported higher-energy savings during secondary stage (fibrillation) of refining of the fungal-treated jute and suggested that this was due to the combined effect of softening of lignin by fungal attack in the layers of cell wall in addition to large lumen size of the bast fibers (Sabharwal et al. 1996).

As far as brightness of the pulps obtained from fungal-treated fiber is concerned, a brightness gain was achieved in the presence of sucrose or corn powder and other additives with the maximum of four points in the presence of sucrose. While in the absence of any additive, that is, with water only, there was a drop in brightness. Reports available in the literature also show a lot of variation in brightness of the fungal-treated pulps. For biomechanical pulps, it was reported that the fungal pretreatment significantly reduces the brightness of the resulting pulps; however, it was also showed that the pulps could be bleached with either alkaline hydrogen peroxide or sodium hydrosulfite and the biomechanical pulps gained more brightness points than did the corresponding untreated control pulps. Thus, the optical properties although get diminished during fungal treatment but brightness can be restored readily with peroxide bleaching (Akhtar et al. 1998) Similar response to bleaching was reported when biomechanical pulps were prepared from aspen chips treated with different strains of *C. subvermispora*, and the pulps obtained from the fungus-treated aspen wood chips responded well to peroxide bleaching, readily reaching the same or higher brightness levels than the pulp obtained from an untreated control. Fungal pretreatment on lodge pole pine for TMP production is also reported to decrease brightness significantly.

On one hand, biomechanical pulp of jute bast is reported to be having lower brightness and that it could not be bleached to the values equivalent to control, that is, refiner mechanical pulp when bleached with similar doses (Sabharwal et al. 1996, 1995) while, on the other hand, the biochemical pulps obtained from soda AQ-pulping of the whole jute chips pretreated with four different fungal strains were reported to show improved brightness values to a level of 4–5 points as compared to the control pulp (Mohiuddin et al. 2003). While a slight increase in the brightness of the biomechanical pulp obtained from fungal-treated kenaf has been reported as compared to the respective control. The wheat straw treated with *Pleurotus* sp. is reported to increase the substrate brightness. While studying the biokraft pulping of whole kenaf and kenaf bast using *C. subvermispora* for pretreatment, it was reported that biokraft pulps could be bleached to higher brightness levels (about 8 points) than did the respective controls for the same amount of chlorine dioxide used, and it was suggested that the white rot fungi might partially reduce the extractives and modify the lignin into a form suitable for removal during bleaching with chlorine dioxide and/or hydrogen peroxide (Ahmed et al. 1998). It was also reported that white rot fungi partially removes the extractives and modifies the lignin structure (Akhtar et al. 1998) in woods.

Thus, different values in brightness of the pulps obtained from fungal-treated fiber in the present study are similar to those reported in literature. However, no report is exclusively available on SSF of the paper mulberry fiber prior to the APP process. The brightness gains realized in the pulps obtained from modified APP process through SSF with white rot fungal cultures might be explained due to partial reduction of extractives and modification of lignin of the paper mulberry bast into a form suitable for either removal or further modification during the APP process, which utilizes hydrogen peroxide as a bleaching aid during pulping itself. Thus, the effect of hydrogen peroxide mentioned in the literature-available reports of better bleachability of the pulps obtained from fungally treated woody and nonwoody as well as bast fibers with the hydrogen peroxide might be functional during APP process also and thus resulting into brightness gains of the pulps obtained from APP of paper mulberry fiber subjected to solid-state fermentation with *P. ostreatus* as compared to that obtained from untreated control fiber.

17.3 Conclusion

In view of the growing public concern about the environment, handmade paper can have a big share in the market due to its ecofriendly attributes. However, for actually realizing the potentials involved, industry people need to face the inherent challenges with fruitful solutions/remedies. Besides the other technical modifications as detailed herewith, biotechnological applications can play a very significant role in addressing such challenges of the handmade paper industry as reflected in the huge potential described. In the specific context of the case study of the solid-state fermentation of the bast fiber of paper mulberry with the white rot fungus, *P. ostreatus*, the biotechnological route has proved to be very effective in terms of brightness gain and improvement in strength properties of the treated fiber as compared to the untreated fiber. Such applications of biotechnology can help in improving the eco-friendliness of handmade papermaking, thereby enabling to move ahead more efficiently with the concept of “green marketing.”

Once the major challenges are tackled, the Indian handmade paper industry should adopt the concept of “green marketing” as a new marketing avenue of handmade paper. The green marketing, which speaks about the growing market for sustainable and socially responsible products and services, can be rightly utilized for handmade paper and products. Green marketing is not simply a “catch phrase,” rather it is a marketing strategy that can help an entity to get more customer improvements in bottom line and thereby an improvement in the value of the enterprise as well. At the point of juncture where excessive pollution has provoked nature and the nature has started behaving in unnatural ways (in the form of global warming vs. global cooling, heavy rains vs. draught, and other natural calamities like frequent earthquakes and tsunami, cyclones, epidemics, etc.), green marketing of handmade paper can boost a new life in the industry. It is time to stop “recycling” our twentieth-century ideas and to start looking at the reality of current situations. To thrive in this dynamic world, a new “mind dynamic” is needed rather than the new “mind-set” so that the huge potentials of the handmade paper industry as a sustainable enterprise can be utilized for the benefit of the mankind and also for the holistic development of the nation.

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18 Exploring the Niche: Real Environment Demonstration and Evaluation of Innovative Nature-Based Sanitation Technologies in a Water-Scarce Community Context in India

Tatjana Schellenberg

Abstract

The exploration of niches through local demonstration of innovative technologies plays a key role in enabling sustainability transition processes.

This chapter is based on investigations carried out under the INNOQUA Research Project and the demonstration of different modular system configurations of nature-based sanitation solutions in water-scarce and marginalized community setting in South India. The four innovative technologies are composed of Lumbrifiltration as primary and secondary treatment technology based on vermifiltration processes, Daphniafiltration via daphnids and BioSolar Purification via microalgae and sunlight as tertiary treatment stages, and UV disinfection as quaternary treatment stage. Besides the overall generic goal in providing an efficient and affordable sanitation solution, sustainability is addressed at a broader horizon by targeting at water reuse in water-scarce environments and nutrient recycling in response to increasing food insecurity and land degradation.

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Schellenberg, T. is the author of this publication and the responsible research engineer for the installation, operation, analysis, and evaluation of the presented INNOQUA demonstration site.

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This study presents the findings after one full year of pilot-scale demonstration in real environmental conditions in a marginalized community based in Bangalore, India. Technology performance of each module was evaluated based on the parameters BOD, COD, TSS, temperature, pH, conductivity, NH_4-N, TN, NO_3-N, NO_2-N, TP, PO_4-P, *E. coli*, Faecal Coliforms, Faecal Streptococci, *Salmonella*, *Legionella*, loading rates, energy footprint, and operation and maintenance aspects.

Keywords

Algae · Daphniafilter · Decentralized · Lumbri-/vermifilter · Nature-based · Sustainability transition · UV disinfection · Wastewater treatment

Abbreviations

A	Area
BOD/COD	Biological oxygen demand/chemical oxygen demand
BSP	BioSolar Purification
cap/d	Capita per day
CDD Society	Centre for DEWATS Dissemination Society
DF	Daphniafilter/Daphniafiltration
dia.	Diameter
FC	Faecal coliform
GHG	Greenhouse gas
hh	Household
HRT	Hydraulic retention time
L	Litre
LF	Lumbrifilter/Lumbrifiltration
LF_{eff}	Lumbrifilter effluent
LF_{in}	Lumbrifilter influent
max./min.	Maximum/minimum
m/mm	Metre/millimetre
mg/g	Milligram/gram
$m^2/m^3/m^{2 \text{ or } 3}$	Square metre/cubic metre/m^2 or m^3; both units valid in this case
mJ/cm^2	Megajoule per square centimetre
N/TN/NH_4-N/TKN	Nitrogen/total nitrogen/ammonia/total Kjeldahl nitrogen
NTU	Nephelometric turbidity units
no.	Number
NO_3-N/NO_2-N	Nitrate/nitrite
O&M	Operation and maintenance
P/TP/PO_4-P	Phosphorus/total phosphorus/phosphate
PE	Population equivalent
pH	Power of hydrogen

ppm	Parts per million
inh	Inhabitant
Q_{in}	Incoming volumetric flow, expressed in volume per time unit
TSS/SS	Total suspended solids/suspended solids
UV	Ultraviolet
°C	Degree Celsius
%	Percent

18.1 Introduction

The persistence and accelerating magnitude of environmental pressures is drastically increasing. Although socio-technical systems of modern societies constantly take up corrective measures to improve environmental performance, the sheer severity and accumulation of today's challenges faced by unsustainable practices leaves an increasingly constrained room for responsive action and the recognition that the core problem lies in profound systemic flaws (Raven et al. 2010; Köhler et al. 2019; Grin et al. 2010; Feola 2020). The concept of technological optimism is not new and has been discussed broadly. As early as 1972, the Club of Rome reported that technological adaptation alone cannot provide a sole solution, while nowadays a growing consensus can be discerned that a radical change in the overall structure of systems is required (Raven et al. 2010; Köhler et al. 2019). In order to understand and enable such major systemic shifts for restructuration, an emerging research field around 'sustainability transitions' has developed.

The necessity for wastewater treatment historically evolved and was driven by rising public health concerns in growing urban environments. Despite representing one of the major milestones for public health, sectoral development continues as a struggle in geographies of the Global South due to lack of funding for heavy investments in infrastructure for a basic social service and limited direct revenue streams. With a return of investment for sanitation typically 'paying off' indirectly and 'diluted' in the form of healthcare benefits, the substantial contribution towards poverty alleviation or importance in regard to economic growth tends to be masked and forgotten (Van Minh and Viet Hung 2011; WHO 2012). Over the pace of time the concept of 'sustainable development' gained political attention, and Berkhout describes that social management of technological systems increasingly focused on the environmental field requesting for a fundamental paradigm shift in the sector (UN 2017; Berkhout et al. 2005). Within this shifting future, the envisioned sector rather transforms to a generic resource hub and key element in addressing rising concerns of water scarcity, growing food insecurity, while contributing to the energy revolution (Schellenberg et al. 2020; POWERSTEP H2020 2018). This deviating perspective does not only hold amongst the most prospering potential towards a sustainability transition but further could enable the faced long-term challenges in overall broad coverage in wastewater treatment infrastructure through additional revenue streams.

Research on sustainability transitions and technology innovation has intensified in order to find solutions and holistic approaches that pave the way for a fundamental transformation. Nature-based and green technologies under poly-centric planning and recycling scenarios are understood to deliver a more sustainable solution (Lüthi et al. 2011; Copadaglio 2017; UN WWAP 2017). However, large technical systems typically are composed of 'seamless webs' under which regimes develop along trajectories into complex interweaving systems of artefacts, networks, institutions, rules, and norms (Hughes 1986, 1987; Geels 2004; Berkhout et al. 2005). Given this high degree of complexity towards a deep structural change, technological innovation is typically confronted with a lack of societal legitimacy and profound scepticism (Binz et al. 2016). In order to overcome these barriers and provide a proof of concept on technology readiness levels, the exploration of niches through real, long-term environment demonstrations becomes a relevant starting point in initiating transitions.

The multinational INNOQUA Research Project represents one such niche investigation, in which innovative nature-based wastewater treatment technologies are analysed over the long term in real environmental conditions across 11 demonstration sites worldwide. The key aim is to integrate individual modular, low-cost, sustainable technologies in configurations, which match the local context and further aim at resource recovery (in the form of water and nutrients) and energy efficiency. This study presents the findings of the Indian demonstration site, where all four investigated INNOQUA technologies, including a Lumbrifilter, Daphniafilter, BioSolar Purification, and UV disinfection, have been analysed over 1 year in a marginalized and water-scarce community setting.

18.2 The INNOQUA Project

The objective of the INNOQUA Research Project is the provision of modular, low-cost, ecological, and decentralized wastewater treatment systems. Varying analysed system configurations range from onsite solutions, community systems to industrial treatment facilities. The four investigated treatment technologies in the project are composed of the Lumbrifilter (LF), Daphniafilter (DF), BioSolar Purification (BSP), and an ultraviolet (UV) disinfection unit (Fig. 18.1).

These different technologies can be combined in different configurations depending on (a) incoming wastewater characteristics, (b) local environmental or climatic conditions, or (c) discharge requirements under varying receiving environment or recycling purposes. Table 18.1 provides an overview of the different treatment schemes and configurations in the various demonstration sites.

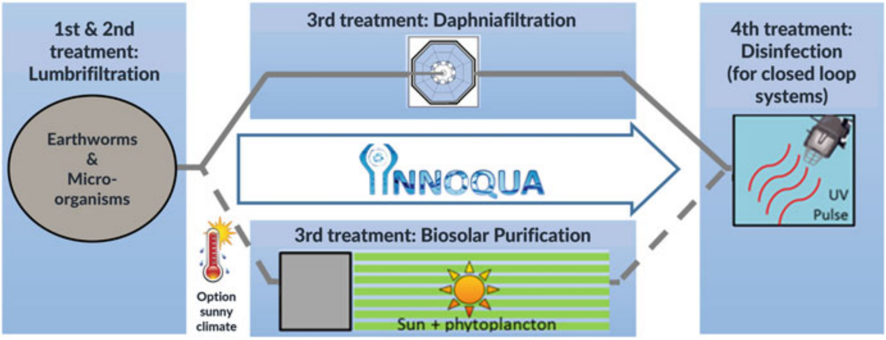


Fig. 18.1 Optional INNOQUA system configuration including all INNOQUA technologies (NOBATEK/INEF4 n.d.-a)

Table 18.1 Overview on system configurations and the demonstration site setting in the different INNOQUA locations

Country	Setting	LF	DF	BSP	UV	Recycling
Ecuador	Domestic apartment complex	Yes	Yes			Irrigation of ornamental plants
France	Office facility	Yes	Yes			Irrigation of ornamental plants
France	Industry, aquaculture sludge	Yes				Lumbricomposting unit
India	Community	Yes	Yes	Yes	Yes	Irrigation in garden
Ireland	Dairy and beef farm	Yes				Agricultural land and year cleaning
Italy	Individual household	Yes			Yes	–
Peru	University	Yes	Yes	Yes	Yes	Irrigation of ornamental plants
Romania	Hotel facility	Yes	Yes			–
Tanzania	Community	Yes	Yes		Yes	Irrigation banana plantation
Turkey	Tourist housing complex	Yes	Yes		Yes	Irrigation of ornamental plants
Scotland	Community	Yes	Yes			–

18.2.1 Lumbrifiltration

The Lumbrifilter is an engineered natural wastewater treatment system, also commonly known as a vermifilter. Lumbrifiltration is a primary and secondary treatment process. The system is a vertical filter composed of sequenced filling materials. The treatment is based on the degrading processes by epigeic worms and bacteria attached to the filling material. The most common earthworm species in typical vermifiltration technologies represent *Eisenia fetida* but can vary according to the given geography. Earthworms play an important role in regulating the excess biomass as they feed on the biofilm attached to filling materials and suspended solids in the active layer. The movement and channelling activities of worms allow to prevent clogging of the filter, while maintaining aeration and percolation functions. The main treatment is performed by bacteria in the biofilm attached to the filling materials respective to treatment processes of a trickling filter. The Lumbrifilter thus is, in comparison to other technologies, understood as 'excess-sludge' free and low odour primary and secondary treatment stage typically used in the treatment of blackwater, greywater, primary (settled) sewage, and industrial effluents (Singh et al. 2019; Sinha et al. 2012; Jeevitha et al. 2016). Apart from major removal rates for common pollutants in wastewater, it holds benefits in three further fields. Studies indicate the effectiveness of worms in bioremediation processes and by this possibly could provide a solution towards the treatment of certain emerging pollutants (Zhu et al. 2017; Markman et al. 2007; Sinha et al. 2012). As an aerobic treatment stage, vermifiltration is reported to reduce GHG emissions, which commonly arise to big concern from wastewater treatment facilities (Luth et al. 2011; Campos et al. 2016) but further also allows to recover nitrogen for further application in a rather more stable form of nitrates with regard to volatilization (Kumar and Ghosh 2019).

18.2.2 Daphniafiltration

The Daphniafilter is a tertiary treatment stage based on natural processes observed for filter feeding daphnid species in water bodies in conjunction with bacterial/ microalgal biofilms (Pous et al. 2020). Daphnia are planktonic crustaceans, more commonly known as water fleas and for their wide application as bioindicators in aquatic toxicology studies (Ebert 2005; Vu Le et al. 2016). Daphnia feed on suspended solids with particle sizes <30 μm that do not settle in a secondary clarifier and thus are associated to the removal of organic matter and to a certain extend also pathogens such as *E. coli*. Resultantly, they can further contribute to the clarification and disinfection of water (Ebert 2005; Serra et al. 2014). Representing a wetland function, microalgal biofilms, or aquatic plants such as *Lemna minor* serve to remove or transform nutrients, while contributing to favouring environmental conditions for the daphnia population (Gersich and Hopkins 1986). The genus of Daphnia covers more than 100 identified species globally with varying sizes from 0.5 mm up to 6 mm. The most commonly studied Daphnia species represent *Daphnia magna* or *Daphnia pulex*. However, daphnia species can vary for the geography with respective environmental or climatic conditions and presumably also feed characteristics. The population of daphnids is dynamic and reported to depend on various factors such as optimum water temperature in the range of 6 °C up to 26 °C, light conditions, or chemical composition of the habitat (Müller et al. 2018; Gersich and Hopkins 1986; Serra et al. 2019a, b; Hathaway and Stefan 1995; INNOQUA Project n.d.). Within the INNOQUA system configuration, Daphniafiltration is sequenced in line with the UV disinfection unit with the aim to further polish the effluent by removing residual particulate COD, nutrients, and reduce turbidity to further improve the performance of the final disinfection stage.

18.2.3 BioSolar Purification

The BioSolar Purification module is a tertiary treatment stage suitable for geographies with higher solar radiation. The treatment is based on the principles of a photobioreactor and metabolic capabilities of microalgae and bacteria. The module is designed in a simplified model of a thin layer cascade to facilitate O&M and affordability. While the attached algal biofilm eliminates nutrients through biomass uptake and their accumulation in the form of biomass, the large surface area of the cascades exposes the circulating water to sunlight aiming at further disinfection of the final effluent. Wastewater treatment with microalgae has a long history of 75 years and finds application in the treatment of sewage, agricultural, or industrial wastewater (Raouf et al. 2012). As a tertiary treatment system, the treated wastewater could be reused in water-scarce environments, whereat the algal biomass can act as alternative fertilizing matter and soil conditioner.

18.2.4 UV Disinfection

Ultraviolet light disinfection represents a quaternary treatment stage aiming to reduce the load of pathogen levels to meet discharge and reuse limits. INNOQUA considers this technological option for environments where treated water is intended for reuse applications and/or where land area may represent a limiting factor.

18.3 Methodology

Within the following, the setup of the Indian demonstration site with given analysis conditions for the evaluation of technology performance is described for the different INNOQUA modules.

18.3.1 Setup of the Indian Demonstration Site and Conditions

The demonstration site is located in an economically weak community in a suburban area of Bangalore, South India. The system configuration in this demonstration site is composed of all investigated INNOQUA technology modules aiming at reusing the treated water in a community garden for irrigation purpose and supporting the full testing of all technologies. The modules in India represent 10PE units designed according to design parameters established during prior lab and pilot-scale testing based on European standard loading rates for PE and a hydraulic load of 1500 L/day (INNOQUA Project n.d.). These consequently differ from conditions in the Indian

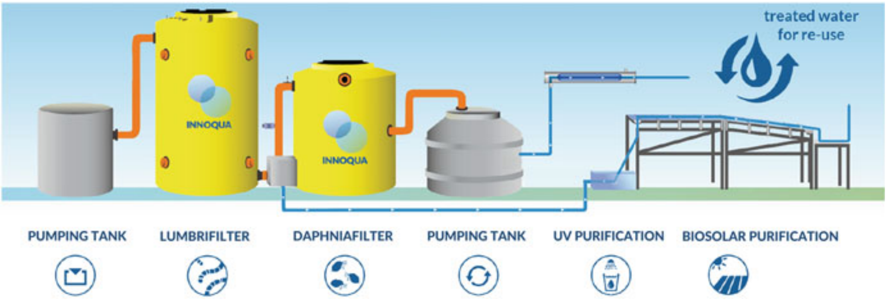


Fig. 18.2 System configuration of the Indian demonstration site (INNOQUA Project n.d.)

demonstration site. The configuration and resulting two treatment streams are depicted in the illustration below (Fig. 18.2).

The system is connected to and receives wastewater generated by nine households of the community. Excess wastewater is bypassed to a pre-existing decentralized treatment plant. As an economically weak community in the suburban borders of Bangalore, a direct connection to drinking water supply or an overall sewerage and treatment system is not existent. Water supply is arranged by community water wells and with increased frequency by water tankers due to rising water scarcity, especially in the dry season. Water use is reported as an approximate of 200 L per household per day and rather defined by the storing capacity of existing water containers used by the households than the number of household members. The total water consumption of the connected households thus amounts in an approximate of up to 1800 L/day. The wastewater source is arising from connected pour flush toilets and a washing room and represents a high-strength wastewater (Benefield 2002).

A preliminary assessment of the wastewater conditions has been carried out in the form of three composite sampling rounds and compared against reported measurements by CDD Society, which is a not-for-profit organization working in the field of sanitation and maintaining a decentralized wastewater treatment unit installed in one cluster of this community. The results for preliminary assessed influent characteristics at the demonstration site and reported influent characteristics to the DEWATS system by CDD Society are shown in Table 18.2.

As indicated in Table 18.2, certain parameters show a wider range as for the case of water consumption, temperature, BOD, COD, and ammonium with generally lower values measured by CDD Society.

18.3.1.1 Lumbrifilter

The Lumbrifilter in India has a surface of 3.63 m^2. The system is semi-buried and is designed with a closing lid and ventilation pipes to minimize the exposure to climatic conditions and higher temperatures. Design recommendations and target wastewater characteristics for the Lumbrifilter are described in Table 18.3.

Table 18.2 Expected wastewater characteristics in the colony; assessed range and data reported by (CDD Society, n.d.)

Parameter	Assessed range	Reported measurements by CDD Society		
		Average	Max	Min
Water consumption	200 L/hh	60 L/cap d		
pH	7.9–8	7.5	8	7
Influent temperature (°C)	23.9–25.5	27	29	25
Suspended solids (mg SS/L)	526–970	250	300	200
COD concentration (mg COD/L)	1219–1894	550	600	500
BOD_5 concentration (mg BOD_5/L)	750–1100	225	250	200
Total nitrogen (mg N/L)	–	200	–	–
Total Kjeldahl nitrogen (mg N/L)	–	120	170	100
Total ammonium (mg NH_4-N/L)	132–140	100	–	–
Total phosphorous (mg P/L)	–	40	70	20

Table 18.3 Recommended range for operation of the Lumbrifilter derived from preliminary testing and related performance (INNOQUA Project n.d.)

Parameter 10PE	Recommended range and performance according to preliminary testing
Worm species	*Eisenia fetida* and/or *Eisenia andreii*
Wood chip	Hard wood chips, max 15–25 mm
Inorganic filter material	Ideally porous stone; 20–30 mm
Hydraulic loading	1500 L based on 150 L/inh day
Dosing frequency	Equally distributed over day; frequency on 1-hourly or 2-hourly basis
Dosing arrangement	Perforated pipe/nozzle/splash plate, pressurized via pump
Temperature (°C)	15–20; min. 5, max. 30
pH	7–8; min. 6, max. 9
BOD (g/day)	600
COD (g/day)	1200
TSS (g/day)	700
NK (g/day)	150
TP (g/day)	40
Removal performance (%)	COD (83%); BOD_5 (94.5%); TKN (93–97%); TN (50%); TP (30–35%)

The filling material in the Indian Lumbrifilter is composed of successive layers of gravel and coconut chips in the diameter range of 15–30 mm. The introduced earthworm species used as inoculum (10,000 worms) were sourced from a local vermicomposting enterprise. A combination of *Eisenia fetida*, *Perionyx excavatus*, and *Eudrillus eugeniae* species was chosen, which are described as key biological agents for vermicomposting in India (Pattnaik and Reddy 2010). Furthermore, the slight differentiation in their characteristics can allow to secure operation under varying conditions covering wider moisture ranges and specifically temperature,

Table 18.4 Characteristics of *Eisenia fetida*, *Perionyx excavatus*, and *Eudrillus eugeniae* (Source Dominguez and Edwards 2011; Pattnaik and Reddy 2010; Singh et al. 2004; Tohidinejad et al. 2011)

Parameter	*Eisenia fetida*	*Perionyx excavatus*	*Eudrillus eugeniae*
Common name	Brandling, tiger worm	Indian blues	African night crawler
Size (mm)	50–123	45–89	80–194
Adult weight (g)	2.42–3.57	1.94–3.12	10.5–12.8
Colour	Brown; banded morph	Reddish brown	Reddish brown
Life cycle (days)	45–51	40–50	50–70
Maturation (days)	28–30	28–42	40–49
Number of cocoons per day	0.35–0.5	1.1–1.4	0.42–0.51
Number of worms per cocoon	2.5–3.8	1–1.1	2–2.7
Temperature optimum (limit) (°C)	25 (0–35)	25–37	25–30 (16–30)
Moisture optimum (limit) (%)	80–85 (70–90)	80	80–82 (70–85)
pH	4–9	–	–
Salt content (%)	$<5\%$	–	–
Consumption per day (mg)	35–50% of body weight	–	–

which represents a critical parameter with elevated ranges for both ambient temperature and incoming wastewater temperature expected at the Indian demonstration site. The characteristics of the given worm species are represented in Table 18.4.

After a starting phase of 1 month, the Lumbrifilter in India, in contrary to other demonstration sites of the INNOQUA Project, received unsettled wastewater with the aim to address faecal sludge management issues, which are prevalent in India. To cope with high TSS, the standard perforated pipe distribution system was changed to a specifically designed splash plate to deliver even distribution of the influent on the surface and minimize potential clogging issues (Fig. 18.3). For analytical purposes, the dosing is controlled by a feeding pump, which further allows a pressurized and even distribution on the LF surface. Dosing intervals were set on either a 1-h or 2-h basis. System performance is evaluated for varying dosing volumes, ranging from 35 to 70 L/dose. Given the high strength wastewater in combination with a dosing of unsettled wastewater, the Lumbrifilter in India is operated and analysed at extreme conditions exceeding the recommended operation ranges as described in Tables 18.2 and 18.3. The effluent of the Lumbrifilter is collected in a pumping tank, which further feeds the tertiary polishing modules: Daphniafilter and BioSolar Purification (Fig. 18.2).

Fig. 18.3 Transversal view Lumbrifilter (NOBATEK/INEF4 n.d.-b)

18.3.1.2 Daphniafilter

The Daphniafilter in the Indian demonstration site has a volume of 1 m^3 and represents a 10PE unit. The recommended operation range derived from preliminary pilot testing is expressed in Table 18.5.

The DF is designed to optimize Daphnia filtration and contains two flat rectangular plates to increase the internal surface area for bacterial and algal biofilm growth (Fig. 18.4). The inflow is located at the centre of the reactor and ensures minimum flow velocities inside it. The outlet runs along the top of the whole cylindrical length of the reactor to ensure a gentle water outflow to the pumping tank of the UV disinfection system. The minimum required hydraulic retention time of 6 h is defined by a limiting upflow velocity for daphnia species. The recommended Daphnia species as to pilot experiments represent *Daphnia magna*, who belongs to the largest species of this genus. *Daphnia magna* is reported to tolerate temperature ranges from 11 to 25 °C (Müller et al. 2018). Located in the climate zone of the tropical savannah, it is highly critical to understand the impact of temperature conditions at the

Table 18.5 Recommended range for operation of Daphniafilter and related reported performance (INNOQUA Project n.d.)

Parameter 10PE	Recommended range according to preliminary testing
Daphnia species	*Daphnia Magna*
Aquatic plants	*Lemna minor*
Hydraulic loading; HRT	1500 L based on 150 L/inh day; min. 6 h, max. 48 h; optimum 24 h
Dosing frequency	Equally distributed over day; 1-hourly dosing intervals
Dosing arrangement	At bottom of tank for upflow; venturi pipe for oxygen
Temperature (°C)	16–23; min. 10, max. 27
pH	7.2–8.5; min. 6.6, max. 9.5
Organic matter (mg COD/L)	<180
TSS (mg/L)	<130
NH_4-N (mg/L)	<40
NO_2-N (mg/L)	<5
PO_4-P (ppm)	<30
Further critical parameters	Cu > 0.4 ppm, N > 250 mg NO_3/L, salinity >3 ppt, avoid turbulence, fish

Fig. 18.4 Daphniafiltration unit at the Indian demonstration site

Bangalore site on the survival of this species. Although Daphnia represent the most studied cladoceran species, studies and markets for this species in India are highly limited.

An investigation by Padhye et al. in 2016 observed three different Daphnia and eight Ctenodaphnia species in India, of which four are reported for the southern region. These entail the subgenus *D. (C.) carinata s. lat.*, *D. (C.) cephalata s. lat.*, *D. (C.) lumholtzi*, and *D. (C.) similoides*. The given species could not have been found in Bangalore. As the only accessible species *Daphnia pulex s. lat*. has been used in the Indian demonstration site. *Pistia stratiotis* and *Lemna minor* have been chosen as aquatic plants. The system is fed on a 1-hourly basis by a pump to allow for regulated inflow volumes in predefined time dosing intervals. As valid for the Lumbrifilter, the Daphniafilter in the Indian demonstration site is analysed under extreme conditions exceeding recommended operation parameters illustrated in Tables 18.2 and 18.5.

18.3.1.3 UV Disinfection

An FS1 ProLine UV System of Berson Milleutechniek BV has been used in the Indian demonstration site as quaternary stage for final disinfection and targeted reuse of treated water in the demonstration garden. The unit is equipped with quartz sleeves and a temperature sensor to protect the system from overheating. The optimum operating temperature range lies within 15–20 °C with a maximum defined at 45 °C. Reported disinfection performance depends majorly on turbidity, the concentration of total suspended solids, and the UV dose as illustrated below (Table 18.6). The possible maximum hydraulic loading is 5 m^3/day. The system is fed by a pump, which was configured to start after a preheating period of 5 min to ensure an optimal UV dose. The system is fed automatically based on the water level in the pumping tank for a defined volume of 500 L.

18.3.1.4 BioSolar Purification

The BioSolar Purification system is composed of two cascading platforms and one circulation tank envisioned to treat 10PE. Compared to other INNOQUA technologies, the BSP has been adapted to the current low-cost configuration within the project lifetime and has been briefly tested and validated at lab scale. Inoculation has been carried out with indigenous microalgae species collected as 40 L of pond water and microalgae biofilm naturally formed inside the Lumbrifilter walls. After a

Table 18.6 UV disinfection levels in relation to TSS, UV transmission (UVT), and UV dose according to the manufacturer (Berson n.d.)

Disinfection level	Incoming load	Theoretical disinfection level	TSS	UVT 10	Average UV dose
FC per 100 mL	No. per 100 mL	Log	mg/L	% per 10 mm	mJ/cm^2
1000	100,000	≥2.0	Max. 10	≥55	Min. 20
			Max. 20		Min. 26
			Max. 30		Min. 30
100	100,000	≥3.0	Max. 5		Min. 30
			Max. 10	≥55	Min. 35
10	100,000	≥4.0	Average 3, max. 5	≥65	Min. 45
2.2	100,000	≥4.6	Average 2, max. 5	≥65	Min. 80

Fig. 18.5 BioSolar Purification, inoculation of algae on Day 0 and Day 10 after inoculation of algae

constant circulation of 10 days, an even algae biofilm has been formed on the surface area of the platforms as illustrated in Fig. 18.5. The BioSolar Purification system receives effluent from the LF, pumped discontinuously to the platform each hour during daytime (11 h) to serve UV disinfection purposes via solar radiation. A small submersible pump located in the circulation tank keeps the water in a constant movement between the two cascades and the circulation tank. The effluent is being discharged through gravitational overflow from the recirculation tank.

18.3.2 Analysis Conditions

All modules have been monitored for varying key performance indicators over a period of 13 months to understand technology performances over long-term, seasonal variability patterns and operation and maintenance requirement. In total, up to 22 sampling campaigns have been conducted for core sampling regime parameters from five different sampling points with adapted sampling frequencies due to unforeseen restrictions caused by the COVID pandemic. The five different sampling points entail the influent to the Lumbrifilter (LFin), the effluent of the Lumbrifilter (LF eff), the effluent of the Daphniafilter (DF eff), the effluent of the UV Disinfection unit (UV eff), and the effluent of the BioSolar Purification (BSP eff). Core sampling regimes have been defined for the different stages and sampling points according to three test lists with related parameters as described in the following:

1. **Core test list:** TSS, BOD, COD, NH_4-N, pH, temperature, conductivity
2. **Test list 1:** TN, NO_3-N, NO_2-N, TP-P, PO_4-P, turbidity
3. **Test list 2:** *E. coli*, Faecal Coliforms, Faecal Streptococci, Legionella, Salmonella

Table 18.7 Sampling points with related sampling procedure and core sampling regime parameters

Sampling point	Sampling procedure	Testing parameters
LF in	24 h-composite	Core test list, Q_{in}
LF eff	24 h-composite	Core test list
DF eff	24 h-composite	Core test list, test list 1, Q_{in}
UV eff	Grab-sample	Core test list, test list 1, test list 2, Q_{in}
BSP eff	24 h-composite	Core test list, test list 1, test list 2, Q_{in}

The relative sampling regimes for the sampling points are described in Table 18.7.

The assessment procedure follows quality protocols, and analysis equipment was calibrated according to standards and manufacturer's instructions. All parameters have been analysed in duplicate according to the following standard analytical methods:

- Total suspended solids (TSS) and chemical oxygen demand (COD) were analysed under APHA method 2540 and 5220; Hach method 8043
- Five-day biochemical oxygen demand (BOD_5) analysed under APHA method 5210
- pH, total nitrogen (TN), and total phosphorus (TP) and orthophosphate (PO_4-P) were analysed under method 4500; Hach methods 10072, 8190, 8048
- Ammonium was analysed with an ammonium probe; Spectroquant Prove 300
- Nitrate and nitrite were analysed with a nitrate probe; Hach method 8039 and standard method 400-NO2; Hach method 8507
- Conductivity was analysed under standard method 2510 B
- *E. coli* were analysed via IS: 1622–1981, Reaff. 2014
- Faecal coliforms were analysed via method IS: 1622–1981, Reaff. 2014
- Legionella sp. were analysed via standard APHA method 9260, GPVA medium
- Salmonella sp. were analysed via IS: 5887 (Part 3)–1999, Reaff. 2018
- Faecal streptococci were analysed via standard method IS: 15186–2002, Reaff. 2014

Key environmental and operation parameters were also monitored during this demonstration phase. These include ambient temperature and weather conditions, the level of the active layer in the Lumbrifilter, operation and maintenance requirements, and operation anomalies or technical complications. Dosing arrangement and thus maximum daily flow was adapted via feeding pumps and estimated as daily feeding volumes for the different modules. Electrical meters have been installed for each electrical appliance to monitor electrical consumption for each module.

18.4 Results

The results in terms of treatment performances for each of the different INNOQUA technologies are evaluated and presented in correlation with observations retrieved from the demonstration site in terms of environmental conditions and operation and maintenance.

18.4.1 Lumbrifilter

The performance of the Lumbrifilter has been evaluated based on the analysis results for the parameters TSS, BOD, COD, and NH_4-N as relative removal rates with a further evaluation of a mass balance in order to gain a better understanding in relation to varying hydraulic loading rates. The overall received volume ranges over the investigated analysis period were 385 L/day up to 1680 L/day and respective hydraulic loading rates of 106–463 L/$m^{2 \text{ or } 3}$ day with observed deviation due to faced water scarcity in the community and resulting lower water consumption. The influent and effluent concentrations for main quality parameters (core test list) with their corresponding variation are presented in Table 18.8.

The overall average removal performance for TSS, BOD, COD, and NH_4-N ranges between 83% and 93% while the standard deviation of 6–10% indicates a relative steady performance over the investigation period. The temperature of the influent showed high variability in the range of 19.7 °C up to 38.8 °C and thus exceeds the optimum and maximum ranges for all three worm species. The pH of the influent ranged between 7.4 and 7.9 with an increase in the effluent of the Lumbrifilter between 7.2 and 8.5, which does not exceed the maximum tolerance limits for earthworms but slightly the optimum conditions. In order to allow a better understanding in relation to varying hydraulic loading rates, a mass balance evaluation of treatment performances has been formed. The results over the sampling period are evaluated and presented in the following with an overview on the sampling and operation timelines (Table 18.9).

The starting phase is represented in the sampling timeline $n = 1$ and 2, while steady-state operation can be observed for $n = 3$–22. During the start of the system, the influent to the Lumbrifilter has been presettled to allow microbial biofilm formation on the coconut chips and adaptation of the earthworm population to the new environment and living conditions. This is also reflected in Fig. 18.6 and explains both the low loading rates and comparably lower performances in terms of TSS, COD, and BOD removal in the starting phase of the system. The overall loading rates for the different analysed parameters show differences over the period of investigation. This is largely a result of variable hydraulic loading and variable concentrations for the various parameters in the receiving influent as depicted in Table 18.8. Average influent loadings with respective deviations after the starting phase ($n = 1, 2$) result in 637 ± 359 g TSS/$m^{2 \text{ or } 3}$ day, 329.5 ± 116.5 g BOD/$m^{2 \text{ or } 3}$ day, 629.4 ± 273.18 g COD/$m^{2 \text{ or } 3}$ day, and 30.3 ± 10.76 g NH_4-N/$m^{2 \text{ or } 3}$ day. The observed maximum was at 1664 g TSS/$m^{2 \text{ or } 3}$ day, 517 g BOD/$m^{2 \text{ or } 3}$ day,

Table 18.8 Results of Lumbrifilter influent and effluent concentrations of main quality parameters (n = 20; excluding starting phase n = 2)

		TSS	BOD	COD	NH_4-N		
		mg/L	mg/L	mg/L	mg N/L	pH	Conductivity
LF_{in}	Average; STD N	2190 ± 951	1165 ± 369	2242 ± 851	104 ± 23.7	7.6 ± 0.1	3228 ± 412
	Max	4030	2000	4190	144.0	7.9	3600
	Min	940	600	1104	60.1	7.4	1680
LF_{eff}	Average; STD N	271 ± 186	90 ± 76	371 ± 217	15.2 ± 8.5	7.9 ± 0.3	2645 ± 348
	Max	615	300	803	37.0	8.5	3100
	Min	36	14	86	2.7	7.2	1596
Efficiency [%]	Average; STD N	88 ± 8	93 ± 6	83 ± 10	85 ± 10	–	–
	Max	98	98	94	98	–	–
	Min	62	75	60	54	–	–

Table 18.9 Lumbrifilter; overview on sampling and operation timeline

Sampling timeline as (a) in no. and operation timeline as (b) in days

(a)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
(b)	30	67	97	122	136	142	149	156	163	177	183	191	198	205	213	219	226	233	240	254	261	422

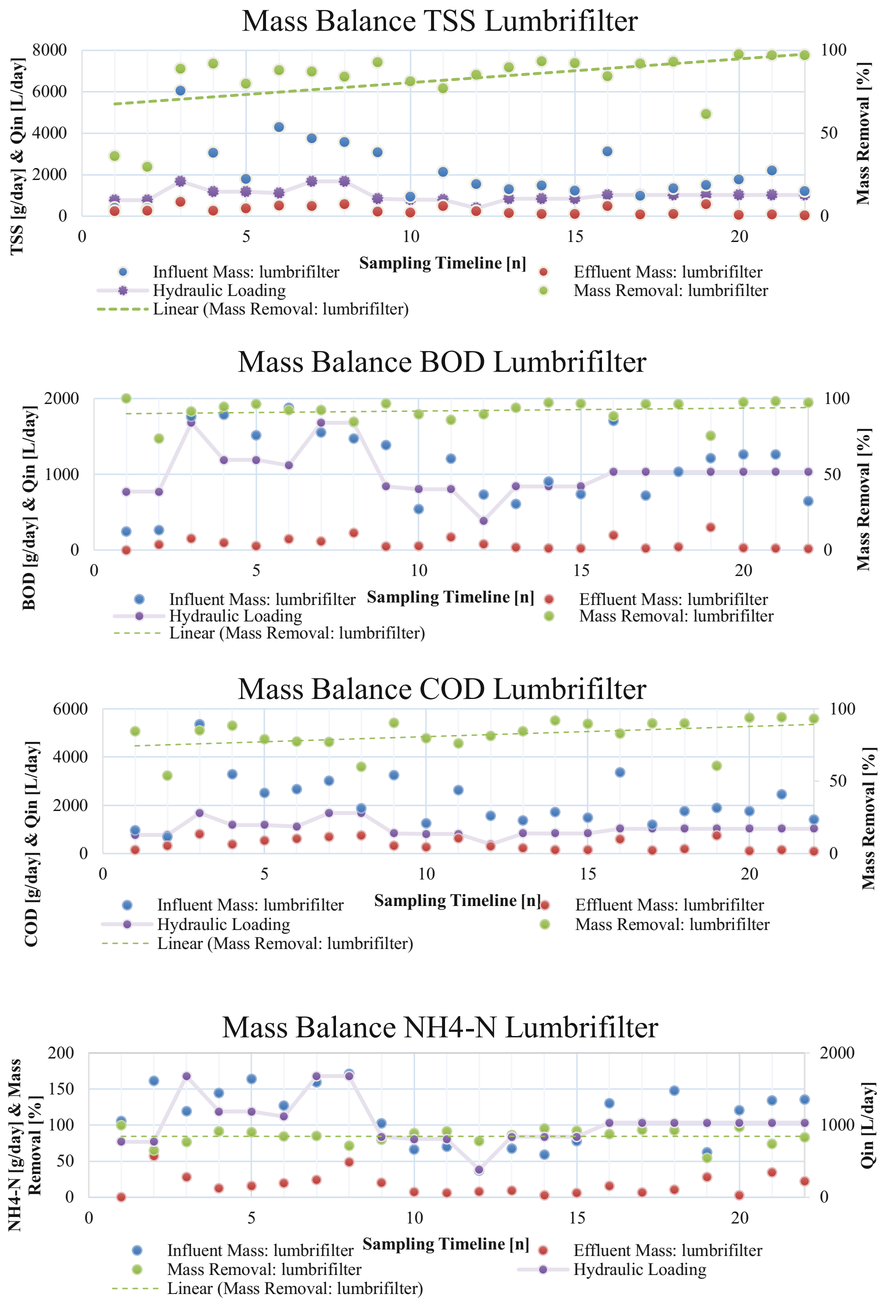


Fig. 18.6 Mass balance and performance Lumbrifilter with $A = 3.63\ \text{m}^2$ and active layer volume of $3.63\ \text{m}^3$

923 g COD/m$^{2 \text{ or } 3}$ day and 47 g NH_4-N/m$^{2 \text{ or } 3}$ day and thus exceeded the recommended loading up to 8.6 times for TSS, 3 times for BOD, and 4.5 times for COD. Despite these high loading rates, the treatment of the Lumbrifilter is observed to perform relatively consistent over the testing period.

A partial coverage with sludge (TSS) or temporal ponding of influent in small compartments of the surface after dosing has been observed over the whole demonstration period. However, visual inspection after racking the surface area below the ponding areas showed an increased number of worms compared to other drier areas in the active layer without temporary influent ponding. A full clogging occurred during the sampling events 8 and 19, which both indicate a divergent decrease in removal performance. During sampling round, eight dead earthworms have been found in the effluent of the LF, which indicated possible unfavourable conditions. Observations and results on loading during this time indicate overall high loading rates and high influent ammonium concentration to the filter, which presumably is the reason. An adaptation has been carried out in the form of reduced inflow volumes from 1.68 to 1.03 m^3/day to decrease the comparatively high loading rates. Between sampling round 21 and 22, the system could not be accessed and maintained for a period of 4.5 months due to lockdown restrictions caused by the COVID19 pandemic. In this period, the system was shut down and did not receive influent. During an initial visit following shutdown, the surface of the Lumbrifilter was dry and covered by vegetation, while no worms could be found in the upper 20 cm layer. After restarting of the feeding pump and operation as to steady state, observations have shown that the worm population reappeared in the upper active layer after 1 week and subsequent testing indicates that the system has not been negatively impacted as treatment performances are comparable to steady-state performances of the system. With temperature and pH as limiting parameters, a further evaluation has been carried out to observe performance ranges in relation to temperature, pH, and conductivity (Fig. 18.7).

A direct correlation between technology performance in relation to temperature, pH, and conductivity cannot be observed. While these parameters are described as influential factors, especially in the case of worm sensitivity to increased temperatures found in the Indian demonstration site, the variability and level of correlation from incoming loading rates for further parameters possibly have a stronger correlation to performance and thus could mask impact levels of these parameters under variable real environment conditions at the demonstration site. The performance at high-temperature conditions does not indicate a decrease as indicated in sampling rounds 6 or 20. Measured values show a correlation of effluent and ambient temperature and thus indicate that ambient temperature influences the temperature gradient within the LF. Further, an increase in the pH and a decrease in the conductivity in the LF effluent are observed.

In regard to the operation and maintenance, the Lumbrifilter required draining while operating under heavy loadings and corresponding observed clogging events. Draining included manual digging of certain parts of the surface area, allowing the stagnated wastewater to pass the filter. Furthermore, the height of the active layer decreased over 10 cm after a period of 6 months and required a refilling in order to

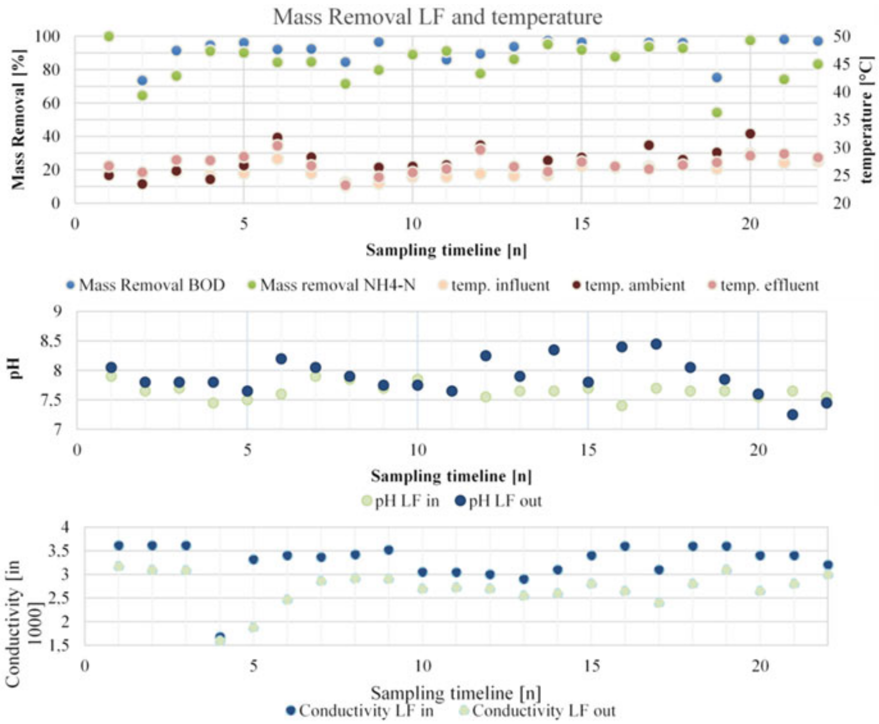


Fig. 18.7 Technology performance in terms of BOD and NH_4-N in relation to pH, temperature, and conductivity

maintain the volume of the active layer. The performance does not indicate a correlated change for the given decreased range of active layer volume. In case of shutdown during the lockdown period of the COVID19 pandemic, growth of plants at the LF surface was observed. A partial clearance has been carried out to restore the condition and facilitate the assessment of the worm population and the surface layer. However, an impact by formation of plants could not be observed. Monitored energy consumption accounts for 0.037 kW h/m^3 of treated wastewater, which results from the operation of the feeding pump.

18.4.2 Daphniafilter

As mentioned above, the Daphniafilter in the Indian demonstration site has been analysed under extreme conditions exceeding recommended influent parameters and critical temperature ranges for daphnia survival. Recent analysis performed by the Eawag observed a high-speed evolution in daphnia species as response to unfavourable conditions (Schaffner et al. 2019). Hence, it was analysed whether the Daphniafilter can perform under given conditions and/or if a possible adaptation can be observed. Site conditions with related achieved performance are presented with the following observations during the investigated period.

As described in the sampling regime, the performance of the Daphniafilter has been analysed for the parameters TSS, BOD, COD, and NH_4-N as to the core test list and TN, NO_3-N, NO_2-N, TP-P, PO_4-P, and turbidity as to test list 1. Relative removal rates were evaluated for core test list parameters, while test list 1 parameters are presented as absolute values. A gradual dosing regime was chosen to allow for possible adaptation of daphnids. The hydraulic load varied over the investigated period from 60 to 390 L/day corresponding to minimum hydraulic retention times of 2.5 days. The envisioned loading of 1.5 m^3 could not be reached due to crashes of the daphnia population as combined effect of unsuitable influent quality and environmental conditions. The concentrations of main quality parameters in the influent and effluent of the Daphniafilter module and removal performances are summarized in Table 18.10.

Influent TSS, BOD, COD, and NH_4-N concentrations show a high variability as presented in Table 18.10. In terms of performance, there was less variability for TSS, BOD, more for COD and extremely variable (in some cases negative) removal for NH_4-N, which presumably result due to analytical concentrations at very low ranges and detection limits. The pH of the LF effluent shows a slight decrease from 7.9 to 7.7 on average in the DF effluent with same observations valid for conductivity. The average influent temperature is 26 °C with a measured minimum of 20.2 °C and a maximum of up to 38.4 °C, which exceeds the reported comfort temperature of 20 °C for daphnia. The measured average effluent temperature of the Daphniafilter was 24.3 °C with an observed critical maximum of 36 °C. Absolute values measured for TP and PO_4-P result in 13.1 mg/L and 11 mg/L, respectively. An outlined value for NO_2-N and NO_3-N (max. of 17 and min. of 0.2 mg/L) has been observed once, which presumably was caused by interchanged reported values by the external laboratory for these two parameters. Within the following, the resulting mass balance is presented with an overview on sampling and operation timelines to facilitate a comparison in relation to respective loading ranges and operation (Fig. 18.8, Table 18.11).

The reactor was inoculated with laboratory breed Daphnia sourced from a local supplier. Initially, the reactor was fed with 30 L of Lumbrifilter effluent daily for a period of 1 week and subsequently changed to a steady operation and dosing of 30 L/h. A first crash of the population has been observed after a month of operation. The dosing volumes have been decreased to 12–15 L/h to allow daphnids to adapt to the environment. A gradual increase to the intended hydraulic loading rates could not be reached. Despite the unfavourable conditions for daphnia at the Indian demonstration site, a stable population has been observed for a period of 2 months during sampling rounds 9 and 16. After sampling event 16, investigations revealed a malfunction of the technical dosing arrangement leading to an increased inflow of 60 L/h, which likely resulted in a washout of the existing daphnia population. The above-presented mass balance indicates slight differences in the effluent values of the parameters TSS, COD, and BOD. While TSS removal indicates a more constant performance during the steady daphnia population, resulting measurements for the

Table 18.10 Influent and effluent concentrations and treatment performance Daphniafilter ($n = 18$, excluding starting phase $n = 2$)

Core testing parameters		TSS	BOD	COD	NH_4-N	pH	Conductivity
		mg/L	mg/L	mg/L	mg N/L		
LF_{eff}	Average; STD N	271 ± 186	90 ± 76	371 ± 217	15.2 ± 8.5	7.9 ± 0.3	2645 ± 348
	Max	615	300	803	37.0	8.5	3100
	Min	36	14	86	2.7	7.2	1596
DF_{eff}	Average; STD N	16.9 ± 11	10.5 ± 4.7	80.7 ± 16.8	8.9 ± 5.7	7.7 ± 0.2	2521 ± 249
	Max	48	28	138	24	8.8	3200
	Min	2	6	54	2.1	7.6	1908
Efficiency [%]	Average; STD N	88 ± 14.8	77 ± 15.9	66 ± 22.9	30 ± 40.7	–	–
	Max	97.9	95.7	89.3	80.5	–	–
	Min	34.2	45.5	1.6	−82.7	–	–
Test list 1		TN	NO_3-N	NO_2-N	TP	PO_4-P	Turbidity
		mg/L	mg/L	mg/L	mg/L	mg/L	NTU
DF_{eff}	Average; STD N	34.5 ± 21.7	20.7 ± 10.9	2.7 ± 3.2	13.1 ± 4.4	11 ± 3.3	88 ± 308.4
	Max	121	55	17	25.1	23	2700
	Min	12	0.2	0.1	8	7	3.0

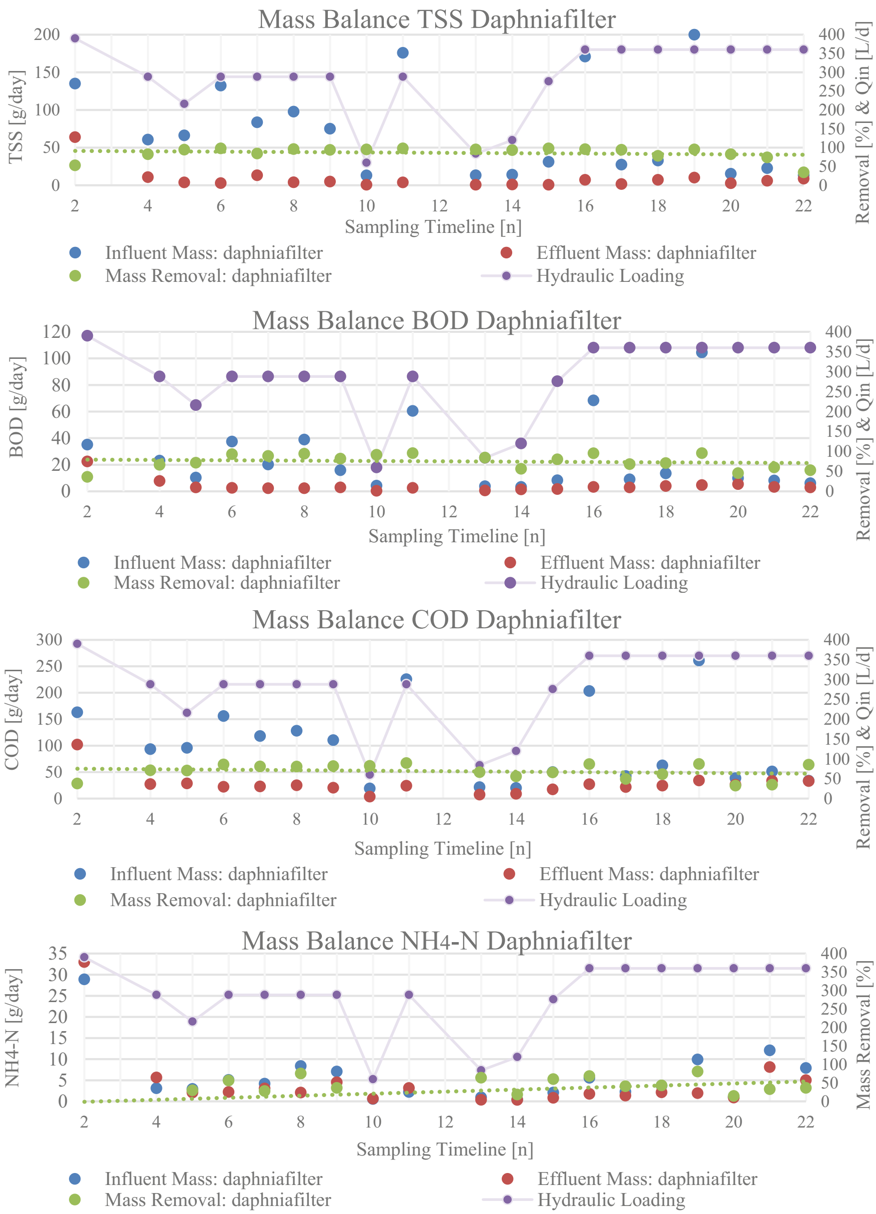


Fig. 18.8 Mass balance and performance of the Daphniafilter for a 1 m^3 system

parameters BOD, COD, and NH_4-N rather show an increase in performance relative to higher loading. NH_4-N results indicate a negative removal and increase in the case of two events at the starting phase and round 11. During this time, a comparably low

Table 18.11 Overview on Daphniafilter sampling and operation

Sampling timeline as (a) in no. and operation timeline as (b) in days

(a)	1	2	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22
(b)	30	67	122	136	142	149	156	163	177	183	198	205	213	219	226	233	240	254	261	422

influent load is observed and thus could result from low concentration levels and related higher error probability in these ranges and system processes. However, considering the high HRT, a direct evaluation on treatment performance cannot be made.

The removal performance in relation to BOD in the Daphniafilter shows a decrease with increasing temperature for the investigation period 8–22, while a correlation between NH_4-N, pH, and conductivity cannot be observed (Fig. 18.9). The effluent temperature shows lower ranges compared to received influent and indicates a cooling effect in the DF tank. All measured ranges lie above the reported optimum of 20 °C for daphnids. The pH shows an increase while conductivity levels are observed to stay in a comparable range.

As the Daphniafilter could not reach steady state, conclusive operation and maintenance requirement could not have been assessed. A fast growth of aquatic plants has been observed, especially in the case of *Pistia stratiotis*. As daphnids require a minimum amount of sunlight, a weekly removal of *Pistia stratiotis* has been carried out to guarantee a surface coverage of aquatic plants of maximum 75%. Furthermore, the Daphniafilter has been emptied and cleaned twice during the investigation period as response to observed dense biofilm formation and deposition of solids at the bottom of the tank in order to prevent unfavourable conditions for

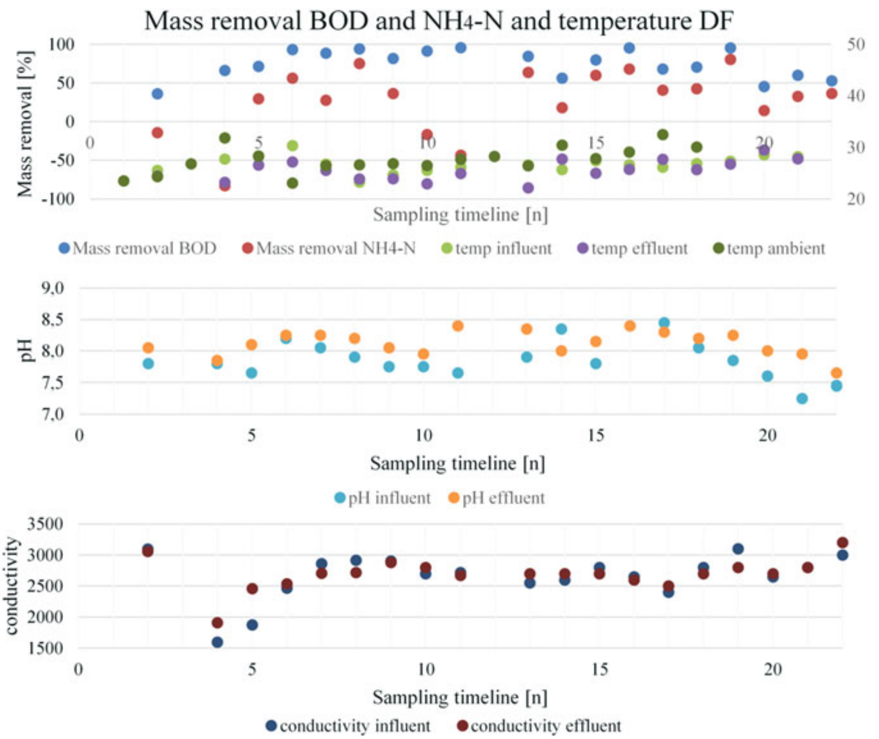


Fig. 18.9 Mass removal Daphniafilter in comparison to temperature, pH, and conductivity

newly inoculated daphnids. However, as daphnia species typically feed on suspended solids and algae/biofilm the maintenance requirement in this regard could possibly be minimized in the case of steady operation. Further investigations would be required to assess operation and maintenance over long-term under steady state. While the effect on the daphnia population during the shutdown events as faced by the lockdown of the COVID pandemic could not have been assessed due to earlier population crash, it was observed that aquatic plants could have been maintained over long term.

18.4.3 BioSolar Purification

As described in the sampling regime, the performance of the BioSolar Purification unit has been assessed in terms of TSS, BOD, COD, and NH_4-N removal as to the core test list; TN, NO_3-N, NO_2-N, TP-P, PO_4-P, and turbidity as to test list 1 and *E. coli*, Faecal Coliform, Faecal Streptococci, Legionella, and *Salmonella* as to test list 2. Relative removal rates were evaluated further in a mass balance in order to gain a better understanding in relation to varying received volumes and loadings, while parameters for test list 1 and test list 2 are presented in absolute terms for measured concentrations. Influent hydraulic loading rates ranged from 98 up to 588 L/day with 588 L/day as predefined dosing volumes in the Indian configuration for a 1.5 m^3 system. The quality of influent and effluent of the BSP module and corresponding removal efficiencies are presented in Table 18.12.

As described in Table 18.12, a general reduction for BOD, COD, and NH_4-N can be observed in the BioSolar Purification unit to varying degrees and a very high deviation for BOD and COD. The amount of TSS is increasing in the BSP effluent. Over the investigation period, it was observed that the effluent of the BioSolar Purification can considerably change from a dark algae layer attached to the bottom surface of the cascades and a clear effluent to rather small microalgae in a suspended form. This presumably is caused by changing algae species and main reason for observed increase of TSS. While this could result from blooming of cyanobacteria, which can be favoured at high nutrient concentrations and improper recirculation, a direct relation to these parameters cannot be observed for measurements. The major removal and conversion performance of the BSP is achieved for nutrient removal. Negative values as indicated in Table 18.12 for the reduction of NH_4-N occurred once during the investigation period as illustrated in the following mass balance. Measurements inform that the effluent values for the LF were highly below average with 2.8 mg/L and thus presumably result due to very low values and comparatively higher error rates in these ranges of system processes. Absolute average values retrieved for TP and PO_4-P resulted in 6.5 mg/L and respectively 4.12 mg/L. Measurements further indicate an average pathogen level resulting in 3481 CFU/100 mL for *E. coli* with a high deviation of ±5369 CFU/100 mL. An outlying extreme maximum of 23,000 CFU/100 mL has been observed whereat the reason is not clear. An adapted evaluation results in an average of 2128 CFU/100 mL with a continuing high deviation of 2064 CFU/100 mL and minimum values as low as

Table 18.12 Results of main intake influent and effluent BioSolar Purification for measured parameters and relative performance

Core testing parameters		TSS	BOD	COD	NH_4-N	pH	Conductivity
		mg/L	mg/L	mg/L	mg N/L		
LF_{eff}	Average; STD N	271 ± 186	90 ± 76	371 ± 217	15.2 ± 8.5	7.9 ± 0.3	2645 ± 348
	Max	615	300	803	37.0	8.5	3100
	Min	36	14	86	2.7	7.2	1596
BSP_{eff}	Average; STD N	224 ± 162	30 ± 19.9	183 ± 83	3.2 ± 2.6	9.2 ± 0.5	2463 ± 263
	Max	497	75	329	11	10	2750
	Min	16	6	64	0	8	1727
efficiency [%]	Average; STD N	−97 ± 253	27 ± 86	14 ± 77	71 ± 25	–	–
	Max	95	98	93	98	–	–
	Min	−821	−233	−162	−7	–	–
Test list 1		TN	NO_3-N	NO_2-N	TP	PO_4-P	Turbidity
		mg/L	mg/L	mg/L	mg/L	mg/L	NTU
BSP_{eff}	Average; STD N	25.2 ± 12.4	11.6 ± 7.26	2.49 ± 2.63	6.48 ± 3.11	4.12 ± 3.1	35.07 ± 22.5
	Max	57	29	11	14	12	82
	Min	2	0.4	0.1	2	0.3	6.8
Test list 2		*E. coli*	Faecal Streptococci	Legionella		Salmonella	Faecal Coliforms
		CFU/100 mL	CFU/100 mL	MPN/10 mL		MPN/25 mL	CFU/100 mL
BSP_{eff}	Average; STD N; adapted	3481 ± 5369	367 ± 132	Absent		Absent	3906 ± 5751
		2128 ± 2064					2465 ± 2413
	Max, adapted	23,000	745	Absent		Absent	24,500
		6200					7900
	min	420	205	Absent		Absent	465

420 CFU/100 mL. The resulting effluent values comply with WHO (2006) water reuse guidelines. Variations in pathogen levels presumably result from changes in solar radiation, incoming loading rates, and changing turbidity due to observed changing algae composition. The evaluation of faecal coliform shows similar characteristics. Legionella and Salmonella were absent in all analysed samples. Within the following, an overview on the sampling and operation timeline is given with further evaluation of the detailed mass balance for a 1.5 m^3 BSP system (Fig. 18.10, Table 18.13).

A major increase in TSS in the BSP effluent can be observed from sampling round 17 after 142 days of operation of the BSP with similar observations for the parameter BOD and COD for the rounds 17, 20, and 21. While the operation until Day 142 rather shows comparably stable and higher removal performances, a higher variability is given for the following investigation period. This indicates that the maximum amount of biomass on the platform has been reached and the equilibrium between biological processes governing the BSP shifted along the uptake of nutrients in the form of biomass, decay of biomass, and nutrients resolubilization. As a consequence, the BSP would require operation and maintenance in the form of cascades cleaning at events where the decay rate of algae biomass exceeds the growth rate. As mentioned earlier, NH_4-N measurements show overall a rather stable performance in conversion with a slightly increasing trend. During the investigation period, no maintenance activities have been carried out. The energy consumption for this unit accounts for 0.3 kW h/m^3 of treated wastewater and is attributable to the feeding and recirculation pump of this module.

18.4.4 UV Disinfection

As described in the sampling regime, the performance of the UV disinfection unit has been analysed for the parameters of the core test list and test list 1 for a better understanding of system processes overall and test list 2 for aimed process results for the given technological unit. Relative removal rates were evaluated further in comparison to TSS concentrations and turbidity to gain a better understanding on relative performances. The dosing volume was set to 500 L/dose. Results for the pathogen concentrations measured in the effluent of the UV unit are presented in Table 18.14.

Pathogen concentrations measured for the effluent of the UV unit show similar ranges as in the case of the BSP unit and result in 2245 CFU/100 mL for *E. coli*, 433 CFU/100 mL for Faecal Streptococci, and 2623 CFU/100 mL for Faecal Coliform. *E. coli* and similarly Faecal Coliforms show high deviations in the range of 2422 and 2756 CFU/100 mL but overall comply with WHO water reuse guidelines for the given application scenarios. Legionella and Salmonella were absent in all analysed samples. With TSS as critical parameter for disinfection performance, an evaluation has been carried out in relation to pathogen concentrations as illustrated in Fig. 18.11.

The TSS levels at the test site were high above the recommended process limitations by the manufacturer (approximately 30 mg/100 mL), which significantly

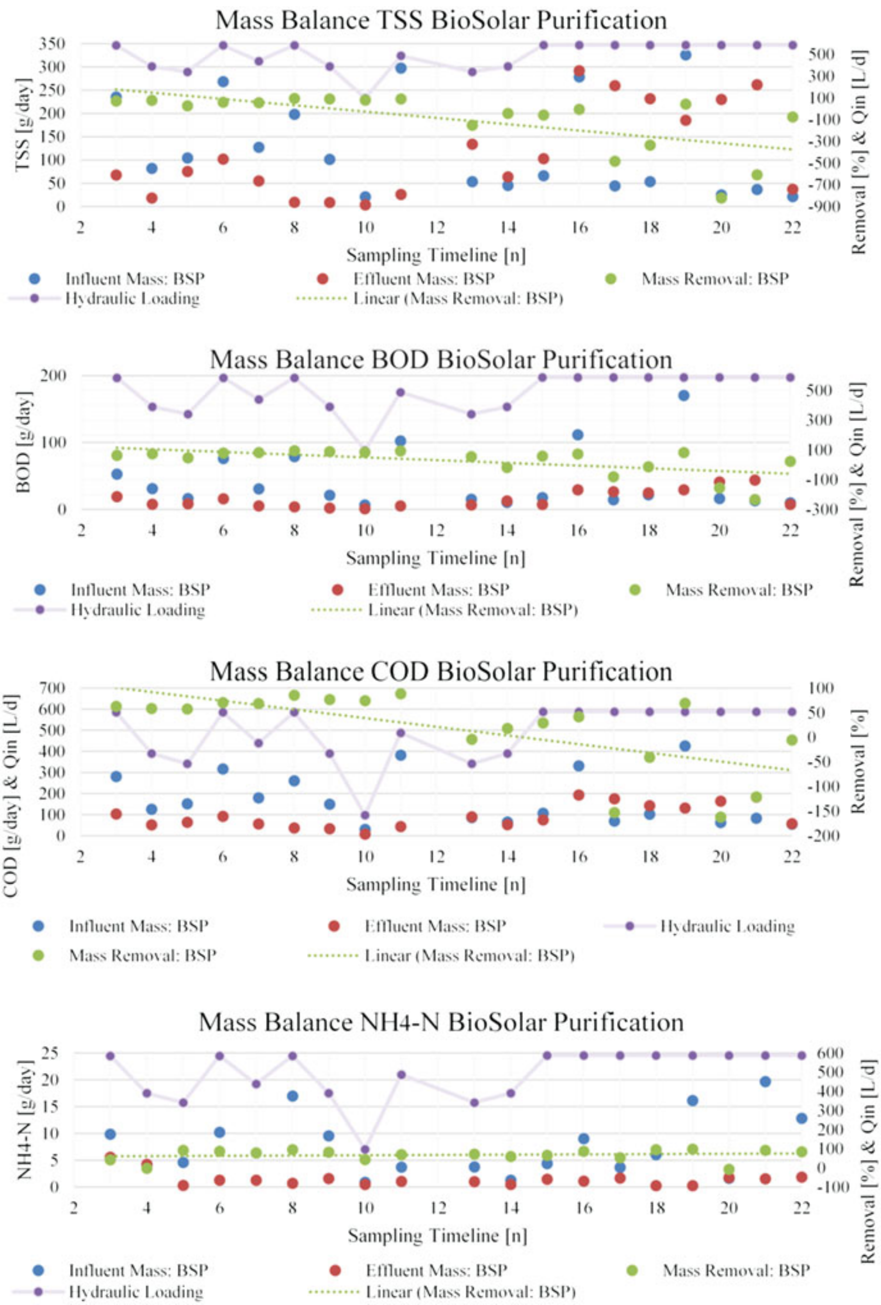


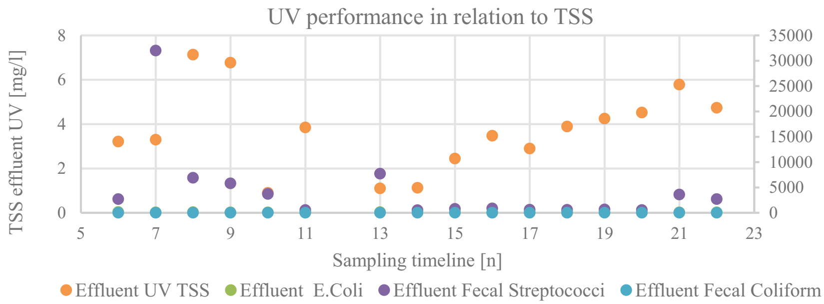
Fig. 18.10 Mass balance and performance of BSP for 1.5 m^3 system (cascade + circulation tank); $n = 20$

Table 18.13 BioSolar Purification overview on sampling

Sampling timeline as (a) in no. and operation timeline as (b) in days																			
(a)	3	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	21	22
(b)	14	39	53	59	66	73	80	94	100	115	122	130	136	142	149	156	170	177	338

Table 18.14 Results of main intake influent and effluent UV disinfection for measured parameters and relative performance

		E. coli	Faecal Streptococci	Legionella	Salmonella	Faecal Coliforms
Test list 2		CFU/100 mL	CFU/100 mL	MPN/10 mL	MPN/25 mL	CFU/100 mL
UVeff	Average; STD N	2385 ± 2356	371 ± 80	Absent	Absent	2623 ± 2756
	Max, adapted	7700	515	Absent	Absent	9100
	Min	510	190	Absent	Absent	580



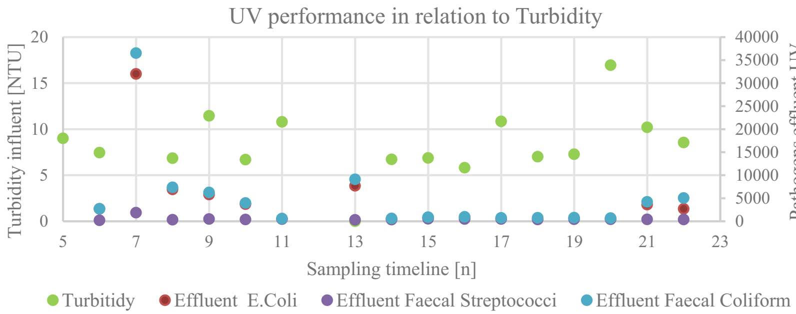


Fig. 18.11 UV unit performance in relation to TSS and turbidity

impacted the UV disinfection performance. Average turbidity of the receiving influent results in 9.1 NTU. However, overall pathogen concentration mostly results in values below 1000 CFU/100 mL. An exemptional analysis was carried out during sampling $n = 5$ for the influent *E. coli* concentration to understand removal performance of the UV unit. With an incoming *E. coli* concentration of 240,000 CFU/100 mL, the UV effluent concentration showed a considerable decrease to 3500 CFU/100 mL. While the slight spike during the sampling event $n = 15$ can correlate to the increase in TSS, values retrieved for sampling $n = 7$ present an outlining value. Overall measurements of pathogen concentrations in relation to TSS or turbidity variability rather indicate a stable performance for all other analysis rounds in regard to absolute values. However, further investigations on removal rates in relation to TSS concentration or turbidity would be required to gain a better understanding on the overall performance of the UV unit.

The maintenance requirements for the UV disinfection unit majorly include the cleaning of quartz sleeves on a regular basis to ensure optimum operation. Cleaning intervals depend on the liquid characteristics of the treated water and can vary between 3 and 6 months. The energy consumption resulting from the operation of a feeding pump and the UV lamp results in 0.0078 kW h/m^3 and respectively 0.37 kW h/m^3 of treated water.

Table 18.15 Global efficiency as removal rates in % of INNOQUA system configuration for core test list parameters; $n = 19$

Treatment stream		TSS	BOD	COD	NH_4-N
LF + DF + UV	Average	97.7	98.9	95.5	94.5
	STD N	1.8	0.9	1.8	3.5
	Min	93.1	95.6	90.4	83.9
	Max	99.8	99.7	98.0	97.9
	Median	98.3	99.1	95.8	95.0
LF + BSP	Average	86.6	97.3	90.6	97.2
	STD N	11.9	1.8	5.6	2
	Min	53.5	93.6	74.3	91
	Max	99.4	99.6	97.7	99.7
	Median	90.4	97.9	92.8	97.4

18.4.5 Global Efficiency

The global efficiency in terms of removal performance of the two different treatment streams in the Indian demonstration site has been evaluated for the core test list parameters TSS, BOD, COD, and NH_4-N and is presented in Table 18.15.

The overall global efficiency for both treatment streams indicates high removal performances for all parameters and treatment streams, whereat an overall evaluation for the LF-DF-UV configuration is difficult due to failed achievement in steady-state operation of the Daphniafilter. Removal performances for the LF-DF-UV configuration result in 98% for TSS, 99% for BOD, 96% for COD, and 95% for NH_4-N and show an overall steady performance over the investigation period with very low deviations for all parameters. With rather extraordinary outlining values, the median indicates increased performance ranges for this stream.

Despite slightly lower performances observed for the LF + BSP treatment stream in regard to TSS with 87% and COD with 91%, the overall removal rate for this configuration remains at high levels and achieves similar performances in regard to BOD with 97% and better performances in regard to NH_4-N with 97%. With the adaptation of operation and maintenance in the form of excess biomass clearing in the BSP system, the LF + BSP configuration could increase the overall performance. A comparatively higher deviation is observed for the parameter TSS and COD, while the median and corrective on outlining measurements as well indicates an increased performance for all presented parameters.

18.5 Discussion and the Way Forward

The INNOQUA modules in the case of the Indian demonstration site have been tested for communal wastewater in an economically weak community. Global trends indicate a growing water scarcity, land degradation, and food insecurity amongst marginalized population. While the city of Bangalore mandates the recycling of water as response to increasingly limited water resources, INNOQUA aims to provide affordable sanitation solutions that enable recycling of both water resources and nutrients. The technologies in the Indian demonstration site have been analysed under extreme conditions and high strength wastewater in addition to dosing of unsettled wastewater exceeding the recommended operation condition from pilot experiments in terms of loading rates and temperature as a further critical parameter for earthworm and daphnids viability. The results on the performance of the different technologies show differences as a nature of the different processes itself and the responsive performance under the given conditions compared to results from preliminary pilots (Tompkins et al. 2019; INNOQUA Project n.d.).

The Lumbrifilter was operated at up to more than three times the loading rate in regard to BOD, more than four times higher loading in regard to COD, and up to almost nine times higher loading in regard to TSS. While ponding has been observed continuously in some parts of the LF surface area, a complete clogging occurred only two times over the investigation period for maximum dosed volumes of 1680 L/day (462 L/m$^{2 \text{ or } 3}$ day) and thus maximum observed loading rates for TSS of 6040 g/day (1664 g/m$^{2 \text{ or } 3}$ day). Observations recommend for a gradual dosing in the starting of the LF and further maximum loading rates of 2000 g/day (550 g/m$^{2 \text{ or } 3}$ day). Despite the extreme and variable conditions over the investigation period, the analysed performance of the LF remains in a relative continuous state and results in high removal rates for TSS, BOD, and COD of 88 ± 8%, 93 ± 6%, and respectively, 83 ± 10% confirming the ranges from preliminary analysis as in the case of BOD while showing a slight increase in removal performance in the case of COD. Conversion/removal of NH_4-N was 85 ± 10%. The lower range in deviation indicates a comparatively stable performance of the LF throughout the investigation period. Further detailed long-term analysis in regard to loading rates and relative worm population dynamics would provide a better understanding on optimum loading rates for respective parameters and prevent clogging events to guarantee a steady operation over long term. In order to understand the performance and operation in a wider sense, broad installation in real environments and long-term monitoring shall provide a clearer picture for on-ground conditions and scenarios. Given the observed growth of vegetation on the LF surface, further analysis could inform whether vegetation can have an impact (such as concentration increase due to evapotranspiration processes) or favour the performance or operation for the designed LF under INNOQUA (as to observed enhancement in nutrient removal and prevention of clogging reported by Samal et al. 2017 and Singh et al. 2021). Current major concerns for the reuse of treated water or sludge are predominantly formed by growing risks attributable to compounds such as heavy metals or emerging pollutants, which typically are not treated in conventional treatment systems. Current studies report bioremediation characteristics of Lumbrifiltration processes. With a growing water scarcity, water recycling is not optional any more in some regions. Further research is needed to assess these processes for a wider spectrum of pollutants and over long term to provide holistic solutions for pollution and risk abatement.

Given the high HRT and failure in achieving a steady daphnia population, an evaluation in regard to performance of Daphniafiltration cannot be made. Despite the extreme climate and operation conditions, a stable daphnia population could have been observed for 2 months during the investigation period and was interrupted because of operative malfunction in the dosing arrangement. This result is indicative that despite the high-temperature conditions the operation of a Daphniafilter possibly could be achieved for climates in the southern region of India. The respective envisioned hydraulic loading could not have been reached under the analysed conditions. Unfortunately, a new inoculation and trial could not have been performed due to lockdown restrictions caused by the COVID19 pandemic. Further assessment would be required to analyse the Daphniafilter as tertiary treatment option coupled to secondary treatment stages under more common effluent strength characteristics. In addition, further research and investigations are required to assess daphnia species in the local environment and their relative performance as filter feeders in terms of optimum temperature ranges, consumption patterns, respective uptake of particle size ranges in relation to varying daphnid species, or possible variation in ecotoxicity for varying parameters.

The BioSolar Purification unit shows a varying range with a high deviation in removal performances, especially in regard to the parameters TSS and BOD. The conversion of NH_4-N, a general reduction of nutrients via uptake in algae biomass, and further the disinfection in a comparable range to the UV unit can be observed, which addresses the intended treatment purpose of a tertiary treatment stage. However, the increase of algae biomass over time and further observations indicating a change of algae species show a high increase in total suspended solids and BOD in the final effluent of the BSP unit. The detailed fraction of TSS caused by algae formation was not assessed. While algae in effluent could be of advantage for irrigation scenarios carrying nutrient-rich biomass, which alternatively can act as fertilizing agent or soil conditioner, it can have disadvantages in regard to possible clogging of irrigation infrastructure, especially in consideration of drip irrigation technologies as safest practice in water-scarce environments and water reuse scenarios. Furthermore, high amounts of TSS and high turbidity decrease the disinfection potential of this unit. It is therefore recommended to further analyse the detailed algae composition over long term and the cause for possible change. With a considerable change observed after 140 days of operation, operation and maintenance could be adapted in the form of removal intervals of algae biomass. An integrated filtration step could allow to further reduce free-flowing particles and coupled with a final biomass separation allow for wider discharge environments such as water bodies with separated harvest of biomass for land application. In the system configuration in India, the stream of the Lumbrifilter has been divided and subsequently resulted in lower hydraulic loading to the BSP unit, which is designed for a potential receiving load of 1500 L/day. Further investigations would be required to analyse the system under increased and varying loading rates.

The overall operation and maintenance requirements and technical knowledge for the operation of the systems are comparably low. While earthworm species are widely available in India, there is recently no market and supply for daphnia species. Breeding of daphnids in comparison to earthworms requires wider technical equipment and is more complex, which could pose difficulties in the operation of the Daphniafilter. In order to address electrical breakdowns, which are a common incidence in India, dosing scenarios without electrical equipment would be of high advantage and would require additional assessment. This could further reduce the CO_2 footprint, costs, and both accessibility of these systems to economically more disadvantaged end-users but also through overall facilitation in terms of reduced technical skills and operation ease the uptake amongst end-users. The energy footprint in terms of electricity consumption for the overall system resulted in 0.73 kW h/m^3 with the UV lamp representing the major consuming unit with 0.39 kW h/m^3. This is comparable to reported median values irrespective to plant size and treatment modules evaluated in 2015 amongst European countries (ENERWATER 2015), which commonly do not include such intensive disinfection stages for recycling purposes. Furthermore, most studies do not consider the broader energy requirements in terms of construction and operation of overall infrastructure and additional materials required for treatment processes, which presumably are high. The INNOQUA demonstration site as a decentralized and nature-based treatment system does not require an extensive infrastructure or the addition of further chemical agents. Further assessment on the overall energy footprint including construction materials or GHG emissions but all also balancing related benefits from recycling activities would provide a better understanding on the overall energy footprint. In regard to energy efficiency, the resulting energy consumption in the case of the Indian INNOQUA site rather represents the consumption per day as pumps for these low feeding ranges are hardly accessible and dosing volumes had to be regulated over bypassing valves. Resultingly, increased hydraulic loading could be achieved at the given consumption levels and, thus, decrease the energy footprint in regard to treatment wastewater volumes.

With increasing limitation in water resources, the UV disinfection unit represents an inevitable technology in the future, especially in dense urban megacities. Bangalore is the first city to introduce a Zero Liquid Discharge Order, which not only applies to the industrial but also the municipal sector. The widely practised and further growing reuse of wastewater will require further safety measures. As a quaternary treatment stage, the UV disinfection technology is installed with the purpose for elimination of pathogens. While a stable operation of the Daphniafilter could not have been reached with observed impact on the influent characteristics to the UV unit, the resulting performance complies with the WHO guidelines and presumably can achieve better removal under steady-state operation of the INNOQUA configuration. Despite the potential increase and requirement for disinfection processes, the treatment via UV disinfection lamps may face challenges observed for technologies relying on electricity and comparably higher costs for these technologies and their operation.

Overall it is observed that the Lumbrifiltration, BSP, and UV disinfection unit show stable and high removal efficiencies during the investigation period. The Daphniafilter will require further analysis under more moderate wastewater influent characteristics and a wider investigation of various daphnia species local to tropical climates. Further analysis on algae composition and changes is recommended for the BSP unit as well as removal and conversion rates for all parameters at all stages to achieve broader insights on overall performances and system functions.

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19

Problems of Increasing Air Pollution and Certain Management Strategies

Arun Arya

Abstract

Man cannot survive without fresh air, it becomes our bitter enemy as and when it gets polluted, since it causes a number of diseases in our body. The human vulnerabilities come as an alarming figure of seven million people that die prematurely every year due to air pollution. The harmful effects caused to human body to the polluted air depend upon the type and concentration of pollutants present in it. The pollutants primarily may cause respiratory and skin diseases. High concentrations of toxic gases like carbon monoxide in indoor pollution and methyl isocyanate released accidently during Bhopal gas tragedy resulted in huge causalities. Gaseous air pollutants do not exist singly in the atmosphere. Therefore, researches on interactive effects of air pollutants must be encouraged to understand the role of air pollutants in combination. Responses of different agricultural crops to major air pollutants such as sulfur dioxide (SO_2), carbon dioxide (CO_2), nitrogen dioxide (NO_2), and aerosols have been evaluated. Among the air pollutants, SO_2 and O_3 are the most reactive and phytotoxic. Air pollutant levels and trends in India as well as plant responses under combined exposure of air pollutants based on Indian studies are reviewed. Carbon dioxide in a limited dose of exposure improves the plant performances; however, other combinations of air pollutants caused damaging effects on productivity of the plants. The physiological and biochemical changes along with growth and yield responses under combined exposure of air pollutants are known. In combinations of air pollutants, responses of agricultural crops are mostly synergistic with respect to physiological, biochemical, yield, and quality traits.

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The COVID-19 pandemic has influenced air pollution because of altered human behavior and change in lifestyle. As the world was brought to a halt in March and April 2020 to contain the spread of Coronavirus, the pollution levels went down, breaking a record of several years and also of several decades in some cities. The review clearly indicates that combinations of air pollutants have the potential to cause a significant unfavorable impact on agricultural plant at comparatively lower concentration. Recent lockdown in countries world over resulting into no or very restricted activities recorded drastic change in quality of air and water. The concentration of pollutants is increasing due to massive industrialization, thermal power plants, and increase in number of vehicles plying on roads. While comprehensive data on pollutant emissions are limited, mobile sources have been identified as the main contributor (70–75%) to urban air pollution. The chapter describes certain strategies adopted to mitigate air pollution. Reducing SO_2 emissions from coal combustion sectors has since become more regulatory since the 1990s. These efforts have led to China's total emissions of SO_2 and primary $PM_{2.5}$ peaking before 2010. At present, tackling the $PM_{2.5}$ issue is the most challenging due to its complex formation, with contributions from multiple precursors and sources. Biopollution caused by cyanobacteria and indoor air pollution caused by fungal organisms like *Chaetomium, Aspergillus,* and *Nigrospora* is described with suitable examples. Mitigation measures and technologies recommended to reduce harmful gaseous pollutants and particulate matter are mentioned in the chapter.

Keywords

Air pollutants · Classification · SO_2 pollution · Ozone · Combined effects · Agricultural crop burning · Phytotoxicity · Biodeterioration · Management

19.1 Introduction

We require food and water to survive, but the role of clean air is still more important than water and soil. Each healthy person requires 11,500 liters of air per day, and the industrialization has affected the pristine resources of air, water, soil, and forests. Depletion of natural resources forces us to suffer with catastrophe of pollution and climate change. The beauty of planet and celebration of life cannot be imagined in the polluted atmosphere. Over the last half-century, many countries have transformed from an agrarian-based rural economy toward an industrial-based urban economy (Cohen 2006). As a consequence, various anthropogenic activities result in the release of harmful particles mentioned as PM_{10} and $PM_{2.5}$ and gases like ozone (O_3), nitrogen dioxide (NO_2), and sulfur dioxide (SO_2) that pollute the air (Duh et al. 2008).

For Rabindranath Tagore, nature was his favorite school, as he recorded in "My Life": "I had a deep sense, almost from infancy, of the beauty of nature, an intimate feeling of companionship with the trees and the clouds, and felt in tune with the musical touch of the seasons in the air. . . . All these craved expression, and naturally I wanted to give them my own expression." He says, "I believe in an ideal life,"

> *I believe that, in a little flower,*
> *there is a divine power hidden in beauty.*
> *which is more potent than a gun.*
> *I believe that in the birds' note.*
> *Nature expresses herself with a force.*
> *which is greater than that revealed.*
> *in the deafening roar of a cannon.*
> (W.W. Pearson, *Santhiniketan*, Epilogue by Rabindranath Tagore, Source: Banerjee 1987)

Air pollution is increasing at an alarming rate in metropolitan cities where there is rapid industrial growth and number of automobiles is also on an increase. Therefore, it is necessary to gather information about the baseline data on existing air quality. The physicochemical monitoring may be carried out by suitably selecting sampling sites in the specified area and collecting periodical samples and analyzing them for different air pollutants. The biological monitoring can be carried out by systematic study of plants, humans, and animals as regards damage or adverse impacts on them. The Government of India enacted the Air Prevention and Control of Pollution Act 1981 to arrest the deterioration in the air quality. The act prescribes various functions for the Central Pollution Control Board at the apex level and State Pollution Control Board at the state level (Gazette of India, Extraordinary 2009). It is interesting to note that during the rule of Maharaj Sayajirao Gaekwad III in Baroda state the sprinkling of water was practiced on roads of Vadodara city to reduce the concentration of particulate pollution in 1920s.

Urbanization and industrialization have resulted in a profound deterioration of air quality. Certain contaminants of air like dust, fumes, gas, mists, odor, smoke, or vapors when inhaled are injurious to human, plant, or animal life as well as to buildings and other properties. The long-term impact of air pollution was reported in Kawasaki City of Japan (Kanada et al. 2013). In terms of CO_2 emissions, the pollution has decreased in China. In 2017, China was consuming 23.2% of global energy and was emitting 27.6% of global CO_2. China experienced its highest annual emissions of sulfur dioxide (SO_2) in 2007, nitrogen oxides (NO_x) in 2012, and primary fine particulate matter ($PM_{2.5}$) in 2006 (Basic statistics of China 2019), at respectively 2.5, 4.6, and 1.5 times the corresponding values for 1990 in China (Wang et al. 2015).

Fumes from paint, hair spray, varnish, aerosol sprays, and other solvents and waste deposition in landfills, which generate methane, add to the pollution. Methane is not toxic; however, it is highly flammable and may form explosive mixtures with air. Methane is also an asphyxiant and may displace oxygen in an enclosed space. There is an increase in methane concentration by 145% since the industrial revolution. Secondary pollutants result due to chemical or photochemical reactions of the primary pollutants with elements or compounds present in the atmosphere. These reactions depend on factors like concentration of reactants, moisture, photoactivation, meteorological parameters, and topography.

The Corona pandemic has made our life miserable. We have to cage ourselves in our houses and a lot of lifestyle changes were automatically adopted in industries, offices, and academic institutions. The war against the invisible virus is on, and we are facing challenges on a daily basis. Millions of people are affected, and many lost their lives because of poor immunity or lack of proper medical attention. But how this virus entered in our body raises many unanswered questions. Did the virus jumped from animal to the human being in search of a stable host for its survival? We have the responsibility of protecting our nature and devise ways to live with harmony with other animals. People in a North Indian city could see the Himalayas after three decades, Kenyans also reported seeing the peaks of Mount Kenya behind the skyscrapers of Nairobi, and the satellite data from NASA indicated dropping pollution levels in the United States' northeast corridor. One of the studies shows that higher levels of $PM_{2.5}$ are directly proportional to the higher death rates from COVID-19. Another study that investigated the relationship between viral disease and $PM_{2.5}$ presented striking findings that it is expected that with a 20% increase in pollution almost 100% rise in the COVID-19 cases would take place in the Netherlands. Previously, a 2003 study found out that patients with severe acute respiratory syndrome (SARS) living in areas with higher pollution were twice as likely to die as those from lowly polluted areas.

Air pollution, ozone depletion, increase in greenhouse gases (GHG), and climate change are few global problems. The CO_2 released from soil carbon in the top matter of soil can increase the temperature by 0.03 °C per year. Climate change induced by increasing greenhouse gases is likely to impact directly on food production across the world, and that can vary from region to region. There is no simple solution for the abatement and adaptation to climate change.

19.1.1 Deteriorating Air Quality

Man cannot survive without fresh air. Air is the most important life-supporting element, sometimes becomes our bitter enemy as and when it gets polluted, since it causes a number of diseases in our body. Air pollution can briefly be derived as the "presence of an unwanted contaminant in atmosphere." A substance in the air that can cause harm to humans and the environment is known as an air pollutant. Pollutants can be in the form of solid particles, liquid droplets, or gases. In addition, they may be natural or manmade. Air quality is defined as a measure of the condition of air relative to the requirements of one or more biotic species or to any human need or purpose. Air quality indices (AQIs) are numbers used by government agencies to characterize the quality of the air at a given location. As the AQI increases, an increasingly large percentage of the population is likely to experience increasingly severe adverse health effects. To compute the AQI requires an air pollutant concentration from a monitor or model. The function used to convert from air pollutant concentration to AQI varies by pollutant and is different in different countries.

Urbanization and industrialization have resulted in a profound deterioration of India's air quality. The polluted air is harmful to man and to all types of life forms including plants, animals, and birds. It also affects nonliving materials like metals, marbles, other stones, wood, paints, etc., which get spoiled by the contact with polluted air, either due to more physical corrosive action of polluted air. Of the three million premature deaths in the world that occur each year due to outdoor and indoor air pollution, the highest number are assessed to occur in India. Surveys indicate that in New Delhi the incidence of respiratory diseases due to air pollution is about 12 times more than the national average and is included in the top ten polluted cities of world according to WHO (2014).

19.2 Sources of Air Pollution

Sources of air pollution refer to the various locations, activities, or factors that are responsible for the releasing of pollutants into the atmosphere. These sources can be classified into two major categories:

19.2.1 Natural Sources

1. Volcanic activity, which produce sulfur, chlorine, and ash particulates.
2. Dust from natural sources, and smoke and carbon monoxide from wildfires.
3. Methane, emitted by the digestion of food by animals, for example, cattle.
4. Vegetation, in some regions, emits environmentally significant amounts of VOCs on warmer days. These VOCs react with primary anthropogenic pollutants—specifically, NO_x, SO_2, and anthropogenic organic carbon compounds—to produce a seasonal haze of secondary pollutants.
5. Radon gas from radioactive decay within the Earth's crust. Radon gas from natural sources can accumulate in buildings, especially in confined areas such as the basement, and it is the second most frequent cause of lung cancer after cigarette smoking.

19.2.2 Anthropogenic Sources

1. Mostly related to burning different kinds of fuel.
   (a) "Stationary sources" include smoke stacks of power plants, manufacturing facilities (factories), and waste incinerators, as well as furnaces and other types of fuel-burning heating devices. In developing and poor countries, traditional biomass burning is the major source of air pollutants; traditional biomass includes wood, crop waste, and dung.
   (b) "Mobile sources" include motor vehicles, marine vessels, aircraft, and the effect of sound, etc.

2. Chemicals, dust, and controlled burn practices in agriculture and forestry management. Controlled or prescribed burning is a technique sometimes used in forest management, farming, prairie restoration, or greenhouse gas abatement. Fire is a natural part of both forest and grassland ecology and controlled fire can be a tool for foresters. Controlled burning stimulates the germination of some desirable forest trees, thus renewing the forest.
3. Fumes from paint, hair spray, varnish, aerosol sprays, and other solvents.
4. Waste deposition in landfills, which generate methane. Methane is not toxic; however, it is highly flammable and may form explosive mixtures with air. Methane is also an asphyxiant and may displace oxygen in an enclosed space. Asphyxia or suffocation may result if the oxygen concentration is reduced to below 19.5% by displacement.
5. Military, such as nuclear weapons, toxic gases, germ warfare, and rocketry.

19.3 Types of Air Pollution

Classification of air pollution has been made in consideration the following points related to pollutants and how they affect the other components of ecosystem.

1. Type of pollution based on occurrence: (a) Indoor, (b) Outdoor.
2. The place of origin: (a) Primary, (b) Secondary.
3. Chemical composition: (a) Organic, (b) Inorganic.
4. State of matter: (a) Particulate, (b) Gaseous.
5. Nature of Pollution: (a) Gaseous/ inorganic, (b) Bio-pollution/pollen, bacteria, fungi, particles of plant or animal origin.

(a) **Primary Pollutants**

These are directly emitted to the atmosphere and remain also in same form in the atmosphere. These primary pollutants are

- Suspended particulate matter like ash, smoke, dust, mist, and spray.
- Inorganic gases like sulfur dioxide, hydrogen sulfide, nitric oxide, carbon monoxide, carbon dioxide, and hydrogen fluoride.
- Olefinic and aromatic hydrocarbon and radioactive substances.

(b) **Secondary Pollutants.**

Secondary pollutants result due to chemical or photochemical reactions of the primary pollutants with elements or compounds present in the atmosphere. These reactions depend on factors like concentration of reactants, moisture, photoactivation, meteorological parameters, and topography like sulfur trioxide and smog. Organic pollutants mainly consist of carbon and hydrogen. However, some may contain oxygen, nitrogen, phosphorous, and sulfur. Hydrocarbons, aldehydes, ketones, alcohol, esters, amines, and organic compounds of sulfur are some of the examples. Inorganic pollutants include carbon monoxide, carbon dioxide, sulfur oxide, carbonates, nitrogen oxides, ozone, hydrogen fluoride, and hydrogen chloride.

Table 19.1 Major air pollution disasters

Time of occurrence/ location	Causes	Effects
Dust clouds in Texas April 1935	Dust storms due to continuous drought	As one "black blizzard" hit after another, harmful dust particles accumulated in people's lungs, causing hundreds of deaths. Dead livestock and wildlife littered the ground
Meuse Valley (Belgium) Dec. 1930	Thermal inversion of gases (SO_2, SO_3, aerosol, and fluorides) from industrial plants	60 deaths More than 1000 affected
Donora (USA) Oct. 1948 called as Donora Smog Museum	Anticyclonic weather, no air movement, temperature inversion, SO_2, and fog with oxidation products and $PM_{2.5}$ were the main cause	The smog killed 20 people and caused respiratory problems for 7000 people of the 14,000 population of Donora, Pennsylvania, a mill town on the Monongahela River 24 miles southeast of Pittsburgh.
Great London smog Dec. 5–9, 1952	Sulfur content of coal was mixed with fog and became dangerous smog	4000 died Many thousands affected
London Jan. 1956	Concentration of smog	1000 died
London Dec. 1962	Shallow inversion, problem due to fog	700 died
Tokyo June 18, 1970	Smog poisoning	6000 affected
Bhopal MIC gas tragedy Dec. 3, 1984	In union carbide pesticide industry, toxic gas methyl isocyanate leaked accidently	2500 died in Bhopal and 10,000 were affected Eyes and other health problems were caused

(c) **Particulates.**

Particulates may be further classified depending upon their physical, chemical, and biological characteristics. Size of the particular is its physical property. Fumes of cement dust fly depending on the chemical properties the particulate may be either organic or inorganic. Organic particulates found in atmosphere are phenols, organic acids, and alcohol while inorganic particulates found in atmosphere are nitrates, sulfates, and metal ash such as iron, lead, manganese, and zinc. Protozoa, bacteria, viruses, fungi, pollen grains, and spores are also biological particulates. The following table provides information on six air pollutants, classified as "criteria air pollutants" by the U.S. Environmental Protection Agency. The Table 19.1 describes each pollutant, including its sources and effects.

19.4 Air Quality Standards and Major Pollution Disasters

There are two areas where air quality is measured.

1. Ambient air quality measurement in which quality of air in ambient atmosphere is measured.
2. Pollutants emitted from different sources.

Air quality standards provide a legal framework for air pollution control. An air quality standard is a description of a level of air quality that is adopted by a regulatory authority as enforceable. It is necessary to assess the present and anticipated air pollution through continuous air quality survey/monitoring programs. Therefore, Central Pollution Control Board had started National Ambient Air Quality Monitoring (NAAQM) Network during 1984–1985 at national level. The program was later on renamed as National Air Monitoring Programme. Table 19.2 describes the occurrence of major pollutants in seven major cities of India during 2001. SPM and RSPM are in critical range in residential areas of Delhi and Ahmedabad (Bhatt and Dhamecha 2009).

19.5 Increasing Pollution Due to SO_2

The Norilsk smelter site in Russia continues to be the largest anthropogenic SO_2 emission hotspot in the world, followed by the Kriel area in Mpumalanga province of South Africa and Zagroz in Iran. Some countries, such as China, have enforced

Table 19.2 Air quality in seven major cities of India during 2001

	Annual mean concentration range (μg/m^3)				
	Industrial (I)			Residential (R)	
Pollution level	SO_2 and NO_2	RSPM	SPM	SO_2, NO_2, RSPM	SPM
Low (L)	0–40	0–60	0–180	0–30	0–70
Moderate (M)	40–80	60–120	180–360	30–60	70–140
High (H)	80–120	120–180	360–540	60–90	140–210
Critical (C)	>120	>180	>540	>90	>210

State/city	SO_2		NO_2		RSPM		SPM	
Area class	I	R	I	R	I	R	I	R
Andhra Pradesh—Hyderabad	L	L	M	M	M	H	M	H
Delhi	I	I	I	M	C	C	H	C
Gujarat—Ahmedabad	L	L	M	M	C	C	H	C
Karnataka—Bangalore	L	L	L	M	H	C	L	H
Maharashtra—Mumbai	L	L	L	L	M	H	M	C
Tamilnadu—Chennai	L	L	L	L	M	H	L	M
West Bengal—Kolkata	L	L	H	H	H	C	M	C

Source: Bhatt and Dhamecha (2009) *Eco-Degradation due to Air Pollution*, p. 129

more stringent emission regulation for coal combustion and other industrial processes, leading to a decrease of SO_2 emissions. Singrauli, Neyveli, Talcher, Jharsuguda, Korba, Kutch, Chennai, Ramagundam, Chandrapur, and Koradi are the major SO_2 emission hotspots in India. in some fast-developing Asian countries such as China and India, SO_2 is still present in a significant amount in the atmosphere (Smith et al. 2011). Gurjar et al. (2016) observed a decreasing trend of SO_2 in all monitoring sites in megacities of India due to the lowering of sulfur content in coal and diesel. Thermal power plants are major users of coal in India, accounting for more than 25% of total emissions from 1973 to 1997.

Billions of tons of agricultural waste are generated each year in the developing and developed countries. Agricultural residue includes all leaves, straw, and husks left in the field after harvest, hulls, and shells removed during processing of crop at the mills, as well as animal dung. The types of crop residue that play a significant role as biomass fuels are relatively few. The single largest category of crops is cereals, with global production of 1800 Tg in 1985 (FAO 1986). Wheat, rice, maize, barley, and millet and sorghum account for 28%, 25%, 27%, 10%, and 6%, respectively, of these crops. The waste products that are the main contributors to biomass burning are wheat residue, rice straw and hulls, barley residue, maize stalks and leaves, and millet and sorghum stalks. Sugarcane (0.95 gigatons) provides the next sizeable residue with two major crop wastes: barbojo, or the leaves and stalk, and bagasse, the crop processing residue. The cotton crop also gives some residue in the form of stalks and husks, both of which are used as biofuels. Four minor crops provide residue from processing that is frequently used as fuel: palm empty fruit bunch and palm fiber, palm shells, coconut residue, groundnut shells, and coffee residue. Geographical distribution of crop residue is skewed by large crop productions in India and China (FAO 1986). The other countries of Southeast Asia have rice and sugarcane as dominant crops. In the Middle East, the crop mixture is more diverse with more cereals and less rice and sugarcane. India produces 14%, China 29%, and Asia 66% agro waste/residue from cereals. Share of cotton, sugarcane, and minor agro industry is 1%, 3%, 0 in India 3%, 1%, and 0 in China, and 5%, 6%, and 1% in Asia, respectively (Yewich and Logan 2003). In the dry lands of the Near East and Mediterranean northern Africa, wheat and barley predominate. In the sub-Saharan Sahel in Africa, millet and sorghum (5%) and wheat and rice (1%) are the main crops. The farmers of the northeastern provinces in India prefer to grow traditional rice with long straw as opposed to the short straw modern varieties because the straw is needed for buffalo fodder. Similarly, farmers throughout India grow wheat varieties that give good returns in straw to provide fodder. In the northwest, the rice straw is mixed with cow dung for use as fuel (Yewich and Logan 2003).

19.5.1 Vehicular Sources as Pollution of SO_2

Sulfur is ubiquitous in the biosphere and often occurs in relatively high concentrations in fossil fuels, with coal and crude oil deposits commonly containing

1–2% sulfur by weight. The widespread combustion of fossil fuels has, therefore, greatly increased sulfur emissions into the atmosphere, with the anthropogenic component now substantially greater than natural emissions on a global basis. The recent data shows that in India the number of motor vehicles has increased from 0.3 million in 1951 to 159.5 million in 2012. Out of these, 32% is concentrated in only 23 metropolitan cities. The use of metro rail is encouraged by provision of an adequate number of feeder buses run on CNG that ply with the desired frequency in Delhi. It is estimated that almost 67% of air pollution in Mumbai city is caused due to automobiles (Chaphekar et al. 2009).

19.5.2 Changes in Pollution Levels of SO_2 and NO_x during COVID-19

Concentrations of SO_2 in polluted areas in India have decreased by around 40% between April 2019 and April 2020. Using data from the Copernicus Sentinel-5P satellite, from the European Union Copernicus program, scientists have produced new maps that show the drop in concentrations across the country in times of COVID-19. In India, emissions of SO_2 have strongly increased over the last 10 years, exacerbating haze problems over large parts of the country. However, owing to the COVID-19 pandemic, human and industrial activity dropped considerably since the beginning of its lockdown on 25 March 2020.

This analysis of available early data suggests that government policies had directly reduced human activities. In general, the significance and consequences of a lockdown were still poorly understood and studied, and could probably play an important role in restoring air quality. The nationwide lockdown during the time of the COVID-19 pandemic provided a unique opportunity to work in this direction. Therefore, a quantitative assessment of air pollution was necessary in order to ultimately take measures to limit air quality, especially when such alternative control measures were necessary. This study is an attempt to evaluate the usefulness of locking as an alternative strategy to reduce air pollution in East China. As for NO_2, the OMI instrument allowed to determine the total NO_2 content in the vertical column of the atmosphere as the total number of NO_2 molecules between the Earth's surface and the tropopause per unit area (Boersma et al. 2004). NO_2 content in the vertical column of the atmosphere was calculated by dividing the inclined content of the air mass value of NO_2, which is depended on a number of parameters, including the geometry of the observations, the surface albedo, the shape of the vertical profile of NO_2, and cloud characteristics (height, density, and sky coverage) (Boersma et al. 2004).

19.5.3 Effect of Sulfur Dioxide on Plants

Gaseous air pollutants do not exist singly in the atmosphere. Therefore, researches on interactive effects of air pollutants must be encouraged to understand the role of air pollutants in combination. The monitoring of pollutant fluxes in different

Fig. 19.1 Effect of SO_2 pollution on wheat leaves. (Source: Photograph by author)

locations also highlights the substantial geographical and temporal variations and the wide-ranging air pollution scenarios. The micromorphological and biochemical changes along with growth and yield responses under combined exposure of air pollutants that varied with pollutant combination, their levels, and plants are under consideration. An accidental release of SO_2 caused burning in boot leaf of wheat crop in Vadodara (Fig. 19.1). Crops like mango and drumstick were more susceptible. Figures 19.2 and 19.3 show less fruiting in drumstick (*Moringa oleifera* Lam.) and black tip of mango in orchards located close to brick kilns. Gases like carbon monoxide, $SO_{2,}$ and ethylene constituting the fumes from brick kiln are known to damage growing tip of mango fruits (Fig. 19.4: Arya 2004). In combinations of air pollutants, responses of agricultural crops are mostly synergistic with respect to physiological, biochemical, yield, and quality traits. Higher concentration of SO_2 may bring acid rains, which is injurious to plants. The increased concentration of pollutant gases made the plants susceptible to pathogenic attacks due to microbes.

19.5.4 Impact of Sulfur Dioxide Pollution on Monuments

Deposition of particles can also stain and damage stone and other materials, including culturally important objects such as statues and monuments. An analysis of water-soluble samples collected from marble and sandstone of monuments for different ions has been done. The combustion, manufacturing, and other polluting operations existing within Agra area have been investigated. The measurements of flue gases amounting to 3.63×10^9 S.C.F. (standard cubic feet) indicate atmospheric contamination and deterioration of archeological monuments of Agra. It has been

Fig. 19.2 Normal fruit setting in drumstick. (Source: Photograph by author)

Fig. 19.3 Reduced fruit setting in drumstick due to SO_2 pollution. (Source: Photograph by author)

found that the principal sources of air contamination are the 325 iron foundries and 3 railway shunting yards located within 0.3–3.0 km of the main monuments. The topographical and micrometeorological conditions of the city have tended to favor and aggravate the concentration of effluents in the surrounding air of the monuments. The annual average existing level of SO_2 ranges from 16 to 20 μg/

Fig. 19.4 Black tip of mango in orchards due to SO_2 emitted by brick kilns. (Source: Photographby author)

m^3. The maximum concentration of SO_4^{2-} and NO_3^- amounting 0.46 and 0.38, respectively, by weight percentage found existing at Red Fort causes efflorescence of sandstone (Sharma and Sharma 1982).

19.6 Pollution Due to Biomass Burning

Biomass burning is a major source of particulate matter and trace gases to the atmosphere and strongly affects global air quality and climate (Akagi et al. 2012). In fire-prone regions such as the western United States, the frequency and intensity of wildfires have increased over the past several decades due to fire management practices and climate change (Westerling et al. 2006), and this trend is expected to continue in the coming decades (Dennison et al. 2014). In particular, biomass burning organic aerosol (BBOA) is subject to atmospheric aging processes that could significantly alter the climate and health-relevant properties of biomass burning emissions. Such processes include oxidation of gas-phase compounds followed by partitioning to the particle phase, forming secondary organic aerosol (SOA); direct oxidation of molecules in the particle phase through heterogeneous reactions; and evaporation of particulate semivolatile molecules upon plume dilution, potentially followed by subsequent gas-phase oxidation. However, despite the potential importance of aging on biomass burning emissions, the effect of aging on BBOA composition and loading over multiday timescales is not well-constrained and usually is not included in global chemical transport models (Shrivastava et al.

2017). Field measurements provide strong evidence that the composition of BBOA changes significantly when photochemically aged. In aircraft measurements of biomass burning plumes, OA consistently becomes more oxidized downwind, relative to the source of emissions (Capes et al. 2008).

Anthropogenic sources were mostly related to burning of different kinds of fuel. The traditional biomass includes wood, crop waste, and dung. According to an estimate, 20 million tons of rice stubble is produced every year in Punjab alone, 80% of which is burnt. A further study estimated that crop residue burning released 149.24 million tons of CO_2, over nine million tons of CO, and 0.25 million tons of oxides of sulfur. One metric ton straw burnt can produce 2 kg of SO_2, 60 kg, CO,1460 kg of CO_2 199 kg ash, and 3 kg PM (Yewich and Logan 2003).

Some studies show that little to no net secondary organic aerosol is formed over the course of several days of aging, or even that a loss of organic mass can occur (Akagi et al. 2012). Even under constrained laboratory experimental conditions, these studies show significant variability in SOA formation between burns of similar or even identical fuels. This variability is often attributed to differences in burning conditions (e.g., flaming and smoldering) (Hennigan et al. 2011) or the presence of unmeasured SOA precursors, but predicting biomass burning SOA across fuel types and burning conditions has remained a challenge. The range of global estimates is thus essentially unconstrained, with some studies ranking biomass burning as an insignificant source of SOA and others ranking it as the major source of global SOA (Shrivastava et al. 2017).

19.7 Pollution Due to Volatile Organic Compounds

The troposphere is the region of the Earth's atmosphere in which we live and into which chemical compounds are generally emitted as a result of human activities (an exception being the exhaust from present and future supersonic transports). As described below, emissions of oxides of nitrogen (NO_x "NO and NO_2"), volatile organic compounds (VOCs), and sulfur compounds lead to a complex series of chemical and physical transformations that result in such effects as the formation of ozone in urban and regional areas (National Research Council 1991) as well as in the global troposphere and the formation of secondary particulate matter through gas/particle partitioning of both emitted chemical compounds and the atmospheric reaction products of VOCs, NO_x, SO_2, and organosulfur compounds (Odum et al. 1997).

19.8 Pollution Due to PM_{10} and $PM_{2.5}$

The PM_{10} standard is generally used to measure air quality (Rizwan et al. 2013). The PM_{10} standard includes particles with a diameter of 10 μm or less. These small particles are likely to be responsible for adverse health effects because of their ability to reach the lower regions of the respiratory tract. According to the air quality guidelines by WHO, the annual mean concentration recommended for PM_{10} was 20 μg/m^3, beyond which the risk for cardiopulmonary health effects is seen to increase. Major concerns for human health from exposure to PM_{10} include effects on breathing and respiratory systems, damage to lung tissue, cancer, and premature death. Elderly persons, children, and people with chronic lung disease, influenza, or asthma are especially sensitive to the effects of particulate matter. The urban air database released by the WHO in September 2011 reported that Delhi has exceeded the maximum PM_{10} limit by almost ten times at 198 μg/m^3, trailing in the third position after Ludhiana and Kanpur (WHO 2014). Vehicular emissions and industrial activities were found to be associated with indoor as well as outdoor air pollution in Delhi (Goyal and Khare 2011). Heavy pollution was observed in Chengdu, China, on 18–21 May, 2012. The episode was the most long-lasting pollution event in the historical record of Chengdu, which was caused by a combination of stagnant dispersion conditions and enhanced $PM_{2.5}$ emission from intensive biomass burning, with peak values surpassing 500 μg m^{-3}. The event was characterized by three night-time peaks, relating to the burning practice and decreased boundary layer height at night (Chen and Xie 2014).

19.9 Pollution Due to Smog

The citizens of Delhi on November 7, 2017, woke up to the first dense smog of the season with the air quality in many areas ranging from poor to hazardous. Air quality is indicated by daily or mean annual concentration of fine particulate matter (PM_{10} and $PM_{2.5}$, i.e., particles smaller than 10 or 2.5 μm, respectively). The safe limits for annual and 24-h $PM_{2.5}$ and PM_{10} particles are 40 and 100, respectively, in India by the CPCB and anything more than 300 is considered as hazardous. The root causes of smog are burning coal, petrol, diesel, gas, biomass in industries, and power plants. Then come smoke from rural kitchens, traffic pollution, increase in vehicle numbers, increase in price of compressed natural gas (CNG), vehicular emissions, car growth, low cost of parking, dieselization of cars, jeopardization of nonpolluting modes of public transports, overpopulation, low investment in public transport, and lack of public infrastructure, large-scale construction activity, burning of residual crop in neighboring states such as Punjab, Haryana, and Uttar Pradesh. A study by Sharma et al. (2010) revealed that during autumn and winter months, about 500 million tons of crop residues burn in Indo-Gangetic plains. According to another study by Sindhwani and Goyal (2014), vehicular emission alone contributes about 72% of the total air pollution load in Delhi. Moreover, in winter months, slow winds and cool temperatures trap dust and pollutants closer to the ground forming smog. The visibility at the Delhi–Chandigarh highway was as low as 50 m. Low visibility has resulted in accidents across the city, notably a 24-vehicle pileup on the Yamuna Expressway. Use of face antipollution masks was suggested when outdoor. New Delhi, one of the most contaminated urban communities on the planet, has made accessible to its occupants some specific veils that can go about as a shield from the fine toxins that make the city's air polluted. "It reminded of the Great London Smog" occurred in December 1952 (Table 19.1) when London was trapped in a deadly cloud of smog and pollution for 5 days. At the time, the city ran on cheap coal for everything from generating power to heating homes. So when an anticyclone caused cold air to stagnate over London, the sulfur dioxide, carbon dioxide, and smoke particles mounted and ended up choking as many as 4000 people to death.

19.10 Indoor Air Pollution

Air pollution indoors is just as relevant to ambient outdoor exposures as about 99,000 deaths in Europe are attributed to indoor exposures, but this is likely to be a gross underestimate. Outdoor pollutants penetrate the home, schools, and workplaces; further, current trends to make buildings energy efficient by sealing those increase the accumulation of pollutants from furnishings, household products, and cooking. In the developing world, the burning of biomass for heating and cooking is a particular problem for women and small children. In indoor air pollution (IAP), from the indoor biomass combustion, the air pollutants that are emitted include SPM, NO_x, CO, C_6H_6, 1,3-butadiene (C_4H_6), methanol (CH_2O), polycyclic aromatic hydrocarbons (PAHs), and several toxic organic compounds (WHO 2006). PM_{10} and $PM_{2.5}$ concentrations over megacity Delhi have reduced by above 50% in comparison to the before-lockdown period in India, that is, improvement of air quality during the COVID-19 lockdown was noticed (Mahato et al. 2020).

Indoor air quality is directly linked to the respiratory system of living peoples and dependence on hard biomass for cooking and burning or heating exposes respiratory health problem of children of developing countries as well as in India due to use of lofty levels of indoor air pollution (Padhi and Padhy 2008). The improvement of cooking stoves and use of cleaner fuels should become a top concern in the high-focus-vulnerable regions. The children in house should be kept away from the kitchen during the cooking and fumes. The post-COVID behavior has shown that kitchen room should be large and people should keep themselves away from smoke as they are not being capable of concentrating within a polluted room for a longer period. Smokers of the family should also stay away from indoor house environment when smoking.

19.11 Problems of Biodeterioration by Biopollutants

Prokaryotes like bacteria and cyanobacteria, algae, fungi, and particles of animal and plant origin constitute biopollutants. Gregory (1973), in his book on Aerobiology, gave a detailed account of these organisms including fungal and pollen grains causing allergy, respiratory, and other health issues. These microbes can damage the objects as well as old buildings and monuments (Fig. 19.5).

Fig. 19.5 Black-colored dome of Baroda College due to microbial growth

19.11.1 Biodeterioration by Bacteria Cyanobacteria and Algae

The Cyanobacteria like *Oscillatoria, Lyngbya, Gloeocapsa,* and bacteria cause damage to old heritage buildings. The black crust coating the surfaces of building materials located in urban/polluted environment may contain all kinds of organic matter. Wet and dry deposition process with the help of compounds present in aerosols and was found to form to black crust formation, with aerosols, spores, pollen, dust, and every class of particulate matter entrapped in the mineral matrix. The chief factor determining microbial growth on constructional materials is moisture. Thus, it is important for conservators to understand the activities of microbes. Damp surfaces are readily colonized by microbial cells settling from the air. Biopollutants were reported on arts faculty of The Maharaja Sayajirao University, the second biggest dome in Asia built in 1881. Algae like *Protococcus* and *Ulothrix* are reported from monuments. These microbes produce acids and damage the building besides imparting an ugly look (Arya and Shah 2009).

19.11.2 Problems of Biodeterioration by Fungal Pollutants

Scientists have studied aeromycoflora of cities like Baroda (Arya and Arya 2007) and Lucknow (Dhawan and Agrawal 1986). Biopollutants can harm the buildings, objects, plants, and human beings. Arya and Arya (2007) reported numerous fungi from different vegetable and fruit markets of Baroda. Biodeterioration phenomena represent a complex of physical and chemical alteration processes in various materials, such as those constituting the objects that represent our cultural heritage.

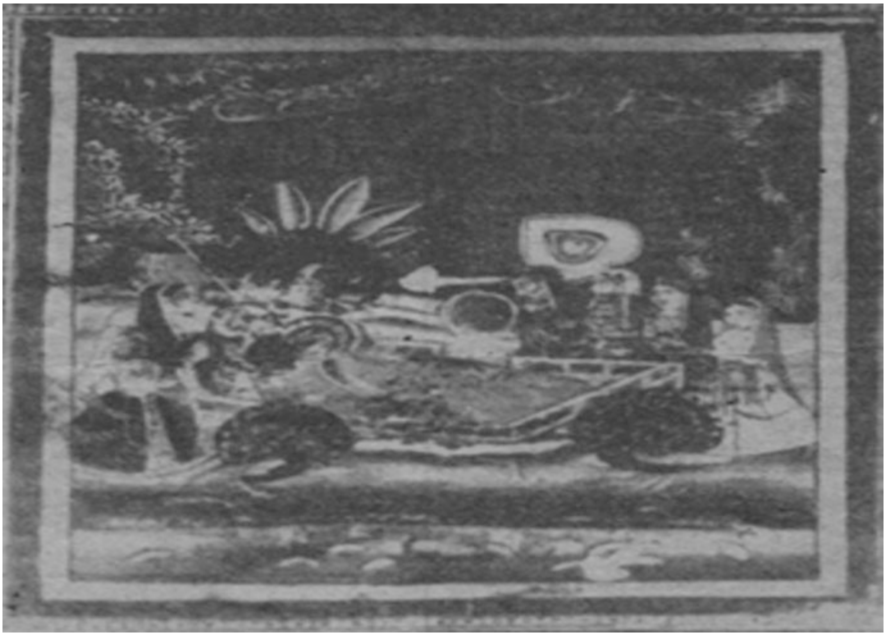

Fig. 19.6 Damaged miniature painting in Baroda Museum showed the presence of *Chaetomium gangligerum*. (Source: author)

A large number of fungi were reported to cause damage of the valuable cellulosic material. Pigments produced by fungi impart brown or black lesions on paper or cloth objects. Studies on damage to anthropogenic objects like miniature paintings, palm leaf manuscript, and Egyptian mummy were undertaken (Arya et al. 1988; Arya 2020). Hydrolyses of cellulose occurs with extracellular enzymes, which leads to degradation of paper. Enzymes cellodextrins and cellobiose break down cellulose. Damaged miniature paintings (Fig. 19.6) in Baroda Museum showed the presence of *C. gangligerum, A. versicolor, A. terreus. Penicillium* sp., and *Streptomyces*. Palm leaf manuscript (Fig. 19.7) showed the occurrence of fungus *Nigrospora* (Fig. 19.8). Mummy placed in Baroda Museum showed the presence of *Emericella nivea* (Figs. 19.9 and 19.10) (Arya 2006). Microbes gain entry through wind currents (Gregory 1973). The biodegradation of paper is conditioned by several variables such as the materials from which cellulose is obtained, the manufacturing processes employed, etc. Two fungal strains, *Aspergillus terreus* Thom and *Chaetomium globosum*, which are cellulolytic species frequently associated with paper spoilage, were used to produce stains with characteristics close to those observable on art objects made from paper.

Fig. 19.7 Blackening of palm leaf manuscript. (Source: Arya 2020)

19.12 Air Pollution and Human Health

To fully appreciate the risks of air pollution to health, further research is urgently required on how pollutant mixtures impact on the body. Beyond lung and cardiovascular disease, research should accommodate systemic effects such as obesity, diabetes, and changes linked to dementia and cancer, as well as effects on the developing fetus and early childhood. The physicians have a duty to speak out about this and raise awareness in our communities and at the same time offer solutions (Kunzil et al. 2014). Since the publication of the RCP/RCPCH report, efforts are being made to achieve this with new initiatives being rolled out from Public Health England, the Mayor of London, and through parliamentary process. Maybe Brexit creates an opportunity to introduce a new Clean Air Act focused on transport as recently called for by Environmental Protection UK. Nonrespiratory effects of air pollution are also seen more in Delhi such as hypertension, chronic headache, eye irritation, sore throat, and skin irritation. Many studies on air pollution and mortality from Delhi found that all-natural-cause mortality and morbidity increased with increased air pollution (Rizwan et al. 2013).

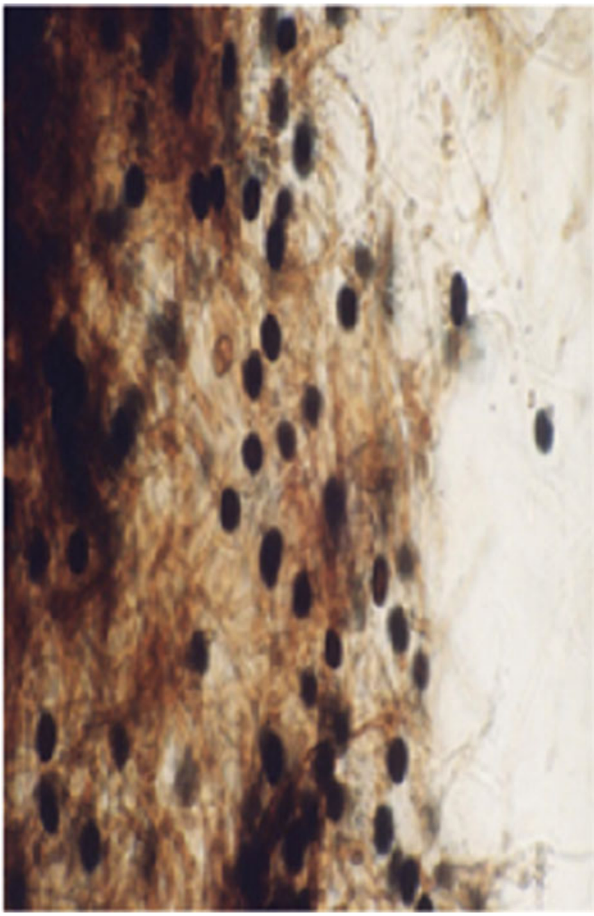

Fig. 19.8 Black conidia *Nigrospora.* (Source: Arya 2006)

A large number of studies in Delhi have examined the effect of air pollution on respiratory functions and the associated morbidity. The findings were compared with a rural control population in West Bengal. It was found that Delhi had 1.7 times higher prevalence of respiratory symptoms in the past 3 months as compared with rural controls ($P < 0.001$); the odds ratio of upper respiratory symptoms in the past 3 months in Delhi was 1.59 (95% CI 1.32–1.91) and for lower respiratory symptoms was 1.67 (95% CI 1.32–1.93). Prevalence of current asthma (in the last 12 months) and physician-diagnosed asthma among the participants of Delhi was significantly higher than in controls (Rizwan et al. 2013). Lung function was reduced in 40.3% individuals of Delhi compared with 20.1% in the control group. Delhi showed a statistically significant ($P < 0.05$) increased prevalence of restrictive (22.5% vs. 11.4% in control), obstructive (10.7% vs. 6.6%), as well as combined (both obstructive and restrictive) type of lung functions deficits (7.1% vs. 2.0%). Metaplasia and dysplasia of airway epithelial cells were more frequent.

Fig. 19.9 Showing the Egyptian mummy kept in Baroda Museum: the two fingers of right foot showed the presence of white-colored growth. (Source: Arya 2020)

Fig. 19.10 Petri dish showing three colonies of fungus: *Emericellanivea*, which is the perfect state of *Aspergillus*. (Source: Arya 2020)

The SO_2 is heavier than air and has a suffocating odor at an atmospheric concentration of around 500 parts per billion (ppb), at which level it can be fatal. Short-term exposures to SO_2 can harm the human respiratory system and make breathing difficult. People with asthma, particularly children, are sensitive to these effects of SO_2. It can react with other compounds in the atmosphere to form small particles. These particles contribute to particulate matter pollution. Small particles may penetrate deeply into the lungs and in sufficient quantity can contribute to health problems. At lower levels, chest pains, breathing problems, eye irritation, and a lowered resistance to heart and lung diseases can be experienced. At 20 ppb or lower, there should be no ill effects to a healthy person (EEA 2008). The normal atmospheric background concentration of SO_2 is generally less than 10 ppb. A secondary effect is the formation of sulfates (and nitrates), in the form of aerosols or very fine airborne particles, linked to increased asthma attacks, heart and lung disease, and respiratory problems in susceptible population groups. A third effect can occur further away from the emission source where the sulfur oxides will have converted to acids by aqueous phase reactions in the atmosphere. These acidic aerosols are eventually precipitated as acid rain, snow, sleet, or fog but only when they encounter the right meteorological conditions. In the absence of manmade pollution, rain water would be slightly acidic, around pH 5, due to the presence of carbonic acid from the interaction of water vapor and naturally occurring levels of CO_2.

However, we do not separately quantify the health impacts from exposure to sulfur oxides and nitrogen oxides alone due to the difficulty of discerning those effects that are due solely to SOx versus its contribution to PM (i.e., sulfate particles) or effects due solely to NO_x versus its contribution to ozone and PM (EEA (2008).

19.13 Mitigation of Air Pollution

19.13.1 Air Pollution Mitigation Through Plants

Although plant growth is affected by soil, water, and air pollution, microbes may inflict diseases in plants, which are considered one of the important components of ecosystem and help in reduction of CO_2 and pollutant gases and produce O_2. “The vibrant livable centre of our towns have allowed to decay beyond recognition, and what is left of the informal places has been ‘tarted up’ by the wall-to-wall fitted-everything brand of planning”. Urban sprawl has been allowed to overspill and then overrun greenbelt legislation, river boards and water authorities have straightened, culverted and covered their natural assets. Plants act as pollution scavengers and formation of greenbelts around industries and power plants was advocated by Chaphekar et al. (2009). Lichens are suitable pollution indicators as these are long-lived organisms with a high habitat specificity and potential to grow throughout the year. Lichens are widespread in a range of habitats from extreme conditions of heat or cold, from deserts to tropical rain forests, from natural to managed environments. They may be found on all types of substrata such as trees, rocks and earth, as well as manmade substrata, allowing their use as biological monitors of environmental conditions in urban and rural situations (Upreti et al. 1998).

19.13.2 Reduction of Air Pollutants in Power Plants and Industries

Before 1990, China’s coal-fired power plants mainly removed PM using a cyclone and water precipitator, and so forth. By around 1990, electrostatic precipitation technology with a PM removal efficiency of 94.2% had been mastered in China. With emission standards becoming more stringent, in the year 2000 coal-fired power plants began to use electrostatic precipitator (ESP), fabric filter (FF), and electrostatic fabric integrated precipitator (EFIP) (Wang et al. 2019); of these, through technological innovation, highly efficient electrostatic precipitation technologies such as high-frequency power supply, low-low temperature electrostatic precipitation, rotating electrode, and wet electrostatic precipitator (WESP) are introduced. Conventional de-dusting devices are generally configured after the air is preheated (APH); however, this would cause the problems of wear and blockage in the selective catalytic reduction (SCR) denitration catalyst and APH. The SO_2 control technology research on SO_2 control technology started late in China, but proceeded more rapidly than in developed countries. Starting in the 1970s, research, lab-scale experiments, and demonstrations in industrial boilers of desulfurization technology were carried out. In the early 1990s, foreign limestone-gypsum wet flue gas desulfurization (WFGD) technology was introduced. Since 2011, China's Ministry of Environmental Protection has further suggested use of desulfurization technology.

NO_x control technology has had a short development time in China, and low NO_x burning (LNB) and flue gas denitration technologies have been the major focus. LNB technology mainly includes air-staged combustion, fuel-staged combustion, and flue gas recirculation, with air-staged combustion technology being the most widely used. In the 1990s, China's coal-fired power plants began to use this technology, and an SCR denitration device was first installed in the 600 MW unit of the Fujian Zhang zhou Houshi Power Plant in 1999 (Basic statistics of China 2019). When a unit operates under a low load, it is easy for the catalyst activity to decrease, increasing NH_3 to escape. For this reason, the following retrofit was made to achieve wide-load denitration. The No. 0 high-pressure heater was configured to increase the feed water temperature, a bypass flue was added at the inlet of the economizer, the water side bypass was configured for the economizer, and the economizer was hierarchically arranged (Chen, and Xie 2014).

19.13.3 Air Pollution Control Devices

Control of particulate pollution is possible with gravity settling chamber, centrifugal collectors, viz., cyclone collector, wet scrubbers, fabric filter, and electrostatic precipitators. Control of gaseous pollutants is possible with spray towers and venturi scrubbers.

19.13.3.1 Cyclone Dust Collectors

These use cyclonic air movement to separate and contain dust particles from the air as part of a process to minimize air particle contamination in workshops, plants, and manufacturing facilities. Cyclone dust collectors use a combination of centrifugal, gravitational, and inertial forces to remove solid particles from the air, making them highly efficient collection devices for industrial air pollution control applications such as filtration and separation. These types of collectors work great for certain applications, but have some downfalls such as a certain amount of dust and debris re-entering the work environment as the air is exhausted. Cyclone dust collectors, on the other hand, are seen as more effective. These are generally larger, fixed units that are more powerful than their single-stage counterparts.

The air with high velocity enters through the inlet pipe into the dust separation chamber.

If the air at such a high velocity is made to impinge on the cartridges directly, it will damage the filter element. The dust particles are made to change its direction, which loses considerable amount of its velocity. The dry flue gas-cleaning processes are based on well-proven circulating fluidized bed technology. The flue gas flows through a turbo reactor from the bottom to the top and then enters a downstream particulate control device, which can be either a fabric filter or an electrostatic precipitator. Fly ash from incineration and fresh additives are dosed into the turbo reactor, while a large part of the solid material from the reactor is fed back to the fluidized bed as recirculate. Water is also injected to lower the flue gas temperature and achieve higher separation performance. If needed, activated carbon serves to provide excellent heavy metal and dioxin removal. As a result of advanced process management with regard to the operational temperature, solids recirculation, and the dosed additives, material consumption and the quantities of residue are kept to a minimum. The end product of the process is a dry, powdery residue that—depending on its composition—can be land filled or used as filler (e.g., road construction) after stabilization.

19.13.3.2 Dry Sorption

In certain cases, especially for smaller plants and for optimized investments, a dry sorption process based on lime or sodium bicarbonate is an interesting alternative to CFB-based flue gas cleaning technology. The process features a reactor, where the dry powdered additive is injected and mixed with the flue gas, and a downstream fabric filter.

19.13.3.3 Sodium Bicarbonate for Efficiency and Low Residue Levels

The sodium bicarbonate process is used wherever residue quantities must be kept as small as possible. Due to the very high reactivity of sodium bicarbonate, only a small amount of it is required. The process is virtually independent of temperature and maximizes energy recovery.

19.13.4 Reduction in Vehicular Pollution

To reduce the vehicular pollution in mega cities, metro trains and buses on BRTS are used as public transport. The use of vehicular fuel is replaced with less pollutive CNG and electric-driven vehicles. The Centre for Science and Environment (CSE) said in 2005 that the diesel has 350 ppm of SO_2 and large amount of polyaromatic hydrocarbons that makes the fumes carcinogenic. In Delhi, odd and even scheme was launched to restrict the vehicles hitting per day on road. The control of air pollution reached an alarming state due to vehicular pollution and burning of waste paddy straw in Delhi NCR area. Use of CNG, which contains methane at 80 atmospheric pressure and costs 70 and 50% less than petrol and diesel, respectively, was tried. Use of ethanol, methanol, and biodiesel were also tried. Up to 50% *Jatropha curcas* oil can be substituted for diesel in a C.I. engine without any major operational difficulties. Pretreatment of oil can further improve the quality of oil. Biodiesel has higher Cetane rating than diesel, which improves engine performance. It has energy content 39.6–41.8 MJ/kg as compared to 42.6–45 MJ/kg in diesel. The sulfur content is 0.13% as compared to 1% in diesel (Pramanik 2003). Biodiesel can be obtained from *Jatropha*, *Pongamia,* and a number of other plants including algae. Use of hybrid vehicles is promoted, and use of hydrogen as fuel is under trial. Hydrogen and solar power are pollution-free fuels. The Indian government has allowed blending of 20% ethanol with petrol. This will reduce pollution as well as import of crude oil.

The Supreme Court of India ordered the shutdown of hazardous, noxious industries and hot-mix plants and brick kilns operating in Delhi. Control measures so far instituted include introduction of unleaded petrol (1998), catalytic converter in passenger cars (1995), reduction of sulfur content in diesel (2000), and reduction of benzene content in fuels (2000). Others include construction of flyovers and subways for smooth traffic flow, introduction of metro rail and CNG for commercial transport vehicles (buses, taxis, auto rickshaws), phasing out of very old commercial vehicles, introduction of mandatory "Pollution Under Control" certificate with 3-month validity, and stringent enforcement of emission norms complying with Bharat Stage emission norms (Bhatt and Dhamecha 2009).

19.13.5 Reduction in Indoor Air Pollution

Indoor air quality is directly linked to the respiratory system of living peoples in towns and villages. The new buildings and building material can also emit VOCs. A large number of places develop sick building syndrome due to pollutive gases of phenomenon of fungal growth on woody structures. The wood or biomass used for cooking in rural and urban India exposes respiratory health problem of children and women of developing countries as well as in India. Pre-existing morbidities like acute respiratory infection (ARI), cold and cough, and fever among under-five children in India are also risk factors of the Coronavirus disease (COVID-19). Coal is also used, which contains CO and SO_2. Itching or burning is felt in highly polluted rooms or kitchen. These are harmful at higher concentrations particularly in winters. From a research viewpoint, there is a prerequisite for epidemiological studies to investigate the relationship between indoor air pollution and pre-existing morbidity, which are associated with the pandemic. More focus needs to be placed on improving cooking stoves, and use of clean indoor cooking fuels should become a top concern in the high-focus-vulnerable region. Children should be kept away from the kitchen during the cooking and burning of fuels. The kitchen room should have extensive space so that air pollutants smokes are not being capable to concentrate within the room for a long period, which reduces indoor air pollution, which is the risk factor of the COVID-19. Smokers of the family should also stay away from indoor house environment when smoking. Finally, well-built public health proceedings, including rapidly searching in high-focus areas and testing of COVID-19, should be performed in vulnerable areas of COVID-19. In Vadodara, the Green Planet Society has provision to use oxygen in theater room where a large number of persons are present to keep the concentration of O_2 within safe limits.

19.13.6 Control of Biopollutants

Any growth of microorganisms if seen should be controlled, particularly in warehouses and vegetable store rooms. Humidity should be maintained in indoor atmosphere. In Vadodara, museum and picture gallery has a trench outside the museum, where water is filled during hot summer months and adequate humidity is maintained. Use of certain botanicals in the form of eucalyptus oil, sweet flag, or Vacha (*Acorus calamus*) rhizome powder and *Cymbopogon* oil was found useful in control of fungi causing biodeterioration (Shah and Arya 2000). The environment can be made moisture free by keeping silica gel powder in chamber. Nonvacuum fumigation chambers are most often used with thymol and orthophenyl phenol vapors as the fumigant. Orthophenyl phenol is considered less toxic than thymol. The fumigation of rooms can be done by formaldehyde gas produced by keeping a mixture of potassium permanganate and formalin.

19.14 Role of Environmental Awareness

The Government of National Capital Territory of Delhi has taken several steps to reduce the level of air pollution in the city during the last 10 years. The benefits of air pollution control measures are showing in the readings. However, more still needs to be done to further reduce the levels of air pollution. The already existing measures need to be strengthened and magnified to a larger scale. The governmental efforts alone are not enough. Participation of the community is crucial in order to make a palpable effect in the reduction of pollution. The use of public transport needs to be promoted. The use of metro rail can be encouraged by provision of an adequate number of feeder buses at metro stations that ply with the desired frequency. More frequent checking of Pollution Under Control Certificates needs to be undertaken by the civic authorities to ensure that vehicles are emitting gases within permissible norms. People need to be educated to switch off their vehicles when waiting at traffic intersections. Moreover, the “upstream” factors responsible for pollution also need to be addressed. The ever-increasing influx of migrants can be reduced by developing and creating job opportunities in the peripheral and suburban areas, and thus prevent further congestion of the already-choked capital city of Delhi.

The success of government efforts and policies designed to resolve environmental issues can only be achieved with citizens’ support for environmental protection.

Awareness of environmental problems has been shown to be positively correlated with factors such as age, education level, health conditions, and parenthood in various studies conducted in developing countries including China. Understanding awareness of air pollution and support for environmental protection from the general public is essential for informing governmental approaches to dealing with this problem (EEA 2008).

19.15 Conclusion

Massive industrialization and anthropogenic activities have led to increase in atmospheric pollution. The air pollution caused by increase of SO_2, ozone, oxides of nitrogen, and particulate matter causes severe health problems. SO_2, and ozone are harmful to plants as well. Major disasters of pollution were caused by increase of SO and other toxic gases released due to industrial activities and accidents. The studies conducted on pollution of indoor environment had shown that use of burning fossil fuel is responsible for ill-health of women and children. There is an increase in respiratory diseases. Not only industrial pollution the vehicular pollution is also responsible for health-related issues. Large-scale government initiatives are taken to control air pollution like use of sulfur-free diesel and Pb-free petrol, alternatives like blending with ethanol and biodiesel, and more promotion of electric and hybrid vehicles. Various measures are suggested to reduce the air pollution. Regular monitoring should be done, and efforts should be made to reduce pollution due to biopollutants.

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Applications of Geographic Information Science and Technology to Monitor and Manage the COVID-19 Pandemic

20

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Abstract

Computerized Geographic Information Systems (GIS) have been in use since the 1960s, but recently the rapid spread of the highly contagious COVID-19 disease, caused by SARS-CoV-2 virus, has led to unprecedented interest in and reliance on geospatial data and visualizations to help monitor and manage the resultant pandemic. Geospatial factors such as human proximity, movement, and interaction play a central role in this pandemic, and the widespread availability of geospatial data from remote sensing and Global Positioning System technologies are fostering GIS analyses and dashboards that communicate information about its spread. Advances in computing technology are now capable of supporting near-real-time visualization of COVID-19 cases where space–time analysis and GIS software limitations were formerly a bottleneck for epidemiological studies. This chapter describes the current status of the COVID-19 pandemic and defines GIS terms that should be considered when reviewing COVID-19 geospatial analysis as many maps have been created hastily. Examples are provided of near-real-time surveillance websites, and other spatial analyses that show the

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impact of COVID-19 lockdowns on the environment, including effects on wildlife, air pollution, noise pollution, and water turbidity. Wastewater-based epidemiology is discussed as traces of virus components in sewage can also be used to monitor COVID-19 cases. Finally, new and emerging technologies such as contact tracing applications using mobile technology, as well as drones, and robots that reduce human exposure to the virus are discussed as applied to the pandemic. Recommendations are made for improving GIS applications for future pandemics.

Keywords

Coronavirus · COVID-19 · Drone · Environment · GIS—Geographic Information Systems · Health · Lockdown · Pandemic · Pollution · Robot · SARS-CoV-2 · Spatial analysis Technology · Virus

20.1 Introduction

A new virus, SARS-CoV-2, the causative agent of COVID-19, suddenly emerged late in 2019 and quickly spread around the world, causing hundreds of thousands of deaths. By March 2020, the World Health Organization (WHO) declared it a pandemic, with the United States regularly appearing on the list of countries with the highest number of confirmed cases (WHO; https://apps.who.int/iris/handle/10665/332196). As a result, many predictive disease forecasting models, near-real-time surveillance websites, contact tracing applications (apps) (computer applications downloaded to mobile devices), and other spatial analyses using Geographic Information Systems (GIS) have been created at an explosive rate to help monitor and manage this pandemic.

Computerized GIS platforms have been available since the 1960s and have previously been applied to epidemiological studies with some software and technological limitations (Ahasan et al. 2020). However, only recently, in alignment with the COVID-19 outbreak, has the technology improved to where geospatial methods are now widely accessible and readily being used, especially on mobile technology, providing informative results (Nguyen et al. 2020). Many health-based apps have emerged in the last several years with the proliferation of smartphones around the world and greater access to geospatial data (Gupta 2013; Carrion et al. 2016). These apps have provided a user-friendly framework to collect and share information about the spread of COVID-19. GIS provides many tools to create maps and other analyses often derived from remote sensing and Global Positioning System (GPS) technologies. Such tools provide statistics about disease propagation, give predictions about future outbreaks, and track changes in human movements and behavior. Many online near-real-time dashboards have been quickly created utilizing these tools; several provide information about spatiotemporal patterns of the pandemic and are useful for predictive modeling to manage outbreaks (Franch-Pardo et al. 2020). These have helped guide healthcare professionals and government officials in making virus-planning decisions, in addition to becoming extremely popular to citizen-scientists who monitor the intensity of an outbreak in their communities.

Medical geography and epidemiological surveillance have historically been used to manage diseases like the plague and Ebola (Koch 2016). Currently existing surveillance tools now provide a backbone to inform healthcare professionals and the public about virus movement and infection (Emch et al. 2017). Healthcare workers can use geospatial analyses to evaluate spatiotemporal patterns of a virus outbreak and create predictive models enabling appropriate management decisions (Franch-Pardo et al. 2020). Little was initially known about COVID-19, and as new information was found and theories changed, misinformation spread both on social media and amongst healthcare professionals and government officials. Many governments are quickly developing online dashboards that can provide COVID-19 statistics in near-real-time, including number of cases, deaths, and recoveries within their region and battle the spread of misinformation. As many citizens have had to restrict their normal day-to-day activities during mandated lockdowns, dashboards have become popular to help determine changing movement patterns and activities.

GIS systems and GPS data are more valuable and important now that mobile technologies and smartphones have begun to proliferate. With the increasing Internet speeds of mobile devices, users can access large datasets quickly, which is critical to monitor this pandemic in 2020 (Ienca and Vayena 2020). Contact tracing apps have been developed to track the movements of people through GPS technology in smart watches, smart phones, and even vehicles; this can help determine if one person encountered another person infected with SARS-CoV-2. One of the current limitations to contact tracing apps are privacy concerns raised from the processes by which large corporations, or government entities, may obtain detailed location or identity information of smart technology users (Ienca and Vayena 2020). New solutions for contact tracing can use wireless interfacing technology (WiFi) in combination with sound sensors on a smartphone to estimate distance by sound, which has fewer false positives than when just Bluetooth, the latest iteration of which is Bluetooth low energy (BLE), is used (Nguyen et al. 2020). WiFi and sound can be used together, or BLE can be used as good intermediaries between privacy and the need for accurate contact tracing without using the GPS sensor on a phone (Nguyen et al. 2020).

Studies have expressed concerns about the limited utilization of GIS and accuracy of the analyses that have been created to visualize the spatial spread of the outbreak; they have reported limitations in sharing real- or near-real-time spatial data between governmental agencies and more diverse enterprises (Ahasan et al. 2020; Field 2020; Franch-Pardo et al. 2020; Smith and Mennis 2020; Zhou et al. 2020). Ahasan et al. (2020) reviewed over 79 different articles that used GIS or geospatial tools for their analysis, of these only nine articles focused on the spatial patterns of COVID-19 disease spread, whereas 25 articles used GIS to focus on environment-related issues to COVID-19. One review of 63 studies criticized many of the online dashboards as overly complex, hard to understand, and slow to load the contents (Franch-Pardo et al. 2020). Nevertheless, there have been many positive aspects and incredible technological developments to the explosive production of GIS analyses for the pandemic.

By 2020, limitations previously considered as bottlenecks for epidemiological analyses were largely eliminated through technological advances. Potential to rapidly obtain considerable information about COVID-19 now is widely available for the public and healthcare professionals, especially with the production of near-real-time online COVID-19 dashboards (Ahasan et al. 2020). This chapter provides a brief description of the COVID-19 pandemic and relevant GIS and GPS technology, and provides examples of GIS applications and technologies used to monitor and manage the pandemic. Examples of specific environmental applications will be given, such as the use of GIS for development of information about changes in movement patterns and behaviors of humans and wildlife during the COVID-19 lockdowns. Future recommendations will be given for improved application of GIS to epidemiological spatial analysis and local monitoring of disease magnitude and rate of spread.

20.2 Description of the Global COVID-19 Pandemic

A novel coronavirus was first discovered in December 2019 when an outbreak of pneumonia-like symptoms, a disease of unknown origin, appeared in Wuhan City, Hubei Province, China (WHO; https://www.who.int/news/item/27-04-2020-who-timeline%2D%2D-covid-19). The disease was linked to persons who had been exposed to the virus at the Seafood Wholesale Market of Huanan in the city. This is a wet market where wild animals are sold and used as food. Scientists are still debating on how the virus originated. However, based on genetic codon usage studies and sequencing data, many experts believe it may have originated from animal hosts (zoonotic origin), such as bats or pangolins, followed by human-to-human transmission. The causative virus was initially called "novel coronavirus 2019" (2019-nCoV) by WHO. Later genome sequencing analysis revealed that the novel virus has a close relation with another coronavirus SARS-CoV (severe acute respiratory syndrome coronavirus), therefore it was renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the international committee of the Coronavirus Study Group (CSG). Since the novel coronavirus spread so quickly around the world and resulted in thousands of deaths, WHO declared it a pandemic on March 12, 2020. WHO named the disease "COVID-19" for the coronavirus disease of 2019 and named the virus "Severe acute respiratory syndrome coronavirus 2" or SARS-CoV-2. As of October 14, 2021, there have been a total of 240,023,070 worldwide COVID-19 cases and 4,891,303 COVID-19 deaths (Worldometers, https://www.worldometers.info/coronavirus/).

Coronaviruses (CoV) are a group of enveloped positive-stranded RNA virus genera, having divergent evolution in relation to their receptor binding S1 subunit of their spike protein (Li 2012). Sequence analyses have shown that SARS-CoV-2 is closely related to SARS-CoV that emerged in 2002–2003 and the Middle East respiratory syndrome coronavirus (MERS-CoV) that emerged in 2012. Original

SARS-CoV-2 has mutated into several variants, including the D614G strain that has increased its infectivity (Korber et al. 2020).

SARS-CoV-2 is highly contagious and results in a mild, moderate, or severe illness and possibly even death. It can spread through close physical contact (usually within 6 ft.) between two people when one has the virus. The respiratory droplets (containing the virus particles) are released from an infected person who is sneezing, coughing, yelling, singing, talking, or just breathing, and they land on the eyes or are inhaled into the body through the nose or the mouth. The tiny droplets can linger for a long time in the air and sometimes they travel further than 6 ft., which facilitates airborne transmission of this disease (Prather et al. 2020). According to the U.-S. Centers for Disease Control and Prevention (CDC), COVID-19 is less likely to spread through contact with contaminated surfaces, although SARS-CoV-2 can survive on surfaces for hours or days. For example, it can survive on copper for 4 h, paper for 1 day, and plastic for 3 days (van Doremalen et al. 2020). Animals that can be infected with SARS-CoV-2 are reported to include cats, dogs, tigers, gorillas and minks. Humans have contracted COVID-19 from animals, such as on mink farms (Oude Munnink et al. 2021). Persons have the highest risk of contracting COVID-19 if they stay indoors with no ventilation or if they are in big crowds and do not follow social distancing guidelines such as wearing a mask.

The CDC has listed COVID-19 symptoms as (1) fever or chills, (2) cough, (3) shortness of breath or difficulty breathing, (4) fatigue, (5) muscle or body aches, (6) headache, (7) recent loss of taste or smell, (8) sore throat, (9) congestion or runny nose, (10) nausea or vomiting, and (11) diarrhea (CDC; https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html). However, up to 40% of individuals can be asymptomatic depending on how long it has been since their initial exposure to the virus, their age, and other factors (CDC; https://www.cdc.gov/coronavirus/2019-ncov/hcp/planning-scenarios.html).

There are two types of diagnostic tests commonly available for COVID-19, a viral antigen test and an antibody test. For the viral antigen test, a RT-qPCR-based method where specimens can be obtained through an anterior nasal swab or saliva is most frequently used. The nasal swab method can put healthcare workers at risk as they must insert a swab into the patient's nose. Specimens also can be collected at home using an at-home-testing kit, which are then processed by a lab or sent to a testing center. RT-qPCR testing usually requires an expensive RT-PCR machine. Analysis of the sample is time-consuming and expensive as chemicals are used. Quicker, less expensive tests are now available, such as for the viral antigen. For example, the US company, Abbott, developed the BinaxNOW™ COVID-19 Ag Card. The test results can be obtained in 15 min and cost only $5 (Abbott; https://www.abbott.com/BinaxNOW-Test-NAVICA-App.html#/). So far, there are no commonly available potent antiviral drugs (including engineered antibody drugs) or vaccines to treat or prevent COVID-19. Lockdowns, wearing masks, social distancing (e.g., 6-ft. apart), and frequent hand washing with soaps are some of the important strategies currently used to reduce the spread of the virus. The lockdowns have had a huge impact on human movements and activities; as workers transitioned to work at home, in-person learning was canceled at schools, and shopping and recreational activities were constrained. As movement patterns changed, GIS began to be used to study the impact. It was observed that movement patterns changed considerably during the lockdown, with both positive and negative impacts on the environment (Teixeira and Lopes 2020; Road Ecology Center 2020).

20.3 Geographic Information Science and Global Positioning System

Goodchild and Longley (1999) said "the computer is no longer part of the research environment—we are rapidly approaching a world in which the computer is the research environment." Geographic information science is an important example of how this process has been transformative as most current GIS systems are now computer-based. GIS uses geospatial data to analyze different geographic or spatial patterns of movement or occurrences of phenomena under study (Maguire 1991). Many different fields of geographic research and analysis use these computer-based systems, or manual systems, as a tool (Maguire 1991). For these reasons, GIS may be considered as a "background technology (more akin to word-processing than, say, spatial interaction modeling)" (Maguire 1991; Goodchild and Longley 1999).

GPS, originally developed by the US military, is a satellite-based global navigation system that can provide coordinates for geographical locations on the Earth (Krenn et al. 2011). Maps are often created by gathering spatial data from GPS technology that generates coordinates for a given location. Mobile GPS units are now widely available, such as those developed by Garmin. Cell phones have built-in GPS, and many cars even have navigation systems that use GPS to show locations mapped on a visual display. With the rise of smartphone technologies, advanced smartphone sensors, and BLE, contact tracing with cellphone use is becoming a more powerful tool for healthcare professionals in pandemics (Nguyen et al. 2020).

GIS includes spatial statistics, visualization, and creation of maps that can utilize remotely sensed imagery (Smith and Mennis 2020), such as those displayed by Google Earth (https://www.google.com/earth/). The Google Earth app has simplified access to GPS coordinates and enabled the general public to add their own coordinates derived from separate mobile GPS units. All of this can be done with very little knowledge and experience about GIS or GPS systems. Environmental Systems Research Institute (ESRI) Inc. (Redlands, CA), the developer of one of the most popular GIS software platforms, has also created online versions for the general public to create online maps. Improved GIS software and increased access to web-based mapping tools have made it easier for the general public and professionals to create maps with little GIS background.

20.4 Benefits of Applying GIS to Monitor and Manage Pandemics

Some of the earliest medical maps were made in the seventeenth century, such as those for the plague in Naples around 1690 when mapping was an expensive pursuit (Koch 2005). Since the 1960s, when GIS became computerized, it became possible to easily visualize disease movement and prevalence (Kamel Boulos and Geraghty 2020). Other examples of prior GIS usage include studies that employed geospatial analyses to map travel times to healthcare facilities, especially for more vulnerable populations in sub-Saharan Africa to help manage Ebola (Hulland et al. 2019). GIS systems can utilize spatial statistics, spatial models, and cartography to describe populations, movements, and other aspects of disease-induced changes to help us monitor and manage the effects of pandemics around the world (Musa et al. 2013). Data is now easily transformed for easy and quick visual interpretation for public health surveillance. GIS is a powerful aid, especially in the current COVID-19 pandemic, to identify hotspot outbreaks, at-risk populations, resource accessibility, to improve information spread, and to create an information system for pandemics (Burton 2020, Roy et al. 2020). Jack Dangermond, the founder of ESRI, reported that he is very proud of the work the GIS community is doing in responding to this pandemic (Burton 2020).

Epidemiology is one of a wide variety of fields to which GIS has been applied (Maguire 1991; Figueiredo et al. 2020). Epidemiological surveillance systems were first developed by the U.S. Peace Corps to help provide important information to field staff starting in 1985. By 1995, they developed GeoSentinel®, which collects global data for surveillance of travel-related morbidity in collaboration with the CDC (Gamble and Lovell-Hawker 2008). Global observation data has been collected for previous epidemiological studies such as measles, human immunodeficiency virus (HIV), and viral hepatitis for patients with hemophilia (Patel and Orenstein 2019; Schieve et al. 2020). Epidemiological surveillance is also used to collect health-related data that can be analyzed and interpreted to aid in planning decisions about disease prevention and control. This data is useful to analyze geographical hotspots of disease cases and outbreaks. The SARS outbreak of 2003 led to development of methods to report disease cases on a global level (Burrell et al. 2016). For example, WHO now maps polio cases yearly while showing trends over time (WHO; http://polioeradication.org/polio-today/polio-now/). Contact tracing was employed to actively identify cases and monitor the spread of infection, such as for Ebola, and is now being applied to COVID-19.

During the COVID-19 pandemic, many sources and researchers have accessed the global surveillance data to produce websites that show many types of daily statistics (Ahasan et al. 2020). Due to the importance of mapping applications in epidemiological studies, increasing numbers of public health papers that employ GIS are likely to appear in the coming months, and 7 have already been documented in a recent review of 79 different geospatial-based research articles on COVID-19 (Ahasan et al. 2020). Unfortunately, for previous epidemiological studies, there were some technical restrictions that greatly limited some of the geospatial analysis that could be produced.

20.5 Previous Limitations to Epidemiological GIS Analysis

Some of the previous epidemiological studies prior to COVID-19 had spatial and data processing limitations that are now less restrictive. For example, medical bioinformatics experience fewer limitations in transferring huge amounts of data to the Cloud as servers grow in their ability to store large amounts of data, which was a limitation dating back to 2012 (Dai et al. 2012). Improvements include fog computing, developed by CISCO in 2014, that helped improve cloud computing by reducing the distance issues of how far away a user was from the cloud server (Yi et al. 2015). Further improvements to high-speed Internet allow the quick download of large quantities of data that can be provided to the general public and researchers for little to no cost.

The term “big data” has been used to refer to datasets that are too large for the typical database to store and analyze (Manyika et al. 2011; Musa et al. 2013). It was suggested that if the healthcare industry could use big data technology effectively then costs would be greatly reduced (Manyika et al. 2011). Starting from the 1990s, medical GIS has been growing in popularity to help forecast public health issues (Musa et al. 2013). The Johns Hopkins COVID-19 dashboard website taps into the big data potential to provide information about COVID-19 to the world (Dong et al. 2020, Johns Hopkins; https://coronavirus.jhu.edu/map.html). GIS and big data technologies have been combined to provide near-real-time visualizations of the pandemic around the world for monitoring purposes. These software and technology improvements over the last decade have paved the path for the rapid production of GIS systems for the pandemic.

Space–time analysis enables examination of distributions of events, such as disease spread over specific time periods and locations (Musa et al. 2013). For surveillance during this pandemic, a space–time scan statistic has been constructed to show the presence of cases and emerging clusters of COVID-19, and track them through space and time (Desjardins et al. 2020). This is a valuable resource in aiding resource allocation, testing, and monitoring of disease spread. Some dashboards for the COVID-19 pandemic are updated daily, allowing for near-real-time predictions and analysis to aid healthcare professionals and the general public (Desjardins et al. 2020). In contrast, space–time data were considerably limited for GIS epidemiological studies between 1999 and 2013 (Moore and Carpenter 1999; Musa et al. 2013).

Previous GIS software versions also limit the size and scope of statistical analysis and space–time analysis. Links between “R,” a programming language, and GIS software or other statistical packages like S-Plus for Arc/Info as well as software that adds GIS techniques into a statistical package like (SAS/GIS) have decreased such limitations (Waller 1996). In 2013 the spatiotemporal dynamics of influenza outbreaks were visualized by using a random network methodology implemented within the R and GIS systems (Ramírez-Ramírez et al. 2013).

20.6 GIS Concepts of Importance for COVID-19 Studies

Combining many layers of discrete and continuous spatial data such as roads, lakes, cities, elevation, and even precipitation measurements results in the creation of GIS maps. Discrete data is one type of spatial data that includes definable mapped areas having fixed locations, such as property boundaries and roads. This data can be represented with either vector or raster data.

Vector data uses a series of *x*–*y* coordinates stored as points, lines, or polygons, which are as accurate as the gathered *x*–*y* coordinates (Price 2004). Vectors can be very precise as they can store up to six decimal places (Price 2004). Raster data is a grid of cells all holding a value for the same attribute; it is similar to a picture of an area where every pixel is a square of the Earth's surface and holds a value to represent some feature of that area such as elevation or temperature (Price 2004).

It is important to consider the resolution of a raster grid as larger cell sizes limit the ability to detect change across the landscape to that cell size. For example, a raster with a 100 m resolution is made up of cells that each represents a 100 m^2 area with one value for some attribute. While it is valuable to have higher resolutions to more accurately portray change across the land, smaller cell sizes can dramatically increase file size and processing time for analysis. It is important to consider the type of data layer that is used when making a map as there are varying advantages and disadvantages based on the research objectives for the analysis. Data layers derived from different sources may have different levels of accuracy, which must be considered when making a map since the lowest level of accuracy dictates the accuracy of the produced map (Goodchild and Longley 1999).

Remote sensing involves measurements of different characteristics of an object from a distance by using electromagnetic energy reflected from, or emitted by, an object and then recorded by an observer or instrument that is not in contact with the object (Mather and Koch 2011). Generally, measurements of objects on the Earth's surface are made from sensors mounted on aircraft or on satellites. Remote sensing systems usually collect raster-based data.

When creating maps, it is important to consider how the data will be displayed. Important components of a map include scale bars, direction labeling, and a legend. The spatial scale, or the map's extent, is important to determine for the study area and desired analysis. Many of the COVID-19 maps use the world as their extent. Luckily, world-scale maps de-emphasize effects of generalization and other map errors (Monmonier 2018). On a world map, usually a small-scale map, a square measuring 1 in. on each side most likely represents a bigger area than for a country map, or a larger-scale map, where a 1-$in.^2$ represents a much smaller area. Thus, the country map will have much greater detail than a world map, therefore having less generalization. Scale bars are added to help measure components on the map to show how the scale of the map compares to real-world measurements (Peterson 2014). A north arrow is a common way to give a sense of direction, which helps the user orient themselves to the map. Finally, the legend lets the reader know the meaning of symbols and colors in the map.

The book *How to Lie with Maps* by Mark Monmonier (2018) discusses how maps are not always accurate as they necessarily make generalizations about what they are presenting. When creating or viewing a map, it must always be considered how the colors, symbols, phrasing, and point of view are influencing the reader's understanding. For example, red is a common color to use for danger and urgency that can influence a reader to feel more alarmed about the data. The value and accuracy of a map also depends on how well the geometric generalization depicts reality (Monmonier 2018). Global scale maps will have more generalization as line and point layers will not be able to show all the details that a country map can show, such as for roads, which may only be shown as interstates on a global map. It is very easy for anybody to make a map and not consider some of the basic GIS concepts in the process (Buckley and Field 2011; Norheim 2012). The result could lead to inaccurate analyses or results.

Several concepts of spatial data are important to consider when searching for data to make a map. The user needs to consider the accuracy of any located data layers as maps produced will only be as accurate as their data. Data mining is the ability to obtain data at high-speed rates for a particular interest (Goodchild and Longley 1999). An example described by Ahasan et al. (2020) considers the mining of Twitter data containing tweets reporting COVID-19 symptoms of users in relation to COVID-19 testing accessibility (Andrade et al. 2020). Geodatabases are a helpful tool when gathering different sources of data or map layers to include in a map. A geodatabase can efficiently store different layers that are collected from a variety of sources (Price 2004). The usefulness of a GIS system depends on choosing and matching the correct world projections to display the data based on the intended analysis (Price 2004). Different projections can change how one assesses the size of different areas relative to one another, which could lead to distortions on how the map is interpreted (Field 2020). The mapmaker then has to re-project or transform all the data layers to have the same projection so that the data accurately lines up for analysis. Conflation is when functions are applied to resolve issues associated with the merging of different datasets (Goodchild and Longley 1999). A geodatabase can be used to help ensure all data layers have the same projection, cell size, or data extent.

GIS can also include network analysis of path systems like roads, streams, sewer pipes, etc. (Price 2004). This is a helpful tool that can be used, for example, to determine the quickest and most efficient path from a home to a COVID-19 testing center. This tool can even incorporate speed limits, traffic, and time of day for travel.

A mateurs who do not know basic GIS concepts, or do not have a background in cartography, have created COVID-19 maps. Such maps can contribute to a pandemic infodemic (an excessive amount of information, in which some of it is accurate and some is not), which makes it hard to analyze what is accurate or representative of the current situation (Mooney and Juhász 2020). ESRI, through ArcGIS online, is hosting COVID-19 online map-making classes, which is a valuable resource, but it can also make it more likely for the layman to make maps (Rosenkrantz et al. 2020). Caution needs to be used in reviewing different models, maps, and other GIS technologies that are being developed hastily now to convey information during the pandemic.

Many choropleth maps have been produced to depict COVID-19 cases around the world and for other related subjects. Choropleth maps, often considered graduated color maps, use a pattern, shading, or a symbol within a set area to show different classifications of data as a summary or generalization of reality; because of the generalizations needed for such representation, there has been considerable debate on their accuracy (Jenks and Caspall 1971; Monmonier 2018). Many maps for the pandemic have used absolute values to show the number of cases across a country with a choropleth thematic mapping technique. This is known to lead to accuracy issues as different areas have different numbers of people in it leading to inaccurate conclusions when looking at a choropleth map (Field 2020; Franch-Pardo et al. 2020). Another source of error for choropleth maps is when the map maker uses the "default" classification scheme for creating classes (or different patterns) for the map, instead of figuring out which type of breaks or classes make the most sense for what is being conveyed (Monmonier 2018). Model accuracy is "the credibility of long-term projections generated by quantitative models" and is constantly debated (Eker et al. 2018). The model should accurately reflect the observed data (Eker 2020).

Population normalization can solve some of the accuracy problems of creating a choropleth map based on raw numbers (Field 2020). It is achieved by dividing the number of cases by the population in a specific area to give a percentage of that area that has been infected. Otherwise when visualizing the data with only raw numbers, some areas can appear to have very few cases, but instead have a high percentage of infection if that area has a low population.

Some types of choropleth maps are more accurate if they are derived from statistical distributions by dividing an area into subregions. The method of classification for the data greatly influences the accuracy. Jenks and Caspall (1971) describe the amount of error of a choropleth map based on a series of error prisms that relate to the real data and the generalized model and can be calculated based on the sum of the volume of these prisms. In this case, a greater number of map classes may increase accuracy (Jenks and Caspall 1971). However, maps can also become so complex that they no longer serve their purpose (Jenks and Caspall 1971).

An isopleth represents a changing attribute over an area where that attribute is the same magnitude between two isopleth lines. A contour map is an example of an isopleth map where the elevation changes, typically in some multiple of 10, with each new contour line. This is similar to a raster where the entire area will hold a value for that attribute, but a raster is more detailed since each "pixel" of area can hold a different value.

Kriging is another method of spatial interpolation that estimates values at unknown points based on the values at known points (Cressie 1986). It assumes that the direction or distance between samples reflects their correlation. Common examples include precipitation maps where rainfall points measured at weather stations are interpolated to produce a raster of continuous rainfall estimates across that area. It creates a raster surface by inputting points that have a recorded z-value. A

z-value is typically an elevation measurement of a surface at location (x, y); however, the z-value can be any measurement that you are studying at that point location. These commonly used terms and interpolation methods should be considered when reviewing maps made for the pandemic.

20.7 Current COVID-19 Near-Real-Time Maps and Dashboards

Space–time representation in maps is one of the current frontiers in the evolution of GIS and is considered by many to be an essential component of the spatial analysis of disease patterns in the future as technology and data sizes grow (Musa et al. 2013). Now with near-real-time mapping of COVID-19 cases and mortalities, the space–time limitations seem to be largely resolved.

Online dashboards are increasingly utilized now as tools to inform the general public and healthcare professionals about the spread of COVID-19. Dashboards have become incredibly popular during the pandemic partly due to the social confinement of staying at home (Mendoza 2020). Many of these dashboards map the number of cases, deaths, or recoveries over time.

Gardner and Dong at Johns Hopkins University, Baltimore, MD, USA, in collaboration with ESRI, created the COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) to track COVID-19 cases in near-real-time since January 2020 (Fig. 20.1) (https://coronavirus.jhu.edu/map.html). The dashboard displays the number of cases, deaths, and recoveries from different

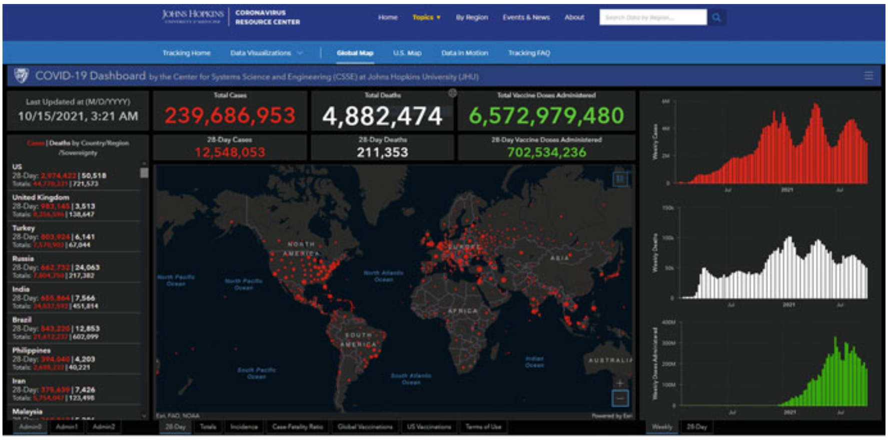

Fig. 20.1 COVID-19 dashboard created by the Johns Hopkins Centers for Civic Impact Engineering (CSSE) tracking COVID-19 in near-real-time in collaboration with ESRI, who acquired the data from WHO, the U.S. Centers for Disease Control and Prevention, the European Center for Disease Prevention and Control, the National Health Commission of the People's Republic of China, 1point3acres, worldmeters.info, BNO, state and national government health departments, local media reports, and the DXY, one of the world's largest online communities for physicians (This screenshot was taken from https://coronavirus.jhu.edu/map.html) on October 15, 2021, with permission to reproduce)

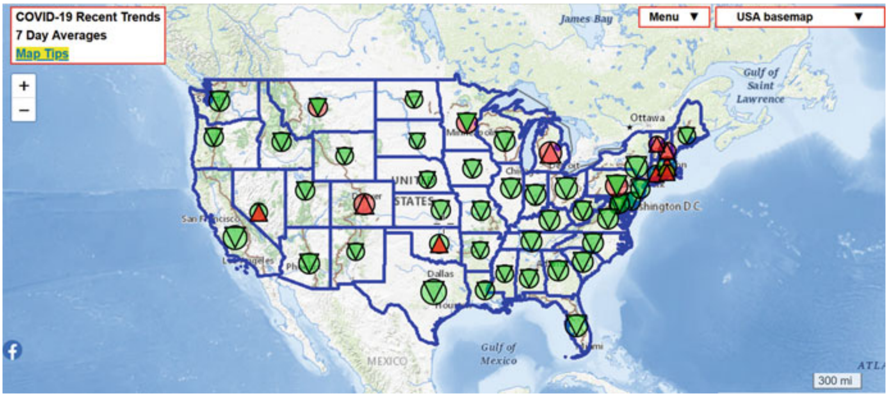

Fig. 20.2 Website shows the COVID-19 daily cases that are averaged over the previous 7 days based on the time series data that Johns Hopkins University updates daily. Based on these averages, the map uses red symbols to show the states where the number of cases is increasing (negative trend) and green symbols to show the states where the number of cases are decreasing (positive trend). This website has different map layers that can be turned on or off, for example, to show the location of testing centers, the number of available hospital beds, and more. (This screenshot was reproduced from (https://bit.ly/36zraWN) on October 15, 2021, with permission from Joseph Elfelt, creator of https://mappingsupport.com/)

countries around the world using a GitHub repository (JHE CSSE) and initially was updated twice a day (Dong et al. 2020). ESRI's ArcGIS Living Atlas team helped to add a "semi-automated living data stream strategy," which aggregates local sources to track cases of COVID-19 in near-real-time with some locations updated in 15-min increments and others updated manually (Dong et al. 2020). The website uses data from many different sources including WHO, the CDC, the European Center for Disease Prevention and Control, the National Health Commission of the People's Republic of China, 1point3acres, Worldmeters.info, BNO (BNO news), state and national government health departments, local media reports, and the DXY, which is one of the world's largest online communities for physicians, healthcare professionals, pharmacies, and facilities (https://coronavirus.jhu.edu/map.html and https://coronavirus.jhu.edu/map-faq). While millions of users have used it to monitor the pandemic, it, unfortunately, does not display maps of the data for previous dates (Kamel Boulos and Geraghty 2020). Other limitations of this website are that it may load slowly and viewing is difficult on smaller mobile devices.

A different perspective on pandemic data is obtained using an online web map produced by Elfelt (2020a). This map uses the time-series data that Johns Hopkins University updates daily and publishes on their GitHub site (Fig. 20.2). The advantage of this website is that, instead of showing the cumulative counts of cases like the John Hopkins website, it can show COVID-19 daily counts that are averaged over the previous 7 days. Averaged counts for the previous 2 weeks are produced by code that automatically runs each night. Based on this averaging, the map uses colored symbols to show if the recent number of cases or deaths is generally increasing or decreasing (Elfelt 2020b). This is calculated by taking the daily counts over the last 7 days and plotting these points on a graph. The *x* values refer to days and can have values 1–7. The *y* values are daily counts. The scripting determines the slope of a line that "best fits" the points. If the slope is positive, the trend is increasing; and if the slope is negative, the trend is decreasing.

Previously, the map did not use the time-series data or plot the average number of cases over 7 days, which led to some errors. Averaging is helpful as some health departments do not report their COVID-19 statistics on a daily basis and the lack of reporting during holidays or weekends may lead to a larger number of cases on a Monday, which can negatively impact the calculation that determines if cases are increasing or decreasing (Elfelt 2020b). For locations that have an increase in cases, there is a red symbol (negative trend), and for areas that have a decreasing number of cases (positive trend) there is a green symbol. This website also contains different map layers that can be added or deleted to show the recent trend data for cases or deaths by county, state, or totals for the United States, as well as testing locations, number of available hospital beds, and more (Fig. 20.2). While some of this information is also widely available from the Johns Hopkins website, it is much faster to load and more mobile-friendly (Elfelt 2020c).

Several statewide dashboards, or near-real-time monitoring websites, have been developed to help monitor the pandemic. The Washington State Department of Health's King County dashboard visualizes a vector-based choropleth map on which confirmed cases, hospitalizations, and deaths are displayed in varying shades of color for each county; representing the magnitude of that category in the county (Washington State Department of Health; https://www.doh.wa.gov/Emergencies/COVID19/DataDashboard). For more detailed information within a county, the user is directed to Local Health Departments, such as the King County Public Health COVID-19 dashboard. Under the geography portion of the King County daily COVID-19 outbreak summary, an interactive map displays data on the most current confirmed positive cases, test positives, hospitalizations, deaths, all test results, and number of people tested by the user-selected areas of city limits, health reporting areas, zip code, or census blocks (King County; https://www.kingcounty.gov/depts/health/covid-19/data.aspx).

Some of the near-real-time online dashboards are less accurate than others. Their temporal accuracy can change over time because the number of cases or deaths may be less accurate over the course of weekends and holidays when the reporting centers update results less frequently (Murray et al. 2020). For example, in King County, Washington, poor air quality, during the wildfires, in the Puget Sound region was listed as a limitation for case and mortality accuracy in September 2020 on the online dashboards since they stated many testing sites were closed (King County; https://www.kingcounty.gov/depts/health/covid-19/data.aspx).

Aside from temporal inaccuracies, the data itself can be a major source of error. Inconsistent health data and reporting discrepancies for mortalities is leading to inaccurate analyses or hard to interpret results (Rosenkrantz et al. 2020). Such as when the State of Washington changed their COVID-19 death reporting methods, which increases the difficulty of following trends over time (Washington State

Department of Health; https://www.doh.wa.gov/Emergencies/COVID19/DataDashboard). Previously, deaths were associated with COVID-19, if the patient tested positive for the virus while they were in the hospital, and even if the patient died of another medical issue like a stroke (Washington State Department of Health; https://www.doh.wa.gov/Emergencies/COVID19/DataDashboard). There are false perceptions that these dashboards or spatial visualizations are completely accurate and their usefulness may be greater than it is (Rosenkrantz et al. 2020).

The *New York Times* interactive COVID-19 maps may be more representative than some of the other dashboards or websites as they normalize the data by reporting the average daily cases per 100,000 over the past week for the world (Almukhtar et al. 2020). They also have several additional tables and charts that further enlighten the public about the COVID-19 pandemic across the globe.

The National Response Portal accesses Google Cloud, which combines data from many public and private sectors, to provide a location for healthcare professionals to access local COVID-19 data in making decisions about the pandemic (Vigliarolo 2020). The portal includes maps that scale down to the county level, are updated daily, and show forecasts for the number of cases and hospitalizations that is normalized per 100,000 people (National Response Portal; https://map.nationalresponseportal.com/portal). It shows mobility data for counties based on different categories like parks and workplaces. Many healthcare agencies help supply the data it provides (Vigliarolo 2020).

20.8 Environmental GIS Applications During the Lockdowns

20.8.1 Impact on Wildlife Movements

University of California Davis's (UC Davis) Road Ecology Center has created detailed reports about how the "stay-at-home," or lockdowns, reduction in traffic patterns has impacted wildlife-related vehicle collisions (https://roadecology.ucdavis.edu/). This center used traffic data from StreetLightdata.com and collision data from California, Idaho, and Maine to determine if vehicle collisions with wildlife were reduced with the stay-at-home orders. Miles traveled by drivers in the United States were reduced from 103 billion miles in March 2020 to 29 billion miles in the second week of April 2020, which resulted in approximately a 71% reduction in travel (UC Davis; https://roadecology.ucdavis.edu/). For the three study states, there was a 63–75% reduction in the amount of driving during this time.

This study also compared the wildlife-related collisions from 4 weeks before the stay-at-home orders were put into place to until 4 weeks afterward (Nguyen et al. 2020). The results were compared to other years as well, and the number of wildlife-related injuries was far fewer as a result from the reduction in traffic. The study predicted that 5700–13,000 fewer large mammals could be killed per year, and in California alone, 50 fewer mountain lions could be killed per year. There was a 58% reduction of mountain lion collisions in California based on the vehicle traffic changes from 10 weeks prior to the issued stay-at-home order until 10 weeks afterward. The results showed a statistically significant reduction in vehicle related wildlife collisions of approximately 21–58% in California, Idaho, and Maine (Nguyen et al. 2020).

There are several caveats the study describes when considering wildlife-related mortalities with vehicles as there is not necessarily a linear relationship between collisions and traffic volumes. The study did not look at collisions with all types of large animals or animals of all sizes. The study did not notice geographical differences in where the number of vehicle collisions and the reduction in collisions decreased. It should also be considered that some individuals are driving faster than normal due to reductions in traffic from stay-at-home orders for the pandemic. Perhaps as a result, some areas are seeing an increase in the number of animal injuries from speeding cars. Between 2013 and 2019, Yosemite reported an average of 24.4 bears were hit per year (National Park Service; https://www.nps.gov/yose/learn/management/statistics.htm). In August 2020 alone, at least four bears were injured from speeding cars in the park (Sanchez 2020).

The pandemic has also led to various reports of changes in animal activity due to changes in human movement patterns from the lockdowns (Rutz et al. 2020). Google has created "COVID-19 community mobility reports" (https://www.google.com/covid19/mobility/), which can be applied to wildlife movement patterns. For example, there appears to be a mobility trend of −5% for humans, after the pandemic started, compared to baseline in park visits in certain areas. These trends are calculated over a several week period and are generally based on the "location history" of someone's mobile technology (Google Community Mobility Report; https://www.gstatic.com/covid19/mobility/2020-11-24_AF_Mobility_Report_en.pdf).

Another initiative is collecting stories about how animals are responding to changes in human activity due to measures used to control the spread of coronavirus. An international consortium has been formed, the "COVID-19 Bio-Logging Initiative" (www.bio-logging.net), which is collaborating with the Movebank online research platform and the Max Plank Institute of Animal Behavior, to use "bio-loggers" to record changes in animal movement and behavior in relation to changes in their environment due to the pandemic (Figs. 20.3a, b) (Kranstauber et al. 2011; Wikelski et al. 2020). The Movebank platform, as of March 2020, included over 2.4 billion locations and over 989 taxa (Kranstauber et al. 2011). Often, data from smartphones is used to derive GPS tracking logs in combination with required field data (Rutz et al. 2020). Most of the studies, unfortunately, are not yet available to the public as it is up to the scientist if they would like them to become available. The Animal Tracker app (available for Android, iPad, and iPhone) allows anyone to see their animal observations with live monitoring and to give permission to allow the general public to see them (Wikelski et al. 2020).

Impacts of the stay-at-home lockdowns on animal movement and behavior are having a positive and negative impact on wildlife. According to Movebank, some species that rely on urban environments, or the protection of humans, will suffer from the lack of people (Max-Planck-Gesellschaft; https://www.mpg.de/15005457/covid-19-lockdown-reveals-human-impact-on-wildlife). Rats, raccoons, birds, and other common city critters that rely on human refuge in urban environments are

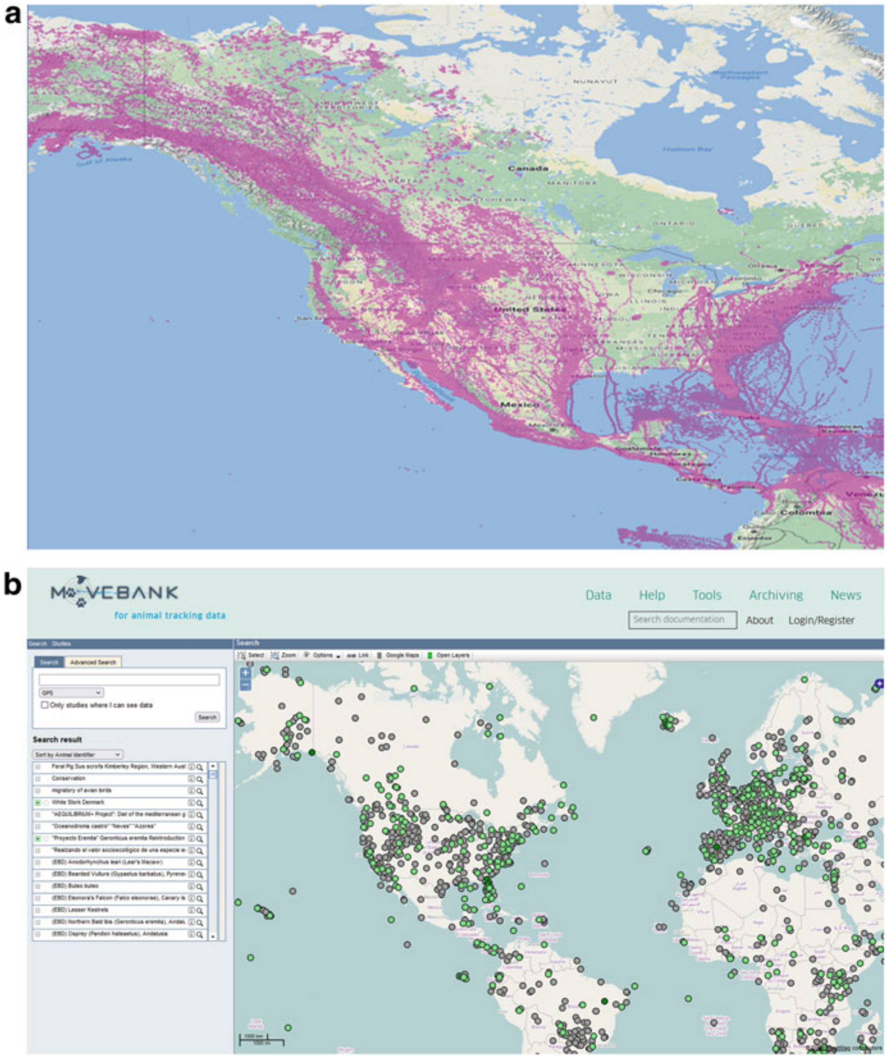

Fig. 20.3 The Movebank online database platform stores and shares animal movement data and bio-logging data, based on a variety of animal studies hosted by the Max Planck Institute of Animal Behavior. Image of the Movebank online platform for animal movement data (Kranstauber et al. 2011). (This screenshot was reproduced from https://www.movebank.org/cms/webapp?gwt_fragment=page=search_map with permission on October 15, 2021)

experiencing increased stress due to the lack of access to food. Endangered species such as rhinos or elephants can be exposed to increased risk of poaching due to the lack of human protection and presence (Max-Planck-Gesellschaft; https://www.mpg.de/15005457/covid-19-lockdown-reveals-human-impact-on-wildlife). Other species have benefited by the reduction of humans in their environment, such as in forest service parks and other popular outdoor tourist areas. Movement of these species and related behavior can be investigated using GPS, and compare their range pre, during, and post stay-at-home initiatives. The mass human behavior changes with COVID-19 have provided a unique time to study the human–wildlife interface, which will be sure to produce valuable research findings, as well as other environmental studies of the lockdowns.

20.8.2 Impact on Air Pollution

Air quality studies have been conducted to compare air pollution levels during the strictest stay-at-home lockdown periods of the pandemic with the pre- or post-lockdown periods. Many of these studies resulted in fewer emissions during the lockdowns. There has been a decrease in air pollution, specifically nitrogen dioxide and carbon dioxide levels during the pandemic (McMahon 2020). The UC Davis Road Ecology Center did an additional study on how much greenhouse gas emissions were reduced based on the stay-at-home orders for COVID-19 (Shilling 2020). The study found that there was a 61–89% reduction in daily miles traveled in the United States during the peak of the stay-at-home orders. California alone saw a 75% reduction in daily travel that reduced greenhouse gas emissions (Shilling 2020). The reduction in greenhouse gas emissions during this time period can aid California toward creating strategies to meet the goal of reducing greenhouse gas emissions by 80% in 2050 (Shilling 2020). A week before the March 2020 lockdown, carbon dioxide emissions were measured at 44 million metric tons (Shilling 2020). Then by the second week of April 2020, after the stay-at-home order was issued, carbon dioxide emissions were down to only 12 million metric tons (Shilling 2020). This was a 71% decrease in emissions based solely on vehicle traffic (Shilling 2020).

Another study in Italy utilizing remote sensing technologies showed a decline in air pollution, particularly nitrogen dioxide, during the lockdown for COVID-19. A Copernicus Sentinel-5P Tropomi satellite showed the imagery in Europe from January 1, 2020, until March 11, 2020, using a 10-day moving average. The study describes this satellite as currently being the most accurate instrument available to measure air pollution from space. The study was confident that the changes seen were from the lockdown restrictions and not likely from cloud cover or weather changes (Fig. 20.4) (European Space Agency; https://www.esa.int/Applications/Observing_the_Earth/Copernicus/Sentinel-5P/Coronavirus_lockdown_leading_to_drop_in_pollution_across_Europe).

Measurements from the European Space Agency's Sentinel-5P satellite showed that nitrogen dioxide levels in Asia and Europe were reduced as much as 40% in January and early February 2020 (Monks 2020). When the United Kingdom announced a nationwide lockdown on March 23, 2020, nitrogen dioxide levels fell as much as 60% compared to 2019 levels (Monks 2020). In the United States, many cities also saw a decrease in nitrogen dioxide. NASA reported a 30% reduction in New York compared to 2015 and 2019 levels (Monks 2020). Los Angeles was down 33%, followed by New York at 22% and Seattle at 19% (Gardiner 2020). A 40%

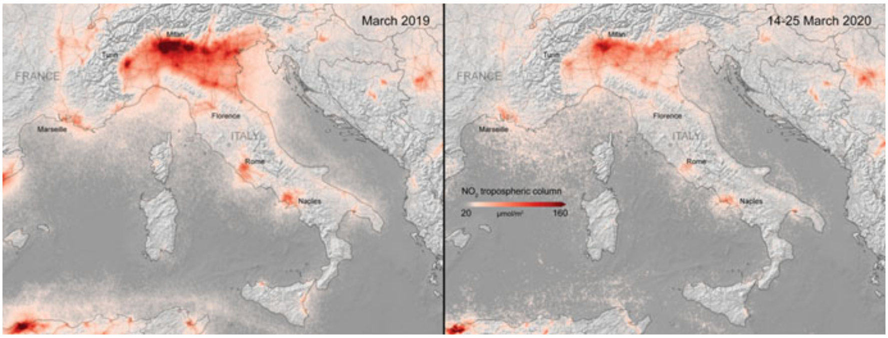

Fig. 20.4 Remotely sensed imagery from the Copernicus Sentinel-5P satellite showing the reduction in monthly average concentrations of nitrogen dioxide (NO) over Italy (**a**) from March 2019 (pre-lockdown) to (**b**) March 2020 (during the lockdown) (ESA 2020). (This figure was reproduced from http://www.esa.int/ESA_Multimedia/Images/2020/03/Nitrogen_dioxide_concentrations_over_Italy with permission)

drop in nitrogen dioxide levels is comparable to removing 192,000 cars from the road (Monks 2020). Areas that averaged even one microgram per cubic meter more PM2.5 particulates were shown by Harvard Universities T.H. Chan School of Public Health to have a 15% higher death rate from COVID-19 (Gardiner 2020). This is relevant to those on the US west coast who are regularly exposed to poor air quality from wildfire smoke pollution.

In Delhi, India, levels of PM2.5 particulates and nitrogen dioxide concentrations were 70% lower (Gardiner 2020). Parts of Europe, South Korea, and China saw reductions in nitrogen dioxide as well. However, the Ukraine, some African countries, and some other European countries saw an increase in these particulates (Gardiner 2020). In addition, China's improvement in air quality was temporary and has already returned to pre-pandemic lockdown levels (Gardiner 2020).

Air quality levels can be impacted by vehicle emissions. A 2017 study removed about 111,000 vehicle commuters from the Stockholm County, Sweden roads, and switched them to commute by bike (Johansson et al. 2020). This resulted in about an extra 449 years of life annually based on improved air quality from reducing vehicle emissions to commute by bike.

StreetLight data provides spatial datasets for different research studies relating to human movement, particularly to varying transportation methods (https://www.StreetLightdata.com/corona-bicycle-metrics/). One study used this data from StreetLight to determine if bike ridership has increased or decreased during the pandemic in the United States (Grogan and Hise 2020). The results were surprising: as bike ridership increased in the smaller metropolitan areas and ridership decreased in major metro areas that would generally have high ridership (Fig. 20.5). For example, some smaller cities not known for bike commuting, Ogden, UT, Lakeland, FL, Knoxville, TN, and other cities saw a huge increase. The results showed that the average trip distance was less than five miles, which is shorter than a typical exercise workout would be (Grogan and Hise 2020). In the small cities, it was found that

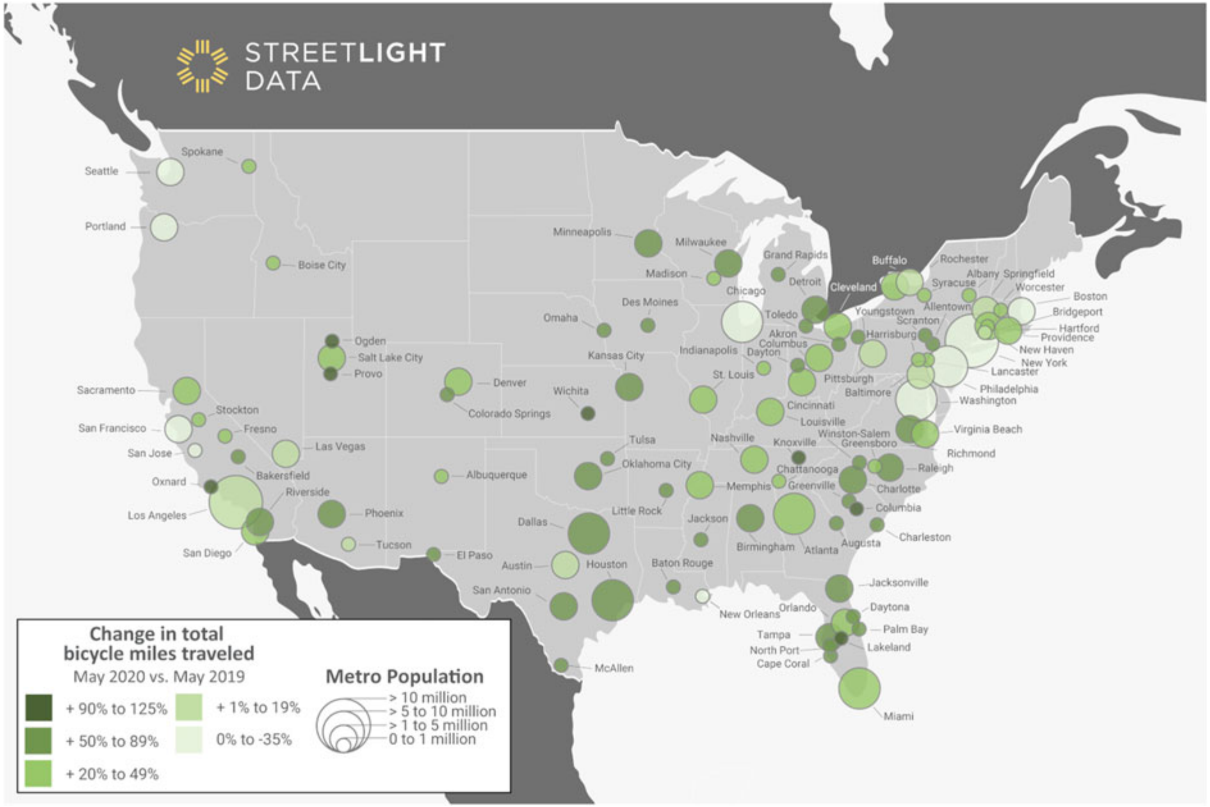

Fig. 20.5 Display of the difference in total bicycle miles traveled between May 2019 and May 2020 to demonstrate how miles traveled changed before COVID-19 lockdowns in 2019 and after lockdowns in 2020. Some of the smaller metro areas nearly doubled in miles traveled and some of the major metro areas declined in bike miles traveled. (This screenshot was reproduced https://www.streetlightdata.com/corona-bicycle-metrics/ with permission from StreetLight Data)

biking increased by the city center, indicating trips may have multiple purposes, such as for shopping to commute to work or other purposes. Whereas larger metropolitan cities, like Portland, OR, saw a decrease in bike trips. Bike trips decreased by 10%, a lot less than for vehicle motor traffic that fell by 35% (Grogan and Hise 2020). A more detailed analysis of bike ridership in Portland showed the biggest increase in ridership was by a mountain biking trailhead and ridership decreased by downtown Portland (Fig. 20.6) (Grogan and Hise 2020). A study in New York found a similar result where bike ridership through the shared bike riding system dropped by about 71%, much less than the subway ridership drop of about 90% (Teixeira and Lopes 2020). Evidently, the stay-at-home orders reduced both bike travel and vehicle travel in some areas.

20.8.3 Impact on Noise Pollution

WHO has said that environmental noise is one of the top environmental risk factors to health (WHO; https://www.euro.who.int/__data/assets/pdf_file/0008/383921/noise-guidelines-eng.pdf). WHO has set different standards to regulate environmental noises and recommend road traffic levels be kept below 53 decibels to reduce negative health impacts (World Health Organization; https://www.euro.who.int/__data/assets/pdf_file/0008/383921/noise-guidelines-eng.pdf?ua=1). In Dublin, Ireland, noise-monitoring stations recorded hourly average equivalent sound levels and maximum sound levels at 5-min intervals at 12 different locations during the pre-lockdown time period of January 1 to March 24, 2020, and during the lockdown from March 25 to May 11, 2020 (Basu et al. 2021). All 12 noise-monitoring stations showed a reduction in sound levels, which may be attributable to a reduction in road and air traffic during the lockdown. However, other environmental factors also could have reduced sound levels (Fig. 20.7) (Basu et al. 2021). Prior to the pandemic (January 1, 2020, to March 24, 2020), all monitoring stations recorded sound levels greater than 55 dB for more than 60% of the recording time (Basu et al. 2021). During the lockdown the percentage of time the sound levels exceeded 55 dB was greatly reduced (Basu et al. 2021) supporting less noisy transportation methods, like bikes in the future to reduce noise levels and associated health issues.

Another sound-level study is currently underway, which allows general citizens to participate by placing recorders on their residence in urban and suburban areas (Challéat et al. 2020). The data will be available to the public (https://osf.io/h285u/) through Open Science Foundation (OSF) (Challéat et al. 2020). The project has recorded sound levels during COVID-19 lockdowns and then during the resumption of normal activities.

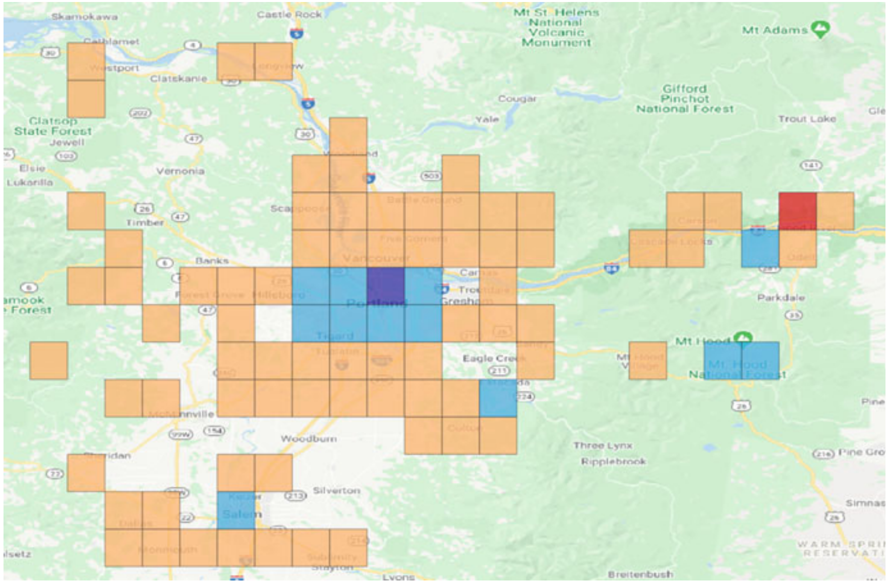

Fig. 20.6 This figure shows changes in bike ridership in the Portland, Oregon, area between May 2019, before the pandemic lockdowns, and in May 2020, during the lockdowns. The red square shows the biggest increase in ridership, where there was a mountain biking trailhead, the dark blue squares show where bike ridership decreased the most in downtown Portland, and the orange squares show where bike ridership increased. (This screenshot was reproduced from https://www.streetlightdata.com/corona-bicycle-metrics/ with permission from StreetLight Data)

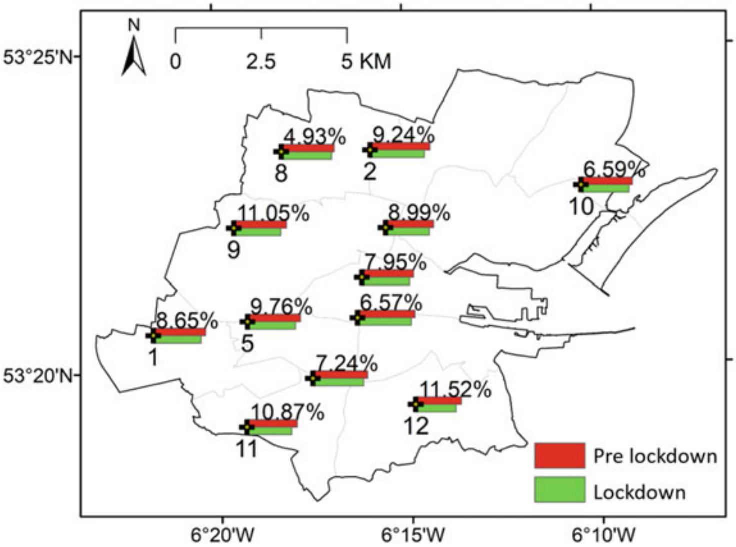

Fig. 20.7 This figure shows mean of hourly average equivalent sound level at monitoring stations in Dublin, Ireland, before the COVID-19 lockdown (January 1, 2020, to March 24, 2020) and during the lockdown (March 25, 2020, to May 11, 2020). (This figure was reproduced from Basu et al. (2021) with permission)

20.8.4 Impact on Water Turbidity

Satellite imagery can measure turbidity, chlorophyll, and colored dissolved organic material (CDOM) concentration in water bodies (Lim and Choi 2015). A recent remote sensing study used satellite data from the Sentinel-2 satellite, during the COVID-19 lockdown, to measure changes in turbidity along some of the most crowded stretches of the Ganga River in India (Garg et al. 2020). The Ganga River originates in the Himalayas and has connections to four surrounding countries, covering nearly one-third of the total geographical area in India. In many areas, the river is not considered clean enough for public drinking and it was found that the turbidity improved for water clarity at multiple locations along the river during the lockdown (Garg et al. 2020). In one region of the river, there was no rainfall to interfere with measurements between approximately mid-March to mid-April 2020, so the reduction in reflectance was likely due to a reduction in pilgrimage along the river (Garg et al. 2020).

20.9 Wastewater-Based Epidemiology

Due to the safety and financial concerns of the general public regularly going to a testing center for COVID-19, alternative or complementary testing methods and indicators of outbreak levels in a community have been explored. Wastewater

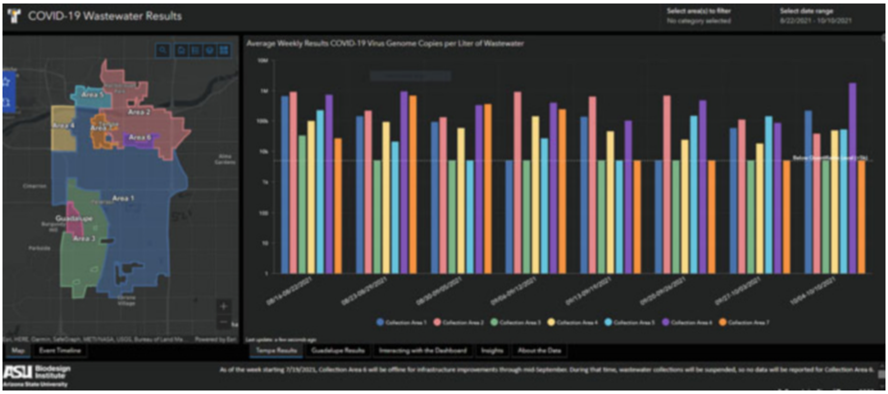

Fig. 20.8 Wastewater-based monitoring system under development in Tempe, AZ, designed to become, an early warning system to alert citizens when COVID-19 case numbers reach dangerous levels in their region. The leftmost panel of the figure shows the Tempe geographical regions, and the rightmost panel of the figure shows the average weekly results for COVID-19 genome copies per liter of wastewater from August 22, 2021, until October 10, 2021. (This screenshot was taken from https://tempegov.maps.arcgis.com/apps/dashboards/45f1871d65824746a46aa25ea5955a5f on October 15, 2021, with permission from the City of Tempe)

provides a unique venue for mass anonymous testing of COVID-19 for community health. Wastewater is the excess water from households or buildings, which includes toilets, showers, and sinks as well as from non-household sources like rain and industrial water that may contain human waste (CDC; https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html). Taking samples at defined points and wastewater treatment plants is inexpensive and uses far fewer medical supplies than the current standard mass testing of individuals (Campbell and Wachal 2020; Hart and Halden 2020). The science behind this procedure is that COVID-19 sheds identifiable biomarkers in human waste that is detectable with regular wastewater testing (Hart and Halden 2020, CDC; https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html). This helps pinpoint hotspots of infection since each test has a known mapped area that flows to that point. Using this method to identify hotspots allows for more efficient use of resources for individual testing and treatment in those areas.

Early in the pandemic, the CDC reported that the number of COVID-19-infected individuals in a region could not be determined based on sewage testing (Landers 2020). However, with new research, scientists determined that SARS-CoV-2 can be detected in the feces of infected individuals several days to a week or more before symptoms develop and can even be detected in asymptomatic individuals (Landers 2020). Now the CDC has a National Wastewater Surveillance System (NWSS) tool to which different states, governments, and tribal departments can submit their wastewater testing data to be input into a national database to help monitor the spread of COVID-19 (CDC; https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html). Wastewater monitoring can complement existing COVID-19 direct testing of patients.

The City of Tempe, Arizona, now tests wastewater to detect COVID-19 biomarkers for different portions of the city (Lindemann 2020). Tempe has developed a publicly available dashboard that shows level of COVID-19 RNA in wastewater, with data collected several times per week (Fig. 20.8) (https://covid19.tempe.gov/). Hart and Halden (2020) are contributing to the knowledge behind COVID-19 wastewater testing with their research. The current working theory is that the COVID-19 biomarker can be detected from one infected individual in the wastewater received from a sample area with up to 2,000,000 people, depending on temperature and travel distance of the wastewater. For their research, Hart and Halden (2020) used the estimated COVID-19 biomarker load to wastewater (ratio) determined by Zhang et al. (2021) and Wölfel et al. (2020), as an input to a decay function for the COVID-19 biomarker. This decay function is used to determine how much of the biomarker remains over a given travel distance with a half-life dependent on the wastewater temperature. The wastewater layout system, structures, and population density were loaded into a GIS and combined with a previously built model that simulates a typical 72-h weekday load. This was used to create a new model to calculate in-sewer travel time, volumetric flow rate, and velocity and then was fed into the ESRI ArcGIS (GIS software) program. This information is used for each segment of pipe for the network analyst tool to analyze the accumulation and time of wastewater from households to the wastewater discharge point. The network analyst tool is used typically in complicated transportation routing problems, but the pipe network behaves similarly to transportation networks and can be treated similarly. Hart and Halden (2020) found that this would require in the worst conditions, at least 0.88% of the study population to be infected to produce successful detection of COVID-19 in the wastewater. With more optimal conditions of cooler temperature and shorter in-sewer travel time, only 0.00005% of the population would need to be infected for successful detection.

Wastewater treatment plants have definable service areas, using a discrete data layer, in the GIS database, so each plant is able to have at least a weekly test done to determine the load of COVID-19 RNA biomarkers present in the wastewater for each service area. While research for determining COVID-19 biomarkers is still in an experimental phase, the current results are promising and Tempe already has the infrastructure to implement this research in a real-world setting (Hart and Halden 2020; Lindemann 2020). For Tempe, there are six wastewater treatment plants each servicing different parts of the city, with the largest area servicing more than 183,000 households. Each service area could be further subdivided for testing to produce more detailed results and concentrations in the city (Lindemann 2020). This method provides a cost-effective, low waste, mass testing result to indicate various communities' health in the city.

Currently, other states in the United States and other countries, including the Netherlands and Australia, have also looked at wastewater detection of the virus in their attempts to reduce the impact of the pandemic. For the state of Massachusetts, the Massachusetts Water Resources Authority website shows Biobot Analytics', one of the first companies in the world to materialize data from sewage, analysis of two million customers in the Boston area (https://www.mwra.com/biobot/biobotdata.htm Hart and Halden 2020). Their measurements show a spike of the viral RNA in wastewater during April 2020, which dropped to half that level by November 2020, with samples taken three times a week (http://www.mwra.com/biobot/biobotdata.htm https://www.mwra.com/biobot/biobotdata.htm). The hope is that an early warning system can be derived to monitor COVID-19 case surges and provide a way to anonymously test for outbreaks. This method could be utilized in every city to help monitor viruses before they reach outbreak levels.

Water utilities continue to function under working environments affected by COVID-19, without disrupting customer needs for clean water and wastewater removal. GIS systems help these utilities manage their human resources to ensure workers stay safe while working remotely and to understand how COVID-19 is impacting their daily duties (Campbell and Wachal 2020). Employee locations and duties can be tracked, up-to-date communications can be provided to customers, and GIS can help identify future problems in service areas (Campbell and Wachal 2020). GIS dashboards, like those provided by ESRI, aid in tracking conditions in business facilities, workforce capacity, and communication needs. Personal and online COVID-19 data is used to visualize where the highest risk of exposure is in a utilities' service area, allowing for efficient resource allocation (Campbell and Wachal 2020).

20.10 Other GIS Applications

Several other GIS applications have been employed during the pandemic. Due to decreased transportation demands during the pandemic, the oil industry has suffered with very low prices for oil. Remote sensing technologies have been used to help determine the spatial location of crude oil reserves, particularly in Canada, to mitigate this effect and increase profits. Remote sensing can also be employed to help ensure that gas emissions from related extraction and processing follow environmental parameters (Gogeomatics; https://gogeomatics.ca/remote-sensing-the-potential-impacts-of-covid-19-on-oil-gas-sector/). Remote sensing has been used to detect underground pipe leaks by comparing daily vegetation health, around the pipes from collected red, blue, green, and near-infrared images (Wiseman 2019).

Another study used GIS and remote sensing to create COVID-19 risk indices based on many factors, such as hotspots, population density, access to clean water, and associated land use/land cover, which were related to COVID-19 levels in India (Kanga et al. 2020). Those that do not have access to clean water in their home have to leave home more often to get it, putting them at greater risk of exposure. ESRI's ArcMap (GIS software) was used to analyze the water well locations with a kriging spatial interpolation technique (Kanga et al. 2020). All of the layers were integrated with a GIS-based weighted overlay analysis. The produced maps showed where the risk was greater for the pandemic.

In the United States, one study determined which of 35 variables influenced COVID-19 cases at the county level across the United States (Mollalo et al. 2020). The variables that seemed to have a high influence on disease incidence were income inequality, median household income, the percentage of nurse practitioners, and the percentage of the black female population (to the total female population) at the county level (Mollalo et al. 2020). Many of the other environmental, socioeconomic, topographic, and demographic variables did not have as strong an influence on the number of cases at the county level (Mollalo et al. 2020).

20.11 Survey of Technologies Used to Monitor and Manage the Pandemic

20.11.1 Contact Tracing Apps

Due to many previous space–time limitations of epidemiological studies, such as limits with distance accuracy of census data, collecting smartphone movement data is currently being considered as a more accurate alternative to monitoring human movement patterns then previously used methods for health risk assessments and contact tracing (Deville et al. 2014). Digital contact tracing, such as through mobile technologies, is less labor intensive than for health departments to manually call potentially infected individuals (Smith and Mennis 2020). Using the data from mobile phones and other mobile technologies is very important when there is limited information available from a newly discovered virus (Ienca and Vayena 2020). There are more than 100,000 smartphone-based health-related apps available (Faezipour and Abuzneid 2020). In 2019, a new mobile program (mHealth) was used to test how easy it was to use mobile phone data to monitor human movements and assess the real-time health risk for infections during travel (Lai et al. 2019).

With the proliferation of smartphone use around the world, the pandemic has resulted in the production of many contact tracing apps around the world for use by the general public. These apps warn individuals that they may have encountered an infected individual. It is warned that these apps may not be as effective in a highly populated region where the virus is widespread compared to a less populous area at an early stage of the outbreak, where quarantining infected individuals is more effective (Servick 2020). Contact tracing is being widely utilized around the world, especially in developed countries, as of November 14, 2020, to help monitor the pandemic (Fig. 20.9).

For example, China has developed a "close contact detector" app that uses big data from the movement of people, such as from transportation data and COVID-19 disease records, to see if someone was in close contact with an infected individual in the last 2 weeks (Kamel Boulos and Geraghty 2020). Data privacy issues were not as much of a concern as they have been in other countries as China considers it a benefit for the greater good (Kamel Boulos and Geraghty 2020).

India, on the other hand, has developed considerably more contact tracing apps (Mitter 2020). The Test Yourself app records information when patients book

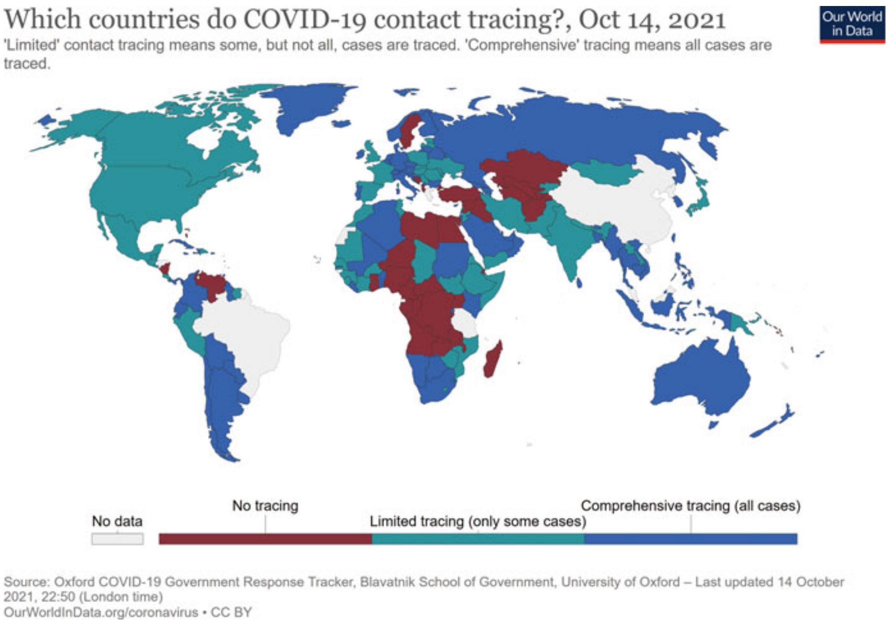

Fig. 20.9 This map shows worldwide usage of contact tracing to warn individuals about encounters with a COVID-19-infected person in their daily activities, which may have exposed them to the virus. Dark blue-colored countries on the map have comprehensive tracing, where tracing is performed for all cases, aqua-colored countries have limited tracing, where tracing is performed for only some cases, and red-colored countries do not perform tracing for any cases. (The figure was reproduced from Our World with Data (https://ourworldindata.org/grapher/covid-contact-tracing?time=2021-10-14 on October 14, 2021, with permission)

medical appointments; users can answer questions about symptoms of the virus they are experiencing and it gives related advice (Mitter 2020). It had at least 50,000 downloads and is available in several languages. The COVA Punjab app helps monitor citizens that are under quarantine and alerts authorities if the person strays over 100 m from their home location (Mitter 2020). It has had over half a million downloads and at one point ranked in the Top 10 "health and fitness" apps on the Google Play Store (Mitter 2020). The Aarogya Setu app reached more than 50 million downloads as a contact tracing app, available in 11 languages, that alerts people when they are within 6 ft. of a known COVID-19 patient (Mitter 2020).

In Germany, the Corona-Warn-App was commissioned by the Robert Koch Institute who partnered with Apple and Google to create it (https://www.coronawarn.app/en/). It uses a mobile device that broadcasts a rolling proximity identifier, or encrypted random codes, and looks for identifiers on other phones using BLE to determine when two people encounter each other, and measure for how long they encountered each other and what their proximity was (Die Bundesregierung; https://www.bundesregierung.de/breg-de/themen/corona-warn-app/corona-warn-app-faq-1758392). Features of this app that protect privacy include (1) identifiers are not stored long term and are created from temporary keys that change every 24 h; (2) the names of the user and locations are kept private, especially after 14 days when the random codes are deleted from the smartphone; and (3) if someone becomes infected, they can choose if they want to share their random codes with others, warning them of the possibility of infection (Die Bundesregierung; https://www.bundesregierung.de/breg-de/themen/corona-warn-app/corona-warn-app-faq-1758392).

Initially, the United Kingdom's own contact tracing app was considered deficient and so an attempt was made to adapt Apple's system for use. Apple and Google combined forces to create APIs for mobile devices to use for contact tracing (Mitter 2020; Lazarević 2020). Unfortunately, the United Kingdom said the Apple system could not accurately measure distance well enough for contact tracing (Doffman 2020).

One of the main problems with these contact tracing apps is getting enough people to use them as many countries have made the contact tracing app voluntary. In the United States, only about 12% of the population had contact tracing apps installed on their phone, so the chance that two people had the app installed on their phones that passed each other was only about 1.44% (Newton 2020). It is thought that at least 60% of the population needs to use the app for it to be effective (Lazarević 2020).

In Europe, several contact tracing apps, such as Germany's Corona-Warn-App, Ireland's COVID tracker, and Italy's Immuni, have been linked together to increase usage (Lazarević 2020, 13WOWK; https://www.wowktv.com/news/u-s-world/eu-to-link-national-covid-19-tracing-apps-together/). As of October 2020, these three apps appeared to be the most used apps in the European Union and have been downloaded by about 30 million people, or about two-thirds of all the contact tracing apps that have been downloaded in Europe; unlike in China, the identity of the user is not stored after the contact tracing is completed (13WOWK; https://www.wowktv.com/news/u-s-world/eu-to-link-national-covid-19-tracing-apps-together/, Kamel Boulos and Geraghty 2020).

Privacy concerns have greatly limited the use and effectiveness of contact tracing apps. Privacy seems to be the biggest concern in the EU and the United States as laws have been developed and employed, which require smartphone users to give their consent to use their location for contact tracing (Johnson 2020; Servick 2020). One study found 30 of 50 different apps required the user to give permission for the apps to access various information such as contacts, photos, location data, camera, and more (Sharma and Bashir 2020). Only 16 of the 50 apps stated the user's data would be kept private, anonymous, or encrypted (Sharma and Bashir 2020). This may be partly why contact tracing apps have not been as widespread in the United States. However, states have started to slowly develop their own apps at a slower rate than in many other countries.

With privacy concerns for contact tracing apps, new apps are using non-location GPS-based tracing methods like Bluetooth or WiFi (Lazarević 2020). Many of the contact tracing apps used BLE technology as an alternative to location-based tracking by GPS. Since BLE has limitations often resulting in false positives such as determining when two smartphones are within 2–6 m of each other, especially indoors, and BLE does not effectively penetrate walls or furniture, other sensor technology contact tracing methods have been suggested (Nguyen et al. 2020). A typical smartphone may have 14 or more common sensors that can be used to improve the ability to detect nearby phones more accurately. Some of the sensors that can be used are the barometer, BLE, magnetometer, microphone, and WiFi signal (Nguyen et al. 2020). The microphone was considered a "fine-grained" sensor for appearance sensing and distance measuring, unlike BLE that was not used for distance measuring. When furniture or other barriers reduced the accuracy of BLE, the microphone and WiFi could both be used to increase accuracy from 25% with BLE only systems to about 65% accuracy (Nguyen et al. 2020). Adding these extra sensors is especially important to reduce distance estimate errors indoors.

20.11.2 Apps That Record the Sounds of COVID-19

Medical apps for use on smartphones have become incredibly popular in recent years and can have a big impact on health care (Gupta 2013; Carrion et al. 2016). One sound-related study records daily respiratory sounds from healthy and unhealthy volunteers, on an app installed on their smartphone or on the website (University of Cambridge; https://www.covid-19-sounds.org/en/app/). The University of Cambridge is trying to determine, based on recordings of healthy and unhealthy volunteers, how their coughs and breathing compare to those with COVID-19 and varying other conditions such as asthma (Brown et al. 2020). This will hopefully lead to an alternative or complementary method to direct testing of patients (Brown et al. 2020). By May 22, 2020, their dataset already consisted of several thousand samples and 235 had tested positive for COVID-19. Preliminary results show even a simple binary machine learning classifier can distinguish between healthy subjects and those with COVID-19 (Brown et al. 2020). Results also have shown distinguishing sounds between healthy subjects, those with a cough, those with asthma, and those who have COVID-19 (Brown et al. 2020). Unlike some contact tracing apps, this study will only collect data when you actively fill out your daily survey, which requests your location, your testing status, if you are in the hospital, your symptoms, and multiple recordings of your breathing, coughing, and talking. As of August 2020, they had not yet derived the best system to create a standalone screening tool (Brown et al. 2020).

20.11.3 Drones

A big concern during the pandemic has been the exposure of healthcare professionals or other employees to infected individuals. Drones are versatile tools that can fill multiple roles while keeping users safe from COVID-19 transmission. They can gather remotely sensed images, deliver goods, provide crime surveillance, and even be used in emergency response situations to deliver aid, assess damages, and locate victims (CBInsights; https://www.cbinsights.com/research/drone-impact-society-uav/). Drone technology has been widely employed in this current pandemic as it provides a safe method of data collection and COVID-19 control (Kumar et al. 2020; Preethika et al. 2020). China and India are using drones to safely sanitize and disinfect public areas in a quarter of the time it takes a human to manually spray an area (Kamel Boulos and Geraghty 2020; Kumar et al. 2020; Preethika et al. 2020).

Combining location technologies with drones can allow numerous advantageous applications of drones to help manage the pandemic. By incorporating GIS with drone technology, users can determine where supplies or disinfecting is needed the most (Kamel Boulos and Geraghty 2020). Drones can spray disinfectant from a height of up to 450 ft. without the risk of COVID-19 transmission to employees (Preethika et al. 2020). Drones are also currently being used as safe and fast delivery systems of COVID-19 medical kits, supplies, and personal protective equipment to rural locations in the United States, the United Kingdom, and even China (Kamel Boulos and Geraghty 2020; Cozzens 2020; Kumar et al. 2020). More recently, drones are being used to deliver test kits to people's homes. In a more traditional role, drones can collect aerial images and data such as thermal imaging in temperature detection. This sort of surveillance is also being tested in monitoring respiratory health, and even heart rates of people in indoor and outdoor public areas and patient care facilities (Kumar et al. 2020).

While utilizing drones effectively can decrease COVID-19 transmission, privacy concerns similar to those with smartphone monitoring can be a barrier with such intimate drone surveillance. Proper regulation of where and when drone surveillance should be used is critical for further implementation of this technology in the public sector.

20.11.4 Robots

The use of robots can help reduce exposure to COVID-19 if it limits the social interaction between humans. The pandemic has led to a wide variety of applications for robots to help decrease virus spread. Brain Navi, a Taiwanese firm, created a robot in just 8 weeks that can test potentially infected humans for COVID-19 without putting healthcare professionals at risk (Fig. 20.10) (Brain Navi; https://brainnavi.com/product/nasalswabrobot/). It is based on Brain Navi's NaoTrac neurosurgical navigation robot that has been used in many medical centers in Taiwan (Medgadget; https://www.medgadget.com/2020/08/robotic-clinicians-for-taking-nasal-swabs-during-covid-pandemic.html). The robot uses facial recognition to accurately pinpoint the location of the nostrils for a sample and has a user-friendly interface design structure that only takes 2–5 min to collect a sample (Brain Navi; https://brainnavi.com/product/nasalswabrobot/). The robot spends at least 10 s to collect the sample and patients report that the specimen collected by the robot is more comfortable than if a human collects it. Finally, the collected specimen is placed in a sanitary closed container that is safe for handling (Medgadget; https://

Fig. 20.10 Brain Navi, a Taiwanese firm, created a robot in just 8 weeks that collects a nasal swab sample, from a potentially infected COVID-19 patient, without putting the healthcare worker at risk, in just 2–5 min and then places the sample in a container for safe handling. (This photo was provided by Zoe Lee from Brain Navi with permission)

www.medgadget.com/2020/08/robotic-clinicians-for-taking-nasal-swabs-during-covid-pandemic.html).

Another application of robots is in China where the first "robot-run" restaurant has debuted (Adams 2020). It has a huge menu and can serve up to 600 customers at a time. The robots even cook the food with some menu options ready in as little as 20 s. Food safety standards will be maintained and it is likely, virus transmission rates will be lower than if humans cooked the food.

Another biotechnology company, Koniku, has designed robots that can help "sniff out" COVID-19 infections. They hope to set protein-based sensors onto a silicon chip to smell pathogens and transmit signals to a microcontroller. The goal is to detect the virus quicker than traditional COVID-19 testing methods (Brown 2020). The device, which is smaller than a Frisbee and looks like a UFO, will light up when it detects what it is designed to sniff out (Brown 2020). Eventually, every American may have a robot in their house to detect COVID-19 or other viruses. The company hopes it will be authorized by the U.S. Food and Drug Administration in the first quarter of 2021 (Brown 2020).

20.12 Future Ways to Improve the Analyses

GIS spatial analyses can only effectively guide healthcare professionals with management strategies for the pandemic if they are accurate. It has been considered that "spatial analysis within GIS is the process of building 'models of models'—whereby the outcome of a 'higher level' spatial analysis is dependent upon its data inputs" (Goodchild and Longley 1999). Since many of the pandemic-related maps were constructed quickly, without spending time to validate them, future further analyses could be done on these maps to improve their accuracy. Validation or quality control measures need to be implemented and applied consistently when the general public constructs maps. Map makers will need to double-check their accuracy as maps based on previously made maps can lead to further error (Monmonier 2018).

It has also been a challenge to quickly provide big spatial data to the health industry during the pandemic (Zhou et al. 2020). Creating and expanding upon publicly accessible data repositories like GitHub might facilitate data sharing in near-real-time for future pandemics. Improving access to sub-county data will help with the production of more finite scale analyses, such as including socioeconomic factors (Mollalo et al. 2020). As Internet speeds increase, greater abilities to access big data, will reduce previous epidemiological restrictions of space–time analysis. 5G mobile broadband network technology will be available soon on many mobile phones, which can enable development of more powerful apps in connection with the Cloud (Lin et al. 2016). With the utilization of 5G wireless technology, two people at different ends of the world can communicate at speeds, likely 100 times faster than the typical data rate. Phone memory is expected to increase up to 120 GB, and huge data files can be transferred at a rate of GB's per second, improving our access to big data in the Cloud. Improvements can still be made in using and accessing geospatial big data for GIS developers to create software applications to visualize pandemics, particularly as source restrictions in commercial enterprises may restrict the use of the data for social management, slowing down its quick online visualization (Franch-Pardo et al. 2020; Zhou et al. 2020). Also, greater participation from other countries, besides the United States and China, who created most of the geospatial analyses according to one survey of 63 studies, will lead to greater global surveillance accuracy for future pandemics (Franch-Pardo et al. 2020).

Faster Internet speeds should allow further utilization of mobile technologies and apps, therefore decreasing delays in transferring and receiving disease-related data. Mobile technologies such as contact tracing apps should be utilized more effectively as many developed countries increase usage of mobile technologies. Only 20% of the studies in one review of 63 studies by Franch-Pardo et al. (2020) used information gathered from smartphones. Privacy concerns are important to consider and data protection is important to prevent data breaches and improve the trust of the public to use contact tracing apps or other mobile technologies (Ienca and Vayena 2020). Contact tracing to provide near-real-time statistics and reduction of disease transmission can be greater utilized and privacy concerns addressed.

Some of the smartphone-designed health apps can be improved to lessen privacy concerns and improve their functionality. Even contact tracing apps using BLE have many false positives when measuring the distance between two users (Nguyen et al. 2020). Distance measurement estimates for contact tracing could be improved in the future by using additional smartphone sensors such as air pressure and the magnetic field, along with BLE, microphone, WiFi, or other sensors (Nguyen et al. 2020). Also improving contact tracing technologies to detect when two people are sharing their air space, rather than two people that are close but in separate rooms, is important for future applications (Nguyen et al. 2020).

In addition, the number and usage of self-risk diagnosis and monitoring apps should be increased to reduce the user's exposure of going to a testing center. Many individuals may be afraid to go to a testing center for fear of getting the virus or for reasons of how much the test may cost them. With the creation and further development of many of the apps and technologies that have been discussed, we will have safer methods available to test individuals in their homes so they do not have to expose themselves or others to infection. Other apps, like the Sickweather app (Sickweather; https://www.sickweather.com/), create local maps based on self-reported symptoms of different illnesses, allergies, and more, which can be further augmented to include more data for current viruses like COVID-19. This and similar apps should be further developed to work in combination with public surveillance dashboards or websites such as the Johns Hopkins website to show more localized data. The general public will need to realize there could be a high degree of error for self-reporting apps. The apps can nevertheless serve as a tool to help individuals select stores or towns that have fewer virus cases to conduct their shopping, exercising, or vacationing. More fine-scale spatial GIS applications are needed. Using mobile technologies like cell phones to gather information directly from the user may reduce reporting lags between governmental agencies, health departments, and other reporters that may be delayed for numerous reasons.

Near-real-time case dashboards may have some inaccuracies as they may only include confirmed cases and not self-reported cases (Desjardins et al. 2020). In addition, these dashboards rely on the reporting techniques of different countries/counties/states, which can vary in time and space, and some have even changed their guidelines for how they report cases and deaths. Further, different data sources should all try to update their data consistently at the same time interval. In the future, emerging clusters of COVID-19 cases can be detected by using space–time scan statistics by public health departments to implement on their near-real-time dashboards to achieve improvements in timeliness (Desjardins et al. 2020). To improve the accuracy of near-real-time mapping of cases, it has been suggested that rapid diagnostic antibody tests could be performed at home with the results quickly given uploaded from individual smartphones. Then image processing and machine-learning methods could link to geospatial information to speed up results (Budd et al. 2020). Combining results from different types of tests, incorporating other testing strategies such as sewage monitoring, and adding different socioeconomic risk factors, such as income inequality and median household income, and pre-existing conditions could lead to better disease forecasting (Franch-Pardo et al. 2020; Kanga et al. 2020; Mollalo et al. 2020; Smith and Mennis 2020).

There is room for improvement, as technology advances, to reduce exposure to viruses and create a safer work environment. Collaborative robots, or cobots, could be used in the workplace to help socially distance employees by acting as an intermediary and removing the need for close contact work. They can work alongside humans to help with isolation and provide a big step forward in manufacturing (Bonomi 2020). Greater utilization of drones can also serve to help monitor and manage pandemics as their purpose is only limited by our imagination.

20.13 Conclusions

The explosive production of GIS analyses, timely production of near-real-time dashboards for disease surveillance, and predictive modeling for the COVID-19 pandemic have been incredible. These near-real-time dashboards and other geospatial websites have become popular around the world as healthcare professionals use them to make disease management decisions and citizens use them to monitor the pandemic. While there were previous limitations in GIS software for major epidemiological research, especially in near-real-time surveillance and data storage, these limitations no longer hinder the widespread use of GIS in epidemiology (Musa et al. 2013). Many of these limitations have been resolved by 2020 as geospatial analyses for COVID-19 cases have improved to near-real-time. The accuracy of these GIS analyses can still be improved upon and the accessibility of big spatial data required for these analyses can be expanded with increasing Internet speeds (Ahasan et al. 2020; Field 2020; Franch-Pardo et al. 2020; Zhou et al. 2020). It has been alleged that, during conflict, data and analytics are critical to understanding the changing environment and capabilities often advance quickly (CBS Interactive Inc.; https://static.cbsileads.com/direct/whitepapers/TR_-_Big_data's_role_in_COVID-19_r1.pdf). The constant development of geospatial tools is essential to making informed disease management decisions and to inform the general public about the current status of the pandemic. Their derivation and utilization will be expected now and in the future as COVID-19 has set a new standard for their use.

The effects of the COVID-19 lockdowns, which restricted movement patterns of humans around the globe, have led to a diverse array of environmental geospatial analyses. The effects of the pandemic lockdowns for the most part reduced vehicle collisions with wildlife, reduced noise pollution, reduced air pollution, and improved the Ganga River clarity. The positive environmental impacts of the lockdowns as human activities were restricted may aid decision makers in making informed decisions to decrease our negative influences on the environment.

Other GIS applications and technologies have had a strong impact on managing the pandemic as well. Contact tracing apps and other health-based apps have had some positive impacts on the pandemic and can be greatly improved upon in the future. Privacy of user information is a big concern that has limited the popularity of many of these apps. Drones and robots have quickly been used to combat this global health crisis and decrease everyday exposure to the virus (Musa et al. 2013). Local agencies have adapted quickly to create personalized COVID-19 monitoring procedures and information dissemination methods to work best for their communities; even utilizing pre-built frameworks such as the City of Tempe, in Arizona, that is adapting wastewater monitoring to detect this virus. Where GIS software and spatiotemporal restrictions were formerly thought to be bottlenecks for the field of epidemiology in 2013, new software components such as fog computing and faster Internet speeds have diminished these limitations with near-real-time case monitoring (Musa et al. 2013). Incorporating multiple monitoring strategies will help to create the most accurate disease surveillance visualizations that are possible. As technology continues to advance, the future of geospatial epidemiological analyses will likewise advance.

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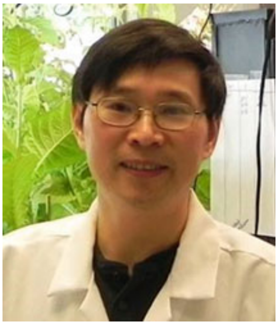

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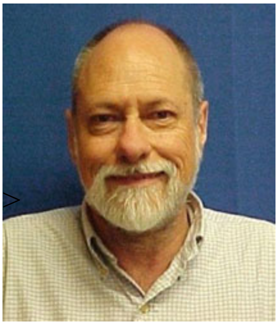

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Part II

Emerging Technologies in Environmental Biotechnology

Check for updates

Emerging Technologies in Environmental Biotechnology

21

Moupriya Nag, Dibyajit Lahiri, Sougata Ghosh, Sayantani Garai, Dipro Mukherjee, and Rina Rani Ray

Abstract

Biotechnology harnesses the most modern cellular and biomolecular approaches to develop technologies and products that help in the sustained development of the environment. The two main objectives of such approaches are total cleaning up of environmental contaminants, precisely the xenobiotic compounds, and restoration of biodiversity. The removal of toxicity created by natural or anthropogenic actions can be accomplished by various biotechnological approaches. These include a wide spectrum of issues like removal of pollutants by bioremediation, denaturation of toxic compounds by biodegradation, and controlling pollution using a bioreactor containing living material to capture and degrade contaminants by biofiltration with subsequent wastewater treatment. Utilization of biodegradable wastes for the production of biofuel and bioenergy is an important aspect. Exploitation of renewable resources like lignocellulosic wastes and agricultural residues for the production of different value-added material is a significant contribution of biotechnological progress with economic interest. These are usually fortified by microorganisms, and at various stages the efficacies of these microbes can be manipulated with the help of genetic engineering. The development of various biosensors to monitor the level of pollution is of immense

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importance. Management of medical wastes and e-wastes, posing a severe challenge for the environment, is attempted to be handled by various biotechnological processes. Today biotechnological approaches blended with artificial intelligence and machine learning are helping to develop cutting-edge technology for cleaning up of contamination and restoration of a pollution-free ecosystem.

Keywords

Biotechnology · Recent trends · Environmental protection · Recent technologies

21.1 Introduction

The regular unfavorable alterations of the environment due to various natural and anthropogenic activities become a serious threat, which attracts the attention of biotechnologists to find out some sustainable way for minimizing the adverse effects. Hence, the domain of environmental biotechnology (EB) plays a pivotal role in developing various strategies to combat the consequences of environmental hazards leading to pollution and loss of biological diversity. Hence, the concept of environmental biotechnology encompasses the science of wastewater treatment, off-gas purification, soil remediation, solid waste disposal techniques involving composting, landfills and bioinorganic recycling, surface, and groundwater cleaning. It is a concept that involves the implementation of multidisciplinary integration of sciences and engineering involving biological systems like microbial cells and plants for sustainable management and restoration of the environment. The concept of EB helps in utilizing and widening the concept of advanced technologies for efficient management of various wastes and toxic materials that are liberated to the environment (Meadows et al. 1973). Environmental pollution is the accretion of toxic substances in the air, water, and land that create contaminations at various levels and makes the affected sites unfit to support life (Manisalidis et al. 2020). In order to have a sustained development in the environment, the toxic wastes and probable pollutants need to be driven out of the system. Hence, the EB attempts to develop the mechanism of satisfying the need of the present without compromising the needs of the future generations allowing considerable economic growth, social well-being, and care for the environment (Anwar et al. 2019). EB brings forth the skills of researchers, industrial participants, and engineers to develop innovative technologies in the field of environmental, industrial, agricultural, veterinary, and medical sciences. Although the early stages EB used the major concept of chemical engineering within a short span of time, this was integrated with the concept of biotechnology to develop the sciences like environmental engineering, environmental microbiology, etc. (Hashim and Uijang 2004), that provide an effective alternate strategy for protecting the environment.

The ever-increasing human population has resulted in the misuse of environmental resources and improper disposal of wastes. The rapid growth in the human population would result in the depletion of the availability of freshwater all over the world (Chakraborty et al. 2013). Another threat is the dumping of wastes liberated from the industries causes the contamination of the freshwater. In addition, the increase in the number of industries due to modernization and globalization has led to an incredible increase in the liberation of untreated sewage, plastic wastes, and even nuclear waste in the environment. Conventional disposal methods involve sanitary landfills, open dumping of wastes, incineration, and decomposition of various toxic contaminants by the application of chemical agents that are found to be uneconomical and possess an adverse effect on the environment such as soil and water.

Here comes the inevitability of biotechnological applications that can protect the environment with the help of biological systems or a part of it. A number of new strategies are being adopted, and new opportunities are explored with the use of sophisticated tools and techniques. The approaches involving genetics and molecular biology for upgrading the new technologies are applied with a special reference on societal benefit and economic viability.

The current chapter deals with various biotechnological approaches to remediate the already contaminated soil and water and recent computational techniques to upgrade them for adding precision.

21.1.1 Sources of Environmental Contamination

Xenobiotics are the groups of abnormal chemical compounds that are not found naturally but are present in high concentrations (Fetzner 2002). These abnormal chemical compounds are produced as a function of toxicity and persist within the environment for a longer period of time. Industrial effluents containing pollutants, heavy metals, and toxic substances are known to cause drastic degradation of air, water, and soil. Heavy metal toxicity rooted by the introduction of toxic effluents in the environment and contamination by recalcitrant compounds due to anthropogenic activities has been a significant environmental issue encountered over the past few decades. Besides these metals, the dyes and inorganic colorants, chemical pesticides, excess of chemical fertilizers, and effluent from pharmaceutical industries tanneries contribute to environmental contamination at large. One of the most deadly sources of environmental pollution in a civilized country is radioactive release during testing of nuclear arms, nuclear explosions, careless handling and clearance of radioactive waste, accidental leakage from nuclear power plants, etc. But the most important threat is attributed to the accumulation of nondegradable plastic products (Mondal and Subramaniam 2020) including PVC, PET, LDPE, and HDPE.

An account of the various xenobiotic compounds, their sources, and toxicological health effects is given in Table 21.1.

Table 21.1 Sources and toxicological health impacts of heavy metals and xenobiotics

Pollutants	Sources	Toxicological health impact	References
Heavy metals			
Lead (Pb(II))	Windblown dust, aerosols, mining activities, smelting, geological and anthropogenic activities	It is considered to be teratogenic and mutagenic in nature bringing about immense damage to the central nervous system and peripheral nervous system. It results in the development of neurodegenerative disorders, hepatitis, encephalitis, renal failures, problems related to reproductive system, various skeletal associated disorders, cancer, mental disorders, and nephritic syndromes	Varsha et al. (2011)
Chromium (Cr(VI))	Chemical industries, electroplating, anthropogenic activities	They are responsible for bringing about various genetic mutations, mutagenic and carcinogenic in nature responsible to bring about damage to DNA	Raskin et al. (1997), Ali et al. (2013)
Cadmium (Cd (II))	Phosphate fertilizers, sewage, manures, sludge, limes	Responsible for the development of problems associated with nervous, cardiovascular, and nervous system. Cause osteoporosis, various problems associated with liver and kidney, anosmia, anemia, activation of oncogenes, and Itai-itai disease	Varsha et al. (2011)
Arsenic (As (III/V))	Sulfide mineral deposits, poultry waste, feed additives, industrial wastes	Causes irritation in lungs, various disorders associated to respiratory systems, anemia, irritation in lungs, miscarriages in woman, suppression of the immune system, development of type II diabetes, vascular and peripheral-associated diseases, coronary artery disease, atherosclerosis, arsenicosis, and development of cancer	Malik et al. (2012)
Mercury (Hg (II))	Surface runoff from rain/ snow, pesticides	They act as neurotoxin and are responsible for the development of various nervous system-associated	Varsha et al. (2011)

(continued)

Table 21.1 (continued)

Pollutants	Sources	Toxicological health impact	References
		disorders along with disorders pertaining to brain, lungs, heart, lungs, and immune system	
Zinc (Zn)	Geological and anthropic activities, coal mining and steel processing industries, pesticides	It is responsible for the development of various neurosensory and neuropsychiatric disorders, causes anemia by improper mechanism iron immobilization, enhances the level of cholesterol, and also causes cardiac dysfunction	Wasi et al. (2010)
Organic wastes			
Dyes		They are highly teratogenic, mutagenic, and carcinogenic and are responsible for the development of allergies	Brown et al. (2008)
Polycyclic aromatic hydrocarbons (PAH)	Dye and dye precursor, combustion, industrial application, coal tar, pesticides	They act as highly toxic substance to liver and kidney, mutagenic, carcinogenic, and hemolyticanemia	Raskin et al. (1997), Ali et al. (2013)
Nitroaromatic compound	Explosive, pesticides	Causes irritation in skin, is highly carcinogenic, immune modulator, and also affect the fertility of the person	Brown et al. (2008)
DDT	Insecticide	Xenoestrogenic, endocrine disruptor	Varsha et al. (2011)
2,4-D	Pesticide	Neurotoxic, carcinogenic	Caldeira et al. (1999)
Inorganic effluents			
Arsenic (As)	Insecticides, preservation of wood	Affects the integumentary, respiratory, nervous, cardiovascular, and reproductive systems. It also affects our immune system	Abdul et al. (2015)
Beryllium (Be)	Coal and nuclear power plants	They are carcinogenic in nature	Thangamalathi and Anuradha (2018)
Cadmium (Cd)	Industrial effluents, Ni–Cd batteries, metal plating	Causes anemia, high blood pressure, and malfunctioning of kidney	Abdul et al. (2015)

(continued)

Table 21.1 (continued)

Pollutants	Sources	Toxicological health impact	References
Chromium (Cr III and Cr VI)	Metal-plating industries	They are carcinogenic in nature	Abdul et al. (2015)
Lead (Pb)	Mining, coal, gasoline, and plumbing activities	Malfunctioning of kidney, development of anemia, and various nervous disorders	Abdul et al. (2015)
Fluorine (F)	Industrial effluents	Mottling of teeth and damage to the bone	Abdul et al. (2015)
Mercury (Hg)	Industrial activities especially by mining activities	Causes toxicity in body	Abdul et al. (2015)

21.2 Conventional Methods to Remove Environmental Contaminants

The mechanism of environmental remediation involves three kinds of approaches: a physical process that involves soil washing, capping, soil vapor extraction, soil mixing, land farming, solidification, soil flushing, and excavation processes, a chemical process that involves chemical immobilization, critical fluid extraction, remediation using actinide chelators, oxidation, in situ catalyzed peroxide remediation and photodegradation (Czupyrna et al. 1989; Gopalan et al. 1993; Gates and Siegrist 1994; Ho et al. 1995), and biological denaturation.

21.2.1 Biodegradation

It is the natural degradation of the complex organic compound to a less complex organic compound with the help of naturally occurring microorganisms. The mechanism of biodegradation involves the process of removing various types of contaminants and pollutants from the environment comprising air, soil, groundwater, sediments, and surface water for reducing the threat to human health as well as to the environment. The mechanism of conversion of pollutants and contaminants into useful metabolites waiving the toxic contaminating substances from the environment is known to environmental remediation (Igiri et al. 2018).

21.2.2 Bioremediation

The concept of bioremediation or biological remediation has been an area of keen interest over the last decade. The accumulation of toxic materials and hazardous substances results in the development of environmental hazards and risks that can be effectively mitigated by the use of the concept of biotechnology in the form of bioremediation or biotreatment. Biological remediation or bioremediation is a growing technology possessing the advanced potentiality in cleaning the toxic pollutants being present within the environment. This process involves various biological systems like plants and microbes, which helps in the detoxification of the environment and converts the complex hazardous substances to simple inorganic substances that can easily mix with the environment (Reshma et al. 2011). The mechanism of degradation aims in reducing the amount of toxic pollutants from various contaminated sites. The mechanism of immobilization on the contrary helps in the transfer of the contaminant to specific receptors without bringing about the degradation of the pollutants. The microbes and plants are predominantly used for the purpose of remediation because of their metabolic potential that helps in effective utilization and transformation of toxic or hazardous wastes to nontoxic substances (Glazer and Nikaido 1995). The process of bioremediation can be characterized into ex situ or in situ depending on the processes involved in the process of remediation. In situ bioremediation is the removal or degradation of toxic or hazardous substances with the help of the biological species in the natural environment, whereas an ex situ bioremediation method involves remediation of toxic chemical substances from the excavated samples (Pandey et al. 2009). In situ bioremediation can be further divided into bioattenuation, biostimulation, and bioaugmentation. Bioattenuation depends on the natural process of biodegradation. In biostimulation, stimulation of degradation of pollutants is accomplished by the addition of electron donors or acceptors to the site for effective bioremediation. In bioaugmentation, the microbes with inherent capabilities of degrading or transformation of the pollutants are added to the site (Madsen 1991). Some basic examples of bioremediation-related technologies are phytoremediation, bioventing, bioleaching, land farming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation, a few of which are discussed herein. The mechanism of biotransformation and biodegradation is either performed by an individual organism or by consortia that play a crucial role in detoxifying the toxic wastes present within the environment. Bioremediation is a broad mechanism that involves a large number of plant and microbial species having an important role in the pharmaceutical and foodstuff industry (Fig. 21.1).

Various types of recent technologies are implemented in the mechanism of removing various toxic and harmful wastes that are present within the environment by degrading them to simpler components, which can easily mix with the environment causing less or no impairment (Olguin 1999; Gavrilescu and Chisti 2005).

21.2.3 Biocomposting

The process that results in the decomposition of complex organic wastes into simple organic materials under a controlled set of conditions so that they get easily absorbed by the environment.

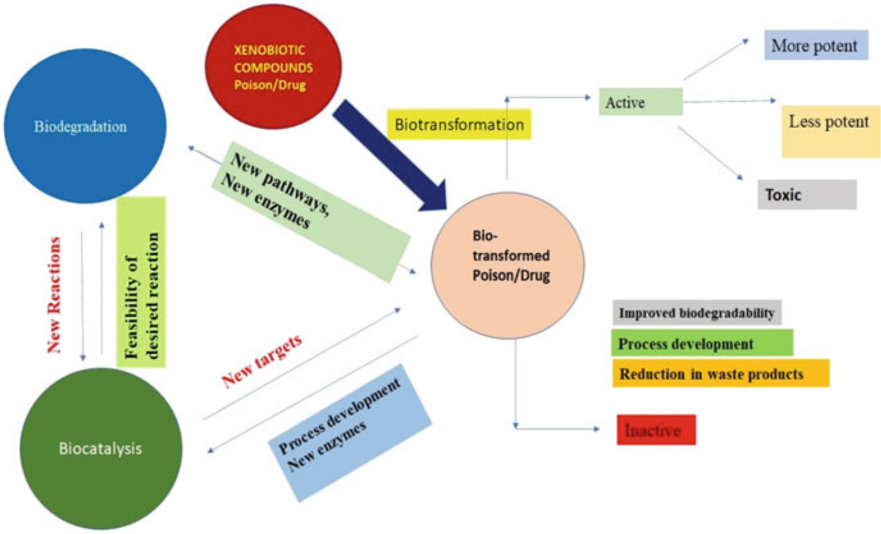

Fig. 21.1 Biotransformation and its relation with biocatalysis and biodegradation

21.2.4 Bioenergy

It is one of the emerging alternatives to the use of fossil fuels and comprises biomass, biogas, and hydrogen that can be used for the household activities and industry. It is also being termed as an alternate source of energy that utilizes biomass and does not cause any harm to the environment.

21.2.5 Biotransformation

This involves the mechanism of transformation from complex organic toxic materials to simpler nontoxic substances.

21.2.6 Biocatalysis

It is the use of biological systems or their parts like natural catalysts or enzymes to enhance chemical reactions.

21.2.7 Biomarkers

The substance that provides a qualitative assessment of toxicity for a chemical being exposed at several degrees to the environment. The biomarkers help in correlating the toxicity effect and the degree of exposure. Various types of conventional techniques are being used to treat the toxic wastes but generate by-products that are still toxic and harmful to the environment (Krieg 1998; Chen et al. 2005).

The interrelations between the aforementioned various techniques depict the mechanism behind the achievement of effective bioremediation of xenobiotic compounds (Fig. 21.1).

21.2.8 Microbial Remediation: Microbes in Remediation

It implements the microbes, mainly bacteria and fungi or their products, in detoxifying the toxic wastes that are available in nature and converts to simpler nontoxic materials having the potency of getting absorbed with the environment easily (Vidali 2009). Studies showed that various microbial species have the potency of removing persistent toxic organic and inorganic wastes in nature (Banat et al. 1996).

21.2.9 Phytoremediation: Plants in Remediation

Plants possess the ability to resist itself to various higher concentrations of xenobiotic organic chemicals without having any toxic impact (Briggs et al. 1982) and also possess the ability to transform the toxic chemical substances to less harmful and toxic metabolites that get easily absorbed within the environment (Kolb and Harms 2002). Phytoremediation refers to the utility of plants for relieving the environmental stress conditions through their metabolic and regulatory actions without the need to carrying the wastes to some other places for facilitating the disposal mechanism.

The mechanism of phytoremediation helps in reducing the concentration of toxic substances found within contaminated air water and soil. Pesticides, solvents, heavy metals, explosives, crude oil, and its derivatives can be mitigated by the applicability of phytoremediation. Green plants obtain nutrients and minerals from the soil, which are carried to all the tissues and cells of the plants by the various transport systems in plants and with the help of adhesive and cohesive forces. There are many metals that are essential for the growth and multiple physiological activities of the plant. This property of plants to utilize certain metals from the soil is employed in reducing excess metal concentration from the soil, and therefore mitigate soil heavy metal contamination. The process of remediating heavy and toxic metals from the natural environment using metal-accumulating plants has been proved to be eco-friendly and cost-effective technology (Raskin et al. 1997; Ali et al. 2013). However, in excess amounts, minerals can cause toxicity in plants, which will eventually lead to the death of plants. Such heavy metal toxicity or metal excess stress can be alleviated in plants by proper gene regulation. miRNAs possess a very strong relationship with the biotic stress responses, the hormonal control in plants and the plant growth, and are known to play a significant role in metal-induced stress alleviation. The mechanism of phytoremediation is still a developing technology to deal with the pollution problem, although it showed its efficacy in remediating heavy metals (Table 21.2)

Table 21.2 Plant species responsible for bringing about heavy metal remediation

Metal	Plants
Lead (Pb)	Water hyacinth *(Eichhornia crassipes),* Hydrilla, *Brassica juncea, (Hydrilla verticillata),* sunflower *(Helianthus annuus), Lemna minor, Salvinia molesta, Spirodela polyrhiza*
Cadmium (Cd)	Alpine pennycress *(Thlaspi caerulescens), Cardaminopsi shalleri,* Eel grass *(Vallisneria spiralis), w*ater hyssop *(Bacopa monnieri),*water hyacinth *(Eichhornia crassipes), Hydrilla verticillata, d*uckweed *(Lemna minor),* giant duckweed *(Spirodela polyrhiza)*
Arsenic (As)	Chinese brake fern *(Pteris vittata), f*ern *(Pteris cretica)*
Chromium (Cr)	Duckweed *(Lemna minor), Ceratophyllum demersum,* giant reed *(Arundo donax),* cattail *(Typha angustifolia),* alfoalbo *(Medicago sativa),* water hyssop *(Bacopa monnieri), Pistia stratiotes,* water fern *(Salvinia molesta), Spirodela polyrhiza*
Copper (Cu)	*Aeolanthus bioformifollus, Lemna minor, Vigna radiata,* creosote bush *(Larrea tridentata),* water hyssop *(Bacopa monnieri),* Indian mustard *(Brassica juncea)*
Nickel (Ni)	*Phyllanthus serpentines, Lemna minor, Salvinia molesta, brassica*
Manganese (Mn)	*Alyxia rubricaulis, Macademia neurophylla*
Zinc (Zn)	*Alpine pennycress (Thlaspi caerulescens), Brassica juncea*
Mercury (Hg)	*Lemna minor,* water lettuce *(Pistia stratiotes),* water hyacinth *(Eichhornia crassipes),* Hydrilla *(Hydrilla verticillata)*

and toxic wastes (Varsha et al. 2011). Recent advancements in research and technology like the use of endophytic bacteria, which are often found genera in soil like *Pseudomonas, Burkholderia, Bacillus,* and *Azospirillum,* are observed to improve phytoremediation and detoxification of contaminants (Ram and Srivastava 2008). Radioactive wastes possess the ability to remain active for many years, thus strategies for remediating the radioactive wastes are necessary for preventing its adverse effect on the environment and mankind. The advent of environmental biotechnology by the utility of phytoremediation is applied in degrading the radioactive wastes from nature.

21.2.10 Microorganisms in Bioremediation

Microorganisms are found to be located at diverse locations that play an important role in geochemistry, breakdown of the accumulated wastes, and recycling of elements. Microbes possess the potential for the usage of pollutants for the purpose of growth. Microbes use the high metabolic capacities possessed by them for the purpose of cleaning up of several contaminations, which are available in the ecosystem (Bernt et al. 2007). Microbial biodegradation has proved to be the major mechanism in remediating the heavy metals and organic wastes that are available within the environment. The microbes possess the ability of acclimatizing with the diverse environmental conditions, and this characteristic helps in the mechanism of

Fig. 21.2 Block diagram showing steps followed by bacteria for bioremediation

reducing concentrated pollutants that are available in nature (Carbajosa et al. 2009). The ability of the microbial cells to perform such activities involves various types of metabolic and genetic diversity having considerable potential for its applicability for the biotechnological purposes (Bergeron et al. 2006). Studies have shown that the toxic wastes that are present within the ecosystem are degraded by the microbial metabolic processes (Bernt et al. 2007). The α-protobacterium is found as one of the most important microorganisms in the process of bioremediation of spilled oil in the bacterial group of pseudomonas with other groups as well and with other proteobacteria. Metal toxicity is an alarming issue for the surrounding ecosystems (Brown et al. 2008). The α-proteobacteria, which has the ability to detoxify the metal toxicity, contains the detoxification processes that are said to be useful for the process of metal bioremediation (Brown et al. 2008). Many microorganisms like the cyanobacterial consortia are capable of degrading crude oil present in soil. The exogenous microorganisms that are adapted to the environment as for the process of increasing the bioremediation also have a significant role. Fungal species play a pivotal role in breaking down numerous toxic substances like petroleum hydrocarbons, polychlorinated biphenyls, heavy metals (by biosorption), phenol derivatives, persistent pesticides, etc. (Varsha et al. 2011). Actually the microbes after taking up of the organic compounds break the complex structure and finally assimilate the breakdown product for metabolism (Fig. 21.2).

A number of microorganisms capable of microbial remediation are known for removing different compounds (Table 21.3).

21.2.11 Various Technologies in Plastic Degradation

Although various classical techniques have been involved in the process of degrading plastics like incineration, landfill, and recycling, but the recent technology that aims in complete polyethylene degradation is by the application of microbial cells (Webb et al. 2012). The diverse groups of microbial cells thriving within the environments makes it an important agent for remediating complex and toxic waste materials that are being present in nature (Iranzo et al. 2001), Various researches have shown that microbial cells are effective in degrading the polymers (Yamada-Onodera et al. 2001; Bonhomme et al. 2003). The polymers are constituted of pure carbon that possess the ability of resisting the methods of degradation, but those constituting of heteroatoms in their backbone possess higher possibility of being degraded (Zheng et al. 2005). The polymers constituted of aromatic groups show resistances in the mechanism of degradation (Müller et al. 2001). Polyethylenethymols (PETs) are the groups of the polymers possessing higher

Table 21.3 Microbial species and their function in remediation (Varsha et al. 2011)

Microbial species	Function
Bacteria	
Pseudomonas sp.	Helps in partial and complete mineralization of organo-phosphorous that are predominantly available within fungicides and pesticides. It also helps in degradation of methyl parathion and morphine
Pseudomonas fluorescens SM1	Helps in remediating various toxic heavy metals and phenolic groups being present at the polluted sites (Wasi et al. 2010)
Pseudomonas pseudomallei	It helps in quenching of phenols from various types of aqueous solutions
P. aeruginosa	Helps in recalibration of various metals and oil that are being present within the soil surface (Mathiyazhagan and Natarajan 2011)
Bacillus sp.	Helps in the mechanism of degradation of various types of benzimidazole compounds being present within the environment
Bacillus subtilis	The biosurfactants produced by these organisms help in recalcitration of oil and thus bringing about the degradation (Amin 2010; Owolabi et al. 2011)
Azotobacter sp.	Helps in removing trivalent chromium by the process of biosorption
Rhodococcus erythropolis	It plays an important role in the mechanism of desulfurization of the crude oil being present in an immobilized state within rotating immobilized cell reactor
Rhodococcus ruber *Pseudomonas aeruginosa*	Helps in the mechanism of degradation of plastics by breaking the CH_2 backbone and utilizing it as a sole carbon source (Hadad et al. 2005)
Anoxybacillus rupiensis	Helps in the degradation of the effluent being liberated from industry. They also decolorization of dyes, which come out as effluent
Anoxybacillus pushchinoensis, Anoxibacillus kamchatkensis and *Anoxibacillus flavithermus*	It helps in the degradation of synthetic dyes being liberated from the industry
Proteus mirabilis, Pseudomonas aeruginosa, and *Micrococcus luteus*	Helps in biomineralization and thus helps in remediation (Reddy et al. 2011)
Methanogenic bacteria and *Methanotrophic bacteria*	Helps in degradation of methane by utilizing oxygen and produces carbon dioxide. The enzyme methane monooxygenase produced by the methanotrophs that degrades methane and other chlorinated hydrocarbon
Cyanobacteria	Helps in degradation of oil being present within the environment

(continued)

Table 21.3 (continued)

Microbial species	Function
Sphingomonads	Helps in the degradation of polyethylene glycol (PEG) and polyvinyl alcohol (PVA)
Fungi	
Saccharomyces cerevisiae	It helps in converting 85% sludge from the paper industry without the treatment of commercial enzymes. Also help in the mechanism of recalcitration of heavy metals like Pb, Co, Cu, and Au (Rajesh et al. 2011)
Trichoderma harzianum	They are predominantly used as the producer of cellulolytic enzymes help in the degradation of the cellulose being produced by the paper and textile industries (El-Bondkly et al. 2010)
Mucorsp, aspergillus sp.	Helps in the bioabsorption of heavy metals
Morchella conica and *Tylospcno fibrilnsa*	Helps in the degradation of organic contaminants being present within the soil (Bennet et al. 2002)
Microalgae	
Chlorococcum sp., *Chroococcus* sp., *Desmococcus* sp., *Dactylococcopsis* sp., *Chlamydomonas* sp.	Helps in the remediation of heavy metals from the environment (Chang et al. 2006)
Spirulina platensis	They act as biosensors in detecting the concentration of accumulated mercury within various solid wastes
Nostoc sp.	Helps in the degradation of DDT and methyl parathion

efficiency of being degraded by a wider number of mechanisms. Microbial lipases and esterases showed higher efficacy in degrading the polymers by acting on the amorphous regions and exposing the polymer crystals (Mueller 2006) Various researches are being done in enhancing the rate of degradation of PET by bringing about modification in the polymer and decreasing the intermolecular cohesive forces (Kondratowicz and Ukielski 2009; La Mantia et al. 2012) (Fig. 21.3).

21.2.12 Microbial Biofilms in Bioremediation Technology

Biofilms are the consortia of sessile groups of microbial cells that remain adhered to the biotic or abiotic surface with the help of extracellular polymeric substances and glycocalyx (Khatoon et al. 2018). Biofilm-forming bacteria are adapted to survive harsh environmental and physicochemical stress factors like toxic and hazardous chemicals, fluctuating temperature, pH, salt concentration, etc. The ability of the biofilm to resist environmental stresses has made it a potential agent for performing bioremediation (Wu et al. 2018). Biofilm-mediated remediation is environment-friendly and cost-effective owing to the nonrequirement of immobilization techniques for industrial usage in bioreactor systems as the microbial cells are

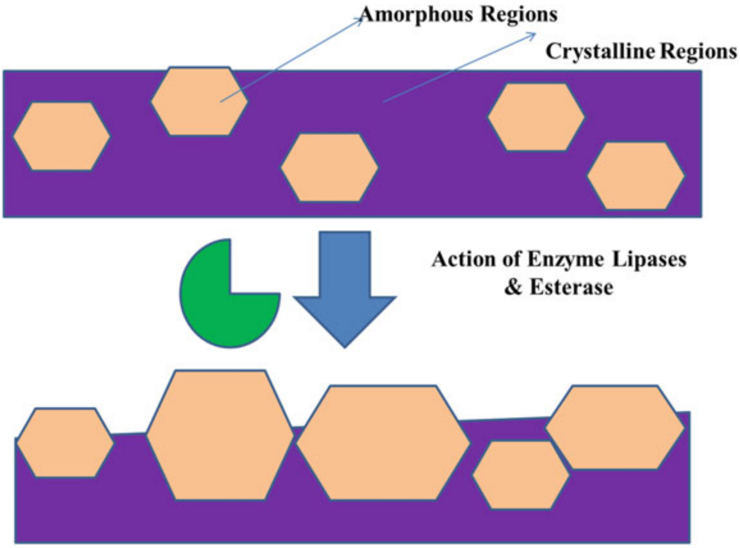

Fig. 21.3 Enzymatic degradation of the polymer

naturally immobilized or embedded in self-produced extracellular polymeric substances (EPS). The production of biosurfactants, which are nontoxic, biodegradable agents, endowed with surface tension-reducing activities (Lindum et al. 1998) in biofilms help to utilize and degrade hydrophobic compounds (Marchant and Banat 2012; Ron and Rosenberg 2002). Use of biofilms is therefore efficient for cleaning up environmental pollutants and xenobiotic agents as biofilms absorb, immobilize, and degrade various environmental pollutants.

Bacterial biofilms exist within indigenous populations near the heavily contaminated sites and show tolerance to toxic materials present in the waste as EPS-containing biofilm matrix acts as a physical barrier for embedded cells. The technique offers (Fig. 21.3) a promising alternative to free-floating planktonic cell-mediated bioremediation as expressions of genes vary within the biofilms and are distinctive. Such differential gene expressions within biofilms owing to variable local concentration of nutrients and oxygen within biofilm matrix and division of labor among microbes may be important for the degradation of varied pollutants by numerous metabolic pathways (Brown et al. 2008).

The community formation between the groups of microorganisms to configure themselves as a biofilm gives them additive benefits apart from their overall robustness. Within a mature biofilm, there exists a heterogeneity (or gradient) in the circulation of metabolic substrate and product (Stewart and Franklin 2008). In mixed culture biofilm, an intermediate (or waste) of one metabolite can serve as a growth substrate for other species (Costerton et al. 1987). Responses such as swimming, swarming, twitching motility, chemotaxis, lateral transfer of genetic material, and quorum sensing in the presence of xenobiotics assist the microbes in

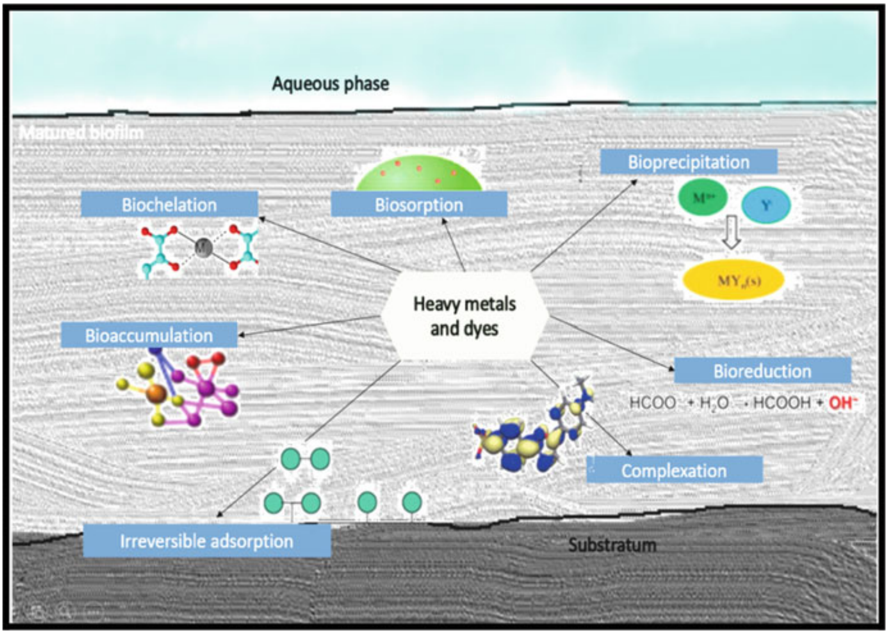

Fig. 21.4 Different mechanisms involved in biofilm-mediated bioremediation of heavy metals

the biofilms to coordinate movement toward pollutant and hence improve biodegradation (Brown et al. 2008) (Fig. 21.4).

21.2.13 Heavy Metal Bioremediation by EPS

Extracellular polymeric substances or EPS function as a protective covering layer as well as a structural framework of the biofilm. The presence of various exopolymeric biomolecular components like carbohydrates, proteins, and lipids accounts for the presence of functional groups such as phosphate ($RHPO_4^-$), carboxylate ($RCOO^-$), amino ($RNH3^+$), sulfhydryl (RSH), and phenol (RC_6H_5OH) groups (Brown et al. 2008), which attribute to the occurrence of multiionic charges in the surface of bacterial biofilms. It has been observed that the number of negatively charged groups in EPS is relatively more compared to the positive ones. This gives a net negative charge to the EPS surface at nearly neutral pH (pH 6.5–7.5) and aids in the formation of organometallic complexes with heavy metals by electrostatic forces of attraction (ImamulHuq et al. 2007).

Metallic ions and ionic dyes from the nearby environment are attracted and entrapped in the EPS owing to its metal-binding capabilities. This helps to maintain the static metabolic level and ion mobility inside the matrix, which ultimately enhances the bioavailability of the toxic metals ions and the remediation process (ImamulHuq et al. 2007). The composition of the EPS and its metal-binding capacity changes according to the surrounding environmental condition and genetic makeup of the biofilm microcolonies (ImamulHuq et al. 2007). Thus, metal detoxification via biofilm bioremediation varies according to the type of biofilm organism and its surroundings. An increase in EPS production can also increase the metal detoxification process (Liao and Li 2013).

21.2.14 Quorum Sensing in Biofilms

Quorum sensing (QS) is one of the most important characteristics of the microbial population in biofilms and plays a vital role in regulating the gene expression and the matrix development within the biofilm (Caldeira et al. 1999). QS helps in the coordination among the sessile colonies dwelling with the polymeric substances by the virtue of cell-to-cell communication with the help of chemicals known as auto-inducers (AI) (Gera and Srivastava 2006). Bacteria use this regulatory mechanism for adjusting itself within the changing environment (Liao and Li 2013) that provides them the stability and adaptability to survive in harsh environmental conditions (Oh 1994).

The mechanism of QS involves the binding of AI like auto-inducing peptides and acyl-homoserine lactones (AHL) with the receptor proteins existing upon the cell surface (Liao and Li 2013) that further helps in the activation of the signal transduction cascades. This further helps in the mechanism of regulating the genes at various levels of transcription factors enhancing the growth of biofilm and the formation of the extracellular polymeric substances (EPS) (Fig. 21.5).

21.2.15 Bioreactor Technology in Bioremediation

Bioreactors have been commercially used in industrial wastewater treatment for decades. Present studies showed that the use of microbial bioreactors helps in efficient bioremediation as various critical parameters can be controlled (Gera and Srivastava 2006). Bioreactors offer many advantages over conventional treatment processes including high concentration and retention of biomass for long periods, enhanced metabolic activity, increased process flow rates, greater tolerance to harsh pollutants, large mass transfer area, the coexistence of anoxic and aerobic metabolic activity, and enhanced volumetric biodegradation capacity (Özkaya et al. 2019). Added to that is the flexibility in the design of the bioreactor in terms of size and configuration to suit application or intended purpose of the reactor. Different configurations and types of bioreactors have been used for organism- and pollutant-specific bioremediation techniques a few of which are enlisted in Tables 21.4 and 21.5.

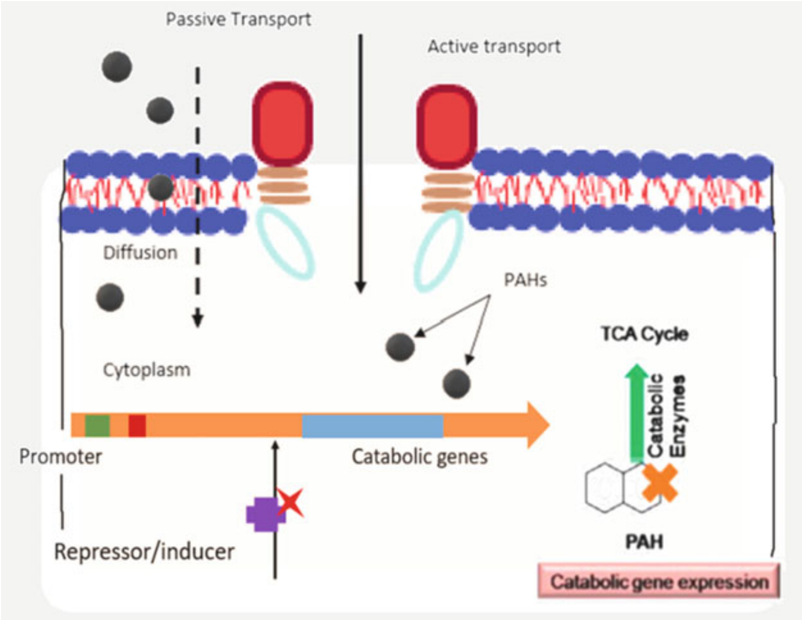

Fig. 21.5 QS-dependent functions useful for bioremediation technology against PAH metabolism

21.2.16 Microbial Enzyme Technology in Bioremediation

Immobilized organisms and/or enzymes are widely used in bioreactor technology for serving the purpose of reusability and cost reduction. Immobilization of enzymes helps in enhancing the enzyme stability across a wide range of temperature, pH, and storage time by providing suitable environmental conditions for the sustenance of the enzyme (Chakraborty et al. 2013). The immobilization of enzymes is performed using a membrane that provides a larger surface area for enhanced enzyme loading and possesses the ability to get operated using convective flow mode. Studies showed that the microbial growth kinetics can be considered to be an analogous process to that of the enzyme kinetics under the immobilized condition.

Immobilization of the enzyme and efficiency of bioreactor outputs are known to be affected greatly by the immobilization techniques (Basile et al. 2013). The enzymes easily get attached to the surface of the solid matrix by the mechanism of adsorption (Huckel et al. 1996; Hanefeld et al. 2009). The mechanism of enzyme adsorption takes place by the existence of difference in charges across the membrane surface and by the development of noncovalent interactions existing between enzyme and matrix (Godjevargova et al. 2000). The mechanism of enzyme entrapment occurs by immobilizing the enzymes within the pores of the matrix and is commonly used within the membrane bioreactor system (Basile et al. 2013). Matrices like PVA-dimethyldimethoxysilane (DMDMOS), poly (vinyl alcohol) (PVA)-tetramethoxysilane (TMOS), polystyrene, PVA-TMOS-DMDMOS,

Table 21.4 Types of bioreactors and their uses in microbial bioremediation

Bioreactor types	Uses	References
Packed bed bioreactor	Helps in the degradation of complex organic wastes that includes PAHs, amines, pharmaceuticals, organochlorine pesticides, and dyes that are emitted from the textile industries	Scott and Karanjkar (1998), Scott (1995)
Fluidized bed	Fungi being present within the bioreactor help in the treatment of the effluents coming out from the industry	Eldin et al. (2011)
Slurry phase	Helps in the mechanism of remediation of various VOC being present within soil, 2,4-dichlorophenoxy acetic acid and PAHs	Chang et al. (2006)
Suspended carrier	Helps in the mechanism of remediation of various persistent organic pesticides, PAHs, and dyes from textile industries using fungi	Chang et al. (2006)
Membrane bioreactor	Remediation of textile dye in waste water; pharmaceuticals, 1,2-dichloroethane, 1,2-dichlorobenzene, and 2-chlorophenol in groundwater; metal recovery	Zhao et al. (2011)
Air lift	Helps in decolorizing the dyes coming out from the textile and cellulose industries by the use of fungi	Jeswani and Mukherji (2012)
Biotrickling filter	Helps in the treatment of various effluents coming as municipal wastewater, wastes from brewery, VOCs, and wastes from various oil mills	Fritzsch et al. (2006)
Continuously stirred tank bioreactor (CSTR)	For hydrocarbon-rich industrial wastewater effluents by mixed microbial cultures, petroleum hydrocarbon	Caldeira et al. (1999), Carvalho et al. (2001)

poly-tetrafluoroethylene (PTFE), cellulose acetate (CA)-PS, PAN, and poly(methyl methacrylate) (PMMA) have been widely used in enzyme entrapment. The covalent binding method is based on the binding of enzymes and membrane by covalent bonds (Ricca et al. 2010). The intermolecular crosslinking of enzymes with one another or with the functional groups results in the process of immobilization (Eldin et al. 2011). Affinity is a mechanism of enzyme immobilization with the help of site-specific group of biomolecules on the matrix. This method allows control of the enzyme orientation in order to avoid enzyme deactivation and/or active site blocking. Figure 21.4 illustrates the different immobilization techniques discussed.

21.2.17 Systemics Biology and Bioremediation

The application of systemic biology and gene-editing tool has brought a new dimension in environmental biotechnology research. In due course of globalization and technological advancements, the pollutants and ecologically toxic compounds

Table 21.5 Types of membrane and biofilm bioreactors used for degradation of pollutants

Bioreactors	Pollutants	References
Silicone tube membrane bioreactor	2-Chlorophenol	Chang et al. (2004), Chang et al. (2003)
Granular activated-carbon biofilm reactor	4-Chlorophenol	Caldeira et al. (1999), Carvalho et al. (2001)
Rotating perforated tube biofilm reactor	2,4-Dichlorophenol	Kargi and Eker (2005)
Hollow-fiber membrane biofilter	Toluene, azo dyes	Arcangeli and Arvin (1995)
Laboratory-scale rotating drum biofilm reactor	Acid Orange 10, 14	Zhang et al. (2010)
Granular activated-carbon biofilm reactor	MCPP; 2,4-D	
Anaerobic–anoxic–oxic (A_2O) biofilm process	Zn, Cd, Ni	Chang et al. (2006)
Biofilm formed on moving bed sand filter	Cu, Zn, Ni, Co	Diels (2003)
Rotary biofilm reactor for algae immobilization	Co	

have evolved multifold in terms of their toxicity and hazard-causing capacity. Degradation of such variable organic and inorganic compounds follows different pathways and needs to be taken care of through specific bioremediation methods for which empathizing on metabolic pathways for gene editing and application of systems biology becomes very important.

Multi-omics and computational biology approaches involving genomics, metagenomics, proteomics, transcriptomics, and metabolomics for determining the mechanism of bioremediation by finding the functional genes being present within the organism. Gene-editing tools such as TALEN, ZFNs, and CRISPR Cas9 are auspicious to get the function-specific microorganisms with particular genes and enzymes responsible for degradation of particular recalcitrant for improved bioremediation (Jaiswal et al. 2019). Identification of community members having genes that encode for enzymes capable of causing biodegradation using second-generation sequencing or identification of biodegrading genes in microbes found in contamination sites by cloning and sequencing of ribosomal DNA has significantly revolutionized the arena of bioremediation (Raman et al. 2018; Dong et al. 2015).

21.2.18 Genomic Technologies in Microbial Bioremediation

In modern times, the genetically modified organisms of the microworld are used for overtaking the charge of the wild type of microbes. The knowledge of various genes responsible for the degradation of compounds helps in developing genetically modified organisms that facilitate the mechanism of bioremediation of complex organic wastes and heavy metals (Varsha et al. 2011). These microorganisms perform more efficiently because of the modifications in their genetic makeup.

These have resulted in them the ability to build improved metabolic pathways, stable catabolic activity, and a diverse range of substrates. The newer biodegradation pathways can be extracted or invented from the existing ones that will help in the process of understanding the process of metabolism in a substrate.

The various groups' microbial cells living within the toxic environment could be predicted by the mechanism of genome enrichment followed by meta-genomic analysis. Screening of microbe by various processes like phenotype-based screening, metabolite-regulated expression profiling, sequence-based screening of biodegradative genes from contaminated sites, etc., can be useful for studying the catabolic potential and dynamics of such biodegrading microbes in situ. Optimization of the media and environmental condition can enhance the rate of remediation and hence pave way to newer research areas wherein the metabolism of such bacteria can be modeled and monitored. Metagenomic approaches, together with emerging high-throughput sequencing technologies, have provided insight into the complex interactions between different microbial communities and their metabolic potentials, which could be exploited in various bioremediation strategies for efficient removal of pollutants from the environment.

21.2.19 In Silico Approach in Bioremediation: Constraint-Based Modeling of Metabolism

Various mechanisms of optimization can be performed by in silico approach, which provides the insight of the extent of biodegradation of various contaminants using biogenic sources and also helps in inferring various types of biochemical pathways that are associated with performing such degradation process. Metabolic capabilities are possible to be observed by utilizing the constraint-based metabolic modeling technology. As the previous processes, this is also used for description and investigation. In the metabolic flux distributions of genome-scale biochemical networks, the kinetic information is needed for performance (Durot et al. 2009). By the process of transformation of the stoichiometric coefficients of almost all the mechanism pathways into a specific microbe, in its numerical matrix (S), the network topology and the different capacity properties should be studied. After some time, it may be used to observe it mathematically (Dutilh et al. 2011). Like this, there are different other parameters on which the bioinformatics network can be operated and can be determined. By making an assumption of steady-state mass balance for all metabolites, that determines in the calculation of ~v, which has a unit of mMolgDW/L/h. In this, the DW accounts for the dry weight. The latter is the flux vector that comes and detects the role of internal and external metabolic fluxes, which has parameters by the lower and upper bounds (Feijao et al. 2006). Several parameters-based modeling applies Flux balance analysis or FBA as a generally accepted technology (Fritzsch et al. 2006).

21.2.20 Nanoremediation: An Emerging Field in Environmental Biotechnology

Nanotechnology deals with the use of very small manufactured particles, called nanoparticles, which are atomic or molecular aggregates with dimension between 1 and 100 nm that undergoes drastic modification in terms of their physicochemical properties compared with the bulk material (Yadav et al. 2017). The application of nanomaterials in environmental detoxification of pollutants is a newfound area of research that is enormously expanding as scientists search for better methods of mitigating toxic pollutants. Nanoremediation methods involve the use of reactive nanoparticles or materials for the environmental remediation of pollutants. Nanomaterials withhold special properties that enable them to facilitate both chemical reduction and catalysis of the different chemicals and a wide variety of compounds that cause pollution in the environment (Karn et al. 2009). Recently, nanoremediation techniques have been suggested as efficient, cost-effective, and environmentally friendly alternatives to existing methods of both resource conservation and environmental remediation. There are many other nanoparticles and nanomaterials that have been proved to be significantly effective against a vast array of pollutants and are widely used for the remediation of such, a few of which are illustrated in Table 21.6.

Traditionally synthesis of nanoparticles involving physical and chemical methods is very costly, which led to the emergence of nanoparticle biosynthesis. The mechanism associated with the synthesis of nanoparticles using microbial enzymes or plant phytocompounds is known as the bottom-up technique. The antioxidant and reducing properties being exhibited by the microbial secretions and plant compounds help in the conversion of metal compounds to their respective nanoparticles (Yadav et al. 2017). Various microbial cells including yeasts, bacteria, fungi, algae, and various plant active compounds are used for the mechanism of synthesizing nanoparticles involving the lesser cost of production (Gurunathan et al. 2009). The removal of environmental contaminants using nanoparticles synthesized by plant and microbial sources is known to be nano-bioremediation (NBR). NBR techniques are very useful for detoxification and bioremediation of soil, water, and other environments in highly polluted conditions owing to their extremely cost-effective biosynthesis and efficient detoxification offered by the greater catalytic surface area of the NPs. Some of the recent research trends and advances reported in nano-bioremediation technology are mentioned in Table 21.7.

21.2.21 Computational Biology in Environmental Biotechnology

The undesired odor that is emitted by various contaminants results in the development of air pollution that has a detrimental effect upon the environment. Thus, monitoring various pollutants being emitted into the environment is needed for understanding the quality of air and its suitability for human being (Zarra et al. 2019). It is very difficult to combat against the pollutants as they are invisible to our

Table 21.6 Types of nanoparticles used for the degradation of pollutants

Nanoparticle/ nanomaterials	Pollutants	References
Nanocrystalline zeolites	Removal of toluene, nitrogen dioxide from wastewater	Theron et al. (2008)
Activated carbon fibers (ACFs)	Ethylbenzene, benzene, xylene, toluene	Theron et al. (2008)
CeO_2 carbon nanotubes (CNTs)	Helps in the mechanism of detoxification of heavy metal	Theron et al. (2008)
TiO_2 nanoparticles	Removal of azo dyes, aromatic pollutants, heavy metal detoxification	Theron et al. (2008)
Amphiphilic polyurethane (APU) nanoparticles	Ability to remediate toxic organic pollutant	Theron et al. (2008)
Pd/Fe nanoparticles	Wastewater treatment of PCBs, chlorinated ethane, chlorinated methanes	Theron et al. (2008)
Ni/Fe nanoparticles and Pd/Au nanoparticles	Chlorinated ethane, brominated organic compounds (BOCs), dichlorophenol, trichlorobenzene	Theron et al. (2008)
Zero-valent iron nanoparticles (nZVI)	Helps in the purpose of remediating Cr (VI)	Yadav et al. (2017)
ZnO nanoparticles	Helps in the removal of complex organic wastes from water by the mechanism of photocatalysis	Theron et al. (2008)
Brush-grafted MNPs	Helps in the remediation of mercury from the environment	Farrukh et al. (2013)

naked eyes and can only be felt with the help of olfactory stimuli. Various strategies and researches are being performed in introducing an intelligent system in monitoring the odor. Such recent technology is the advent of the artificial neural network (ANN) that helps in managing the various environmental odors. The basic factor that governs the mechanism of optimization by ANN is elements, algorithms, and structure. The mechanism of managing the environmental odors can be divided as measurement, characterization, control, and treatment. For each of these individual components, ANN is evaluated critically in understanding the weaknesses and strengths.

Table 21.7 Nanoparticles synthesized by microbes and plants (Yadav et al. 2017)

Nanoparticle	Bacteria	Fungi	Plants
Silver nanoparticles	*Oscillatory willei*NTDMO1, *Escherichia coli, Pseudomonas stuzeri, Bacillus subtilis, Bacillus* sp., *Lactobacillus* sp.	*Aspergillus* sp., *Cladosporium cladosporioides, Fusarium* sp., *Trichoderma viride*	*Ocimum* sp., *Capsicum annum, Opuntiaficus indica, Azadirachta indica, Brassica juncea, Pelargoneum graveolens Rhizophora mucronata*
PbS nanoparticles		*Torilopsis species, Rhodospiridium dibovatum*	*Jatropha curcas L.,Vitus vinifera* L.
CdS nanoparticles	*Klebsiella aerogens, Clostridicum thermoaceticum, Escherichia coli*	*Candida glabrata, Schizosaccharomyces pombe*	
Gold nanoparticles	*Lactobacillus strain, Thermomonospora* sp. *Pseudomonas aeruginosa Alkalo-thermophilic actinomycete*	*Verticillium* sp. and *fusarium oxysporum*	*Cinnamomum zeylanicum, Medicago sativa, Dyopiros kaki, Allium cepa L., Mucuna pruriens, Magnolia kobus, Azadirachta indica* A. Juss.

21.3 Conclusion

The rapid enhancement of the human population and the rise of the need for better living have resulted in depleting the quality of the environment by virtue of the accumulation of various toxic organic compounds and heavy metals being liberated by daily human activities and as industrial emissions. The traditional techniques for waste reduction were not found to be effective as they are unable to bring about complete breakdown and produce by-products, which also create toxicity to the environment. Thus, newer technique in the field of EB has resulted in remediating the toxic wastes to simpler substances so that they get easily absorbed with the environment. The techniques usually use biogenic sources that result in cost-effective and green technique.

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Advanced and Ecofriendly Technologies for the Treatment of Industrial Wastewater to Constrain Environmental Pollution

22

Izharul Haq and Ajay S. Kalamdhad

Abstract

The environmental contamination in the present situation is of great importance to industrial wastewater. Industries consume huge amounts of water and toxic chemicals during product formation and generate large amounts of wastewater loaded with toxic and lethal pollutants, which discharge into the environment without proper treatment and cause major threat to receiving ecosystem. The discharged wastewaters are often characterized by high BOD, COD, TDS, heavy metals, phenolics, and organic and inorganic materials. The conventional wastewater treatment is not efficient to remove these pollutants due to its low biodegradability and toxicity. Several physicochemical methods are available that have been employed for the treatment of wastewater. However, at industrial scale the application of these processes is not suitable due to high cost. Alternatively, nowadays biological treatment process has been considered because of its low charge, energy consumption, and environment-friendly nature. Therefore, the chapter provides updated information of industrial wastewater and their characteristics. Besides this, the chapter also describes various biological treatment processes of industrial wastewater.

Keywords

Industrial wastewater · Environmental pollution · Biodegradation · Toxicity

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22.1 Introduction

The modern life and environment are continuously affected by the involvement of industrial development that discharge huge amounts of noxious and lethal pollutants containing wastewaters into the environment. The rapid industrial activities like paper, tannery, distillery, textile, pharmaceuticals, etc., are polluting drinking water consumed by animals and humans in their daily routine. The wastewaters generated from these industries encumbered with various toxic pollutants including organics, phenolics, dyes, metals and inorganic pollutants, etc., which enter into the environment and cause hazardous effect on aquatic flora and fauna (Haq et al. 2016a, b, c; Raj et al. 2014a, b). Massive production and discharge of untreated industrial wastewater (IWW) are continuously contaminating natural resources and posing a significant challenge to the preservation of a clean and healthy ecosystem (Gricic et al. 2009). The usual management of wastewater including physicochemical process is employed for the management of wastewater (Narmadha and Kavitha 2012; Shivajirao 2012). However, due to their high cost and energy requirements, these methods are not ideal at industrial level. Thus, there is an urgent need of advanced and sophisticated technologies being developed, which should be compatible at industrial level. Because of their low cost and eco-friendliness, many biological wastewater treatment systems are used for IWW remediation, and they are likely to be much more proficient in waste disposal (Rajasulochana and Preethy 2016; Haq and Raj 2020). Therefore, this chapter provides updated information regarding industrial wastewater, their contaminants, and treatment process for sufficient management of wastewater before disposal into the environment.

22.2 Industrial Wastewater and Their Contamination in Environment

Huge amounts of various chemicals are used in factories, mills, and industries for product formation, resulting in the production of huge quantity of toxic and hazardous wastewater that are released without proper treatment into the environment (Dsikowitzky and Schwarzbauer 2013; Haq et al. 2017, 2018). The waste generated from industries is divided into two main categories such as biodegradable and nonbiodegradable on the basis of their nature. Households, textile, distilleries, leather, fruit, and paper processing industries can produce biodegradable waste that can be transformed or disintegrated into nontoxic products with the aid of microbial system. Nonbiodegradable waste, on the other hand, is waste that cannot be degraded by microorganisms, which is created by plastic, glass factories, fertilizers, iron and steel industries, nuclear operations, pharmaceutical and drug industries, and is fully harmful, causing environmental contamination and life-threatening disease to living organisms. Because of their harmful and unsafe consequences, this type of hazardous waste necessitates the use of appropriate management treatment technologies. Some of the most toxic industries that release their wastewater in environment are discussed here.

22.2.1 Pulp and Paper Industry

Pulp and paper industry is globally one of the topmost socioeconomic sectors and plays a key role in economic growth. Paper industry uses plant materials and huge amounts of water and chemicals for paper production. The chemicals that are used in the paper industries are highly toxic and rich in persistent organic pollutants (POPs), creating a major hazard to the environment. The paper industry is a highly water-utilizing industry and requires enormous quantity of water: 4000–12,000 gallons per ton of pulp (Ince et al. 2011). It is estimated that about 75% of consumed water during paper production is discharging as wastewater (Yadav and Garg 2011). The pulp and paper production involve mainly two key steps, namely, pulping followed by bleaching. These two process are highly water consuming and wastewater generated during these steps loaded with highly toxic contaminants. A large amount of various chemicals, such as sodium hydroxide, sodium carbonates, sodium sulfide, bisulfites, elemental chlorine or chlorine dioxide, calcium oxide, hydrochloric acid, and so on, are used in the paper manufacturing process, consequently in the creation of massive amounts of wastewater filled with organic and inorganic salts and radioactive contaminants, which are released after unfinished management (Haq and Raj 2020). During pulp and paper production process, industry release large amount of wastewater containing high concentration of toxic processing chemicals, which is characterized by high BOD, COD, phenolics, metals, and dark brown color of the effluent. The discharged effluent from the paper industry is highly toxic and poses threat to aquatic flora and fauna (EPA 2002). The effluent is loaded with organic and chlorinated hydrocarbons, and organic absorbable halides (AOXs), which affect people and animals in the atmosphere. Different chemicals from the paper industries are gathered through the food chain and are impossible to break down because of the chemical complexity and reactivity. These contaminants are extremely damaging and can lead to serious health and impairments, including neurological, neuronal, and carcinogenic problems (Ince et al. 2011).

22.2.2 Tannery Industry

The tannery industry has been categorized as a red category industry and is tremendously contaminating the environment by discharge of contaminated effluent during lather production. Tanning industry is a major contributor to a country's socioeconomic equity due to its large export and job opportunities (Kumari et al. 2014, 2016). The tannery industry consume large quantities of freshwater (~35–40 L) for the processing of raw skin/hides and utilize huge amounts of toxic chemicals for the exchange of hides into precious products. During the production of lather, this industry discharges huge amounts of wastewater loaded with toxic and lethal contaminants, which are discharged into the environment, posing threat to aquatic animals and human being (Kumari et al. 2014, 2016; Haq and Raj 2018). Substantial sulfates, chlorides, suspended components, toxic metals (mainly chrome), colored complexes, and other contaminants are included in wastewater produced by the tanneries industry (Kumari et al. 2016; Raj et al. 2014b). But the chrome emission is caused mostly by the tanning industry as chrome tanning is used for inexpensive leather manufacturing. The tannery effluents, which make the essential body structure, are serious carcinogenic and transmutagenic changes and are highly toxic to marine and human life ecology (Kumari et al. 2016; Haq and Raj 2018). High chromium toxicity is significantly harmful and has detrimental consequences for living organisms (Kumari et al. 2014; Raj et al. 2014b).

22.2.3 Distillery Industry

Due to its widespread use in pesticides, pharmaceutics, cosmetics, food and perfumery industries, etc., alcohol manufacturing industries are growing considerably. The distillery are highly water-consuming and pollution-causing industry and manufacturing ~8–15 L wastewater/L of overall liquor generation. It is estimated that in India alone there are about 300 distillery industries that are accountable for the production of about 40 billion liters of wastewater annually (CPCB Central Pollution Control Board 2011). In general, molasses is used as a major raw material in the distiller industry, producing waste known as molasses-spent wash (MSW). The main problem of MSW is the presence of melanoidins, organics, and low biodegradability causing severe environmental pollution. The distillery effluent is described by their brown color due to melanoidins (a recalcitrant compound), acidic pH (3–5), elevated COD (80,000–160,000 mg/L), organic and inorganic compounds, phenolic compound, and mineral salt (Basu et al. 2015; Mohana et al. 2009). Due to high pollution load and improper treatment of distillery industry wastewater, it directs to elevated height of water and soil pollution, eutrophication of stream, and inhibition of photosynthesis due to dark brown color of effluent. Due to acid nature of distillery wastewater, it causes reduction of soil alkalinity and deficiency of manganese ion, which leads to soil infertility, seed germination inhibition, and also damage the agricultural crops. Due to the presence of putrified organics, sulfur, and phenolic compounds, the considerable amount of wastewater generated by distilleries indicates fatalities to microbial communities, fishes, and other planktons due to the presence of poisonous and permanent contaminants, which can become harmful for the marine and soil ecosystems.

22.3 Advance Wastewater Treatment Technologies

22.3.1 Biological Treatment

Biological remediation is an ecofriendly method used for the detoxification of wastewater having organic and inorganic toxins using microorganisms such as microbes, fungi, algae, and enzymes (Kumar et al. 2017a, b, c; Haq et al. 2017, 2018; Haq and Raj 2018, 2020). In bioremediation process, complex toxic waste material is converted into simpler less/nontoxic compounds, therefore, direct to mineralization and detoxification (Reshma et al. 2011; Singh et al. 2014). The process of bioremediation mainly depends on the catabolic capability of microorganism to degrade the pollutants, which is also affected by the accessibility of pollutants and their bioavailability (Antizar-Ladislao 2010). Bioremediation technique involves various techniques like bioattenuation, biostimulation, and bioaugmentation (Maszenan et al. 2011). Various literatures are accessible on the topic of bioremediation and biodegradation of industrial wastewater by means of single microbe and their consortia (Singh et al. 2011; Megharaj et al. 2011; Maszenan et al. 2011; Paisio et al. 2012; Haq et al. 2016a).

Bioremediation of paper industry wastewater using bacterial spp. has been employed, and some of the bacterial cultures are available commercially for this purpose. Large number of bacterial spp. (*Micrococcus luteus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Pseudomonas putida*, *Acinetobacter calcoaceticus*, *Ancylobacter*, *Bacillus cereus*, *Citrobater*, *Methylobacterium*, *Enterobacter*) were used for the treatment at small scale and they recorded color reduction (50–97%), BOD (80–96%), COD (80–97%), and lignin (50–97%) (Tyagi et al. 2014; Tiku et al. 2010; Raj et al. 2007). Similarly, several researchers have reported that individual or consortia of bacteria have the potential to degrade kraft lignin as well as its derivatives produced from diverse pulping processes (Chandra et al. 2011; Chandra and Bharagava 2013). The study of Chandra and Singh (2012) suggested that during bioremediation of paper mill effluent using consortium of bacteria (*Pseudochrobactrum glaciale*, *Providencia rettgeri*, and *Pantoea* sp.) the ligninolytic enzyme (LiP, Lac, and MnP) induction was observed. Within 216 h after treatment, the bacterial consortia successfully eliminated lignin and chlorophenol (90%). Raj et al. (2014a) and Haq et al. (2016a, b, c) confirmed generation of laccase and lignin peroxidase enzyme during the degradation of paper industry wastewater by *Paenibacillus* sp. and *Serratia liquefaciens,* respectively. In addition to color and lignin, the bacterial treatment methods also eliminate the effluent BOD and COD. Bioremediation of tannery effluent has been performed by many workers (Kumari et al. 2016). The study of Kim et al. (2014) reports the reduction of COD (98.3%) and Cr (88.5%) from tannery wastewater (TWW). In another study, the reductions in Cr, BOD, and COD were 63.8% and 90%, respectively, using *E. coli* and reductions in Cr, BOD, and COD were 73.5% and 95.4%, respectively, using *Bacillus* sp. during the treatment of tannery wastewater (Noorjahan 2014). Yusuf et al. (2013) reported removal of 85.2% of COD using *P. fragi* and 87.6% of COD using *B. subtilis* and from tannery effluent. El-Bestawy et al. (2013) used the consortium of five different bacterial spp. for the removal of COD (79.16%), BOD (94.14%), and Cr (93.66) from tannery effluent. The consortium of bacterial spp. (*P. aeruginosa, B. flexus, E. homiense,* and *S. aureus*) reduced 80% COD from wastewater of tannery industry. The bioremediation of distillery wastewater (DWW) using bacterial system has been performed by many researchers. Kaushik and Thakur (2009) isolated five separate bacterial strains from polluted distillery wastewater sites in order to evaluate COD and color elimination of wastewater for distillery wastewater degradation analysis.

The one bacterium (*Bacillus* sp.) of the five bacterial spp. was able to reduce COD (30%) and color (21%) significantly from distillery wastewater. After optimization of various environmental and physiological factors by Taguchi approach, the reduction of COD and color was increased up to 90% and 85%, respectively, by *Bacillus* sp. within 12 h of incubation period. In another study, David et al. (2015) found the bacterium *Pseudomonas aeruginosa* to have the efficiency to remove the color (92.77%) from DWW and while producing polyhydroxy butyrate in the presence of excess carbohydrate source. Melanoidins, a recalcitrant organic pollutant present in DWW, are the main coloring agent of effluent that are tough to be degraded by natural treatment system. Santal et al. (2016) isolated and identified a bacterium known as *Paracoccus pantotrophus* for the evaluation of melanoidins degradation. They found that the bacterium has the potential to decolorize melanoidins up to 81.2 ± 2.43% with aid of glucose and NH_4NO_3 as carbon and nitrogen sources. Further, Krzywonos et al. (2017) used *Bacillus megaterium* ATCC 14581 for color removal study. After optimization of organic and inorganic nutrient source, the bacterium was able to remove 38% color of the DWW. In other study, Georgiou et al. (2016) immobilized the ligninolytic Lac using two different matrices as alumina or controlled pore glass-uncoated particles and found the distiller wastewater decolorization percent as 71% and 74%, respectively, after 2 days of incubation period. In the study of Chen et al. (2013), they reported the combined biological process with microelectrolysis can achieve 97.1% color reduction from distillery wastewater. The result of this study also showed the important role played by ligninolytic enzyme and fungal biomass during decolorization process.

22.3.2 Phytoremediation

Phytoremediation is an ecofriendly and low-cost treatment technology. Phytoremediation is beneficial for the environment over the physicochemical cleanup methods that require chemicals, high cost, energy, disturbance of soil structure, and microflora. Phytoremediation is a technique that involves the association between plants and microbes for the removal of contaminants to preserve the health of living organisms and environment. It requires a different strategy for removing metal pollutant from polluted areas, for example, phytoextraction, psycho-stabilization, phytostimulation, phytovolatization, and rhizofiltration (Lee et al. 2013; Chirakkara et al. 2016). There are several articles present on phytoremediation and phytoremediation of municipal wastewater pollution.

Phytoremediation of paper mill pollutants has been reported by many workers like Kumar and Chopra (2016), who used water caltrop (*Trapa natans*) for the reduction of pollution load of paper mill effluent through phytoremediation technique. The result of the study showed that water caltrop (*T. natans*) have the potential to remove TDS, BOD, COD, TKN, and metals like Ca^{2+}, Mg^{2+} and K^+, Cd, Cu, Fe, Ni, Pb, and Zn of the paper mill effluent after the phytoremediation. In the study of Das and Mazumdar (2016), they reported that the plant *Salvinia cucullata* can reduce BOD, COD, TS, TSS, TDS, P, hardness and chloride, and several heavy metals (Cd, Cu, Cr, Ni, Pb, Mg, Mn, Fe, and Zn) present in paper mill effluent. Kumar et al. (2014) also proved the removal efficiency of plant *Croton sparsiflorus* for the treatment of pulp wastewater as it can remove the heavy metals such as Pb, Cr, Cd, and Zn. Johnson et al. (2019) studied the treatment of pulp and paper mill industrial effluent by phytoremediation using *Ludwigia abyssinica*, *Hydrolea glabra,* and *Ceratophyllum demersum*. The results of the study showed that *Ludwigia abyssinica* was highly efficient in the removal of Pb, Cr, Mn, and Zn, BOD, and COD as it was reduced to 69.42%, 64.60%, 80.40%, 57.48%, 82.09%, and 77.77%, respectively. Secondly, *Hydrolea glabra* has the potential to remove these parameters in terms of percentage as 54.85%, 52.10%, 53.30%, 54.99%, 76.12%, and 58.73%. *Ceratophyllum demersum* was less efficient compared to both plants, but it also reduces the pollutants significantly as 38.83%, 35.60%, 31.20%, 52.49%, 65.67%, and 57.67%, respectively.

Gupta et al. (2018) treated tannery wastewater contaminated soil using microbial-associated phytoremediation process. For treatment, Cr6+-resistant PGP *Pseudomonas* sp. (CPSB21 strain) were isolated from the effluent polluted soil. They applied the bacterium for the evaluation of Cr^{6+} toxicity reduction in soil. The result of the study showed that after bacterial treatment the toxicity of the soil was reduced and the growth of the sunflower plant grown in soil was increased due to enhanced nutrient uptake during pot experiment. In another study, Kassaye et al. (2017) used papyrus (*Cyperus papyrus*), Para grass (*Brachiaria mutica*), and swamp smartweed (*Polygonum coccineum*) for the treatment of TWW. The study's results indicate that the Cr present in TWW was shifted to root and shoot of plants from TWW. The Cr removal efficiency of the Para grass and papyrus was high compared to swamp smartweed from TWW. The bacterial-aided phytoremediation of organic contaminants in TWW from a traditional wastewater tanning plant has been documented by Gregorio et al. (2015).

The problems associated with distillery wastewater and a detailed study of existing biological treatment approaches have been reviewed by Kharayat (2012). Billore et al. (2001) used *Typha latipholia* and *Phragmites karka* for the treatment of distillery effluent and the result showed 64%, 85%, 42%, and 79% reduction in COD, BOD, total solids, and phosphorus, respectively. One of the categories of phytoremediation, known as phytoextraction, has been used for the removal of heavy metals from soil, which are essential for plant growth (Fe, Mn, Zn, Cu, Mg, Mo, and Ni). Some of the metals like Cd, Cr, Pb, Co, Ag, Se, and Hg with unknown biological function can also be accumulated (Cho-Ruk et al. 2006). Hegazy et al. (2009) reported the bioaccumulations of the four heavy metals like Cr, Cu, Pb, and Zn using *Lemna gibba* (duckweed)-contaminated industrial wastewater. The result of the study showed that *L. gibba* has the potential to accumulate the heavy metals and was ranked as Zn came in the first place followed by Cr, Pb, and Cu with bioaccumulation factors 13.9, 6.3, 5.5, and 2.5, respectively.

22.3.3 Electrokinetic Phytoremediation

The combination of phytoremediation with electrokinetics known as electrokinetic phytoremediation could be a promising approach for the remediation of metals in contaminated soil and wastewater (Saxena et al. 2020). The study of Mao et al. (2016) showed the removal of heavy metals from paddy soil using electrokinetic phytoremediation approaches. The findings show that the electrokinetic field (EKF) greatly increases the absorption of As and Cs in roots and shootings of the plant and thus increases the efficacy of phytoremediation. The use of electrokinetic phytoremediation for pollutant removal is the latest approach in this field and needs atomization of different parameters such as current application, intensity of electrical field, time period, etc., and their effect on pollutants removal is still a challenge (Mao et al. 2016). Further, the use of mixed pollutants (organic and inorganic) and electrical kinetic phytoremediation has also not been identified.

22.3.4 Microbial Fuel Cells

Microbial fuel cell (MFC) is a new and suitable technology for the treatment of industrial wastewaters. MCF is a bioelectrochemical system that utilizes microorganism for the conversion of organic pollutant into electrical energy. The use of electricity and excess sludge generation may be an effective replacement for traditional activated sludge treatment. MFCs have many advantages over conventional energy systems (such as direct generation of electricity, energy savings due to anaerobic treatment, low slot yield), environmental benefits (water recovery, low carbon footprint, reduced slot generation), economic benefits (power and value-added chemicals), and low operational costs and operating cost (self-generation of microorganisms, good resistance to environmental stress, and amenable to real-time monitoring and control) (Li et al. 2010; Gude 2016). MFCs are ecofriendly technologies for organic substrate electricity manufacturing in industrial wastewater that are free of processes downstream and work under mild operational conditions especially at ambient temperatures (Gude 2016). Primary sludge or processed wastewater was projected to contain 1.43 kWh/m^3 or 1.8 kWh/m^3 using MCFs, respectively (Ge et al. 2015). MFCs consume only about one order of aerobic processes on sludge basis (~0.3 kW or €0.6 kWh/kg of COD in average) of 0.024 kW, or 0.076 kWh/kg COD (primarily for feeding and blending the reactor) (Zhang et al. 2013a, b). This implies, in relation to traditional activated sludge processes that display great energy savings and potential for energy recovery from waste water treatment, that the MFCs consume only approximately 10% of external energy (Gude 2016).

22.4 Challenges and Future Prospects

Each and every industry faces challenges during wastewater treatment process in which the key issues are cost of treatment system, energy consumption, and safe disposal of waste. The degradation/detoxification of pollutants present in industrial wastewater is a serious concern and requires an appropriate treatment technology to remove the toxic contaminants for the safeguard of environment. In our previous articles, we have described various physicochemical and biological treatment technologies for wastewater treatment process (Haq and Raj 2018, 2020). Biological treatment system with its various aspects has emerged as a low-cost and ecofriendly system compared to conventional and other physicochemical processes that are costly and environmentally destructive. However, bioremediation also has some restriction, which involves low level of pollutant to microbes or high concentration of toxic compound that inhibit the microorganism and plants, inhibition of enzymatic system that is responsible for degradation, and many more.

To elaborate the future scope of the bioremediation, the research should be done on the following topics:

1. Isolation and identification of highly potential microorganism for the degradation of ROPs
2. Identification of prominent carbolic gene responsible for efficient enzyme production to enhance the degradation/detoxification of environmental pollutants
3. Development by recombination DNA technology (RDT) for successful bio- and phytoremediation of transgenic microorganisms and of plants
4. Use of appropriate plant species for phytoremediation.
5. Investigation of new rhizobacteria and endophytes for microbe-assisted phytoremediation.

But attempts remain in order, including phytoremediation on the ground, to achieve economic viability of bioremediation technologies.

22.5 Conclusions

On the basis of available literature, finally it is concluded that the wastewater of industrial origin is a chief source of toxic and hazardous pollutant that contaminates the environment. Therefore, industrial wastewater treatment system requires a cost-effective and environmental-friendly treatment technique that treats industrial wastewater holistically. In addition of this, the literature also shows that biological treatment system (microorganism and plants) is a promising approach for the removal of contaminants of industrial wastewater. But still there is gap of knowledge regarding the health hazard of industrial wastewater on aquatic and terrestrial ecosystem. In addition, it seems that priority should be paid to limiting and providing appropriate solutions for the current care environment. Therefore, this chapter includes comprehensive information on industrial wastewater, its characteristics, and environmental processing technologies.

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23 Vermifiltration Technology as a Sustainable Solution for Wastewater Treatment: Performance Evaluation, Applicability, and Opportunities

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Abstract

Vermifiltration technology is a nature-based sanitation solution for wastewater treatment, where polluted wastewater is treated with the help of earthworms. It is an extension of the vermicomposting process, adopted as a biofilter with earthworms, which takes care of almost all the sustainable and economic criteria for its effective implementation. The mechanism behind the vermifilters includes the earthworm's and microorganism's symbiotic interactions, which digest the suspended particles screened on the filter bed and degrade organic matter through enzymatic activity, and in the process of ingestion, it passively aerates the system by burrowing action and removes pathogens. In this context, the present chapter envisages the current state of the knowledge of the vermifiltration process, factors affecting the process and performance of vermifilters under different scenarios related to the treatment mechanisms, and effective applications and advantages of the technology. This technology is a stand-alone technology providing tremendous benefits and can be a new paradigm for wastewater treatment processes.

Keywords

Biofilter · Earthworms · Wastewater treatment process · Vermifiltration

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23.1 Introduction

There is plenty of water without life in the world, but there is no life without water anywhere. Water, clearly known as the elixir of existence, is one of the most important resources for our survival available on earth. It is important for the production and economic growth of food. However, countries all over the world have been facing the issue of extreme freshwater shortage in recent years. The main reasons for the rise in demand for freshwater are urbanization, population growth, and increased economic activity. As freshwater demands are increasing and scarce water resources are increasingly stressed, it is nothing less than unthinkable in the context of a circular economy to ignore the opportunities leading to resource recovery in securely managed wastewater systems (The United Nations World Water Development 2017). Municipal and industrial wastewater, once stigmatized as waste, is now more and more recognized as a valuable source of water, nutrients and energy. Safely managed wastewater is been regarded as the key to water-related sustainable development (Guppy et al. 2019). The reclaimed water can be used to irrigate new areas or replace valuable freshwater where crops are already irrigated. This is already happening as farmers use treated and untreated wastewater directly for irrigation or indirectly when it is discharged into freshwater bodies where it becomes diluted and diverted to the agricultural farms. The populations of some countries, namely Australia, Namibia, the USA, and Singapore, are also drinking treated wastewater (Ghernaout 2018).

Despite this, there is a dire need of setting up treatment facilities for reclamation of wastewater in most of the developing and underdeveloped nations around the globe. The need is to facilitate and expedite implementation of resource recovery innovations where most municipal wastewater still goes into the environment untreated (Drechsel et al. 2015). This untreated wastewater adds to the pollutants such as organics and nutrients into the aquatic ecosystem, posing a threat to the marine life and further depleting valuable water resources by the phenomenon of eutrophication. Wastewater treatment can be tailored to meet the water quality requirements for a planned reuse, which includes the treatment of wastewater in an environmentally sound and economically effective manner (Arora and Kazmi 2015). A lot of conventional and centralized technologies have been incorporated for the treatment of wastewater such as activated sludge system, anaerobic treatment system, moving bed biofilm reactor, etc. However, the technologies come with their own drawbacks as stated in Fig. 23.1. Furthermore, most of the population living in rural and urban areas of developing countries depends upon onsite systems for the treatment of domestic wastewater. The treatment systems that require relatively low costs, energy, and maintenance are preferable for the treatment of domestic wastewater. Numerous solutions have been adopted for the treatment of domestic wastewater such as constructed wetlands, soil infiltration trenches, and vegetation-based wastewater treatment and vermifiltration. Among these technologies, vermifiltration has represented its efficacy as other technologies are restricted to large occupying area. Vermifiltration technology for wastewater treatment represents a techno-economically feasible and emerging solution for water pollution control, water

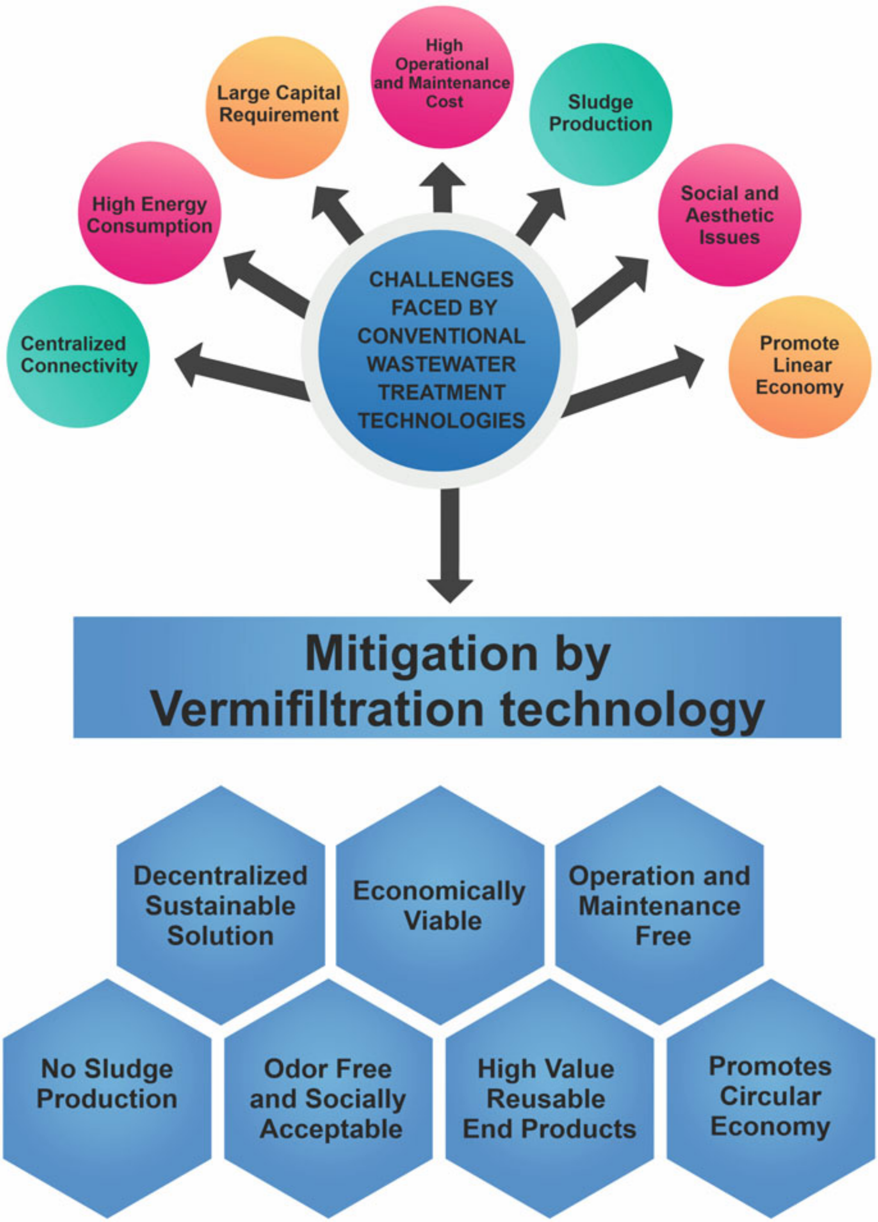

Fig. 23.1 Challenges faced by conventional wastewater treatment technologies and their mitigation by vermifiltration technology

conservation, and reuse of water for non-potable purposes placing them in the suitable alternative for appropriate technology. Throughout the world, it has been researched upon successfully by the scientists for the treatment of domestic wastewater prevailing in various conditions. The results from industrial applications such as dairy industry, gelatin industry, textile industry, palm oil industry, herbal pharmaceutical industry, hospitals, and clinical laboratory are also encouraging and proving its worth for industrial wastewater remediation.

23.2 Design Operation

There are three basic stages in a typical wastewater treatment process. It starts with a preliminary screening by mechanical grids to exclude large material and grit. Grit is removed to protect the pumps and ensure free movement of the water through the plant. This is followed by the first stage of primary treatment, which involves the settlement and sedimentation of fine solids, resulting in removal of suspended organic solid content from the water by 50%. Secondary treatment involves the processes of oxidation and biodegradation. Aerobic bacteria, thriving in the optimized conditions provided, lead to the significant reduction of BOD, COD, N, and ammonia (NH_4^+-N) levels in the effluent. This is the main biological aspect of the process and involves the two essentially linked steps of initial bioprocessing and the subsequent removal of solids due to enhanced biological activity. The high concentrations of nitrogen (N) and phosphorous (P) lead to eutrophication of waterways. Therefore, in order to achieve a successful reduction, after secondary treatment, the effluent is moved to the tertiary treatment unit. Tertiary treatment is required in most cases as an advanced final step in the removal of trace organic substances or in the disinfection of waste, which adds dramatically to the cost of sewage management. The treated water may be suitable for reuse at the end of the process, but it may be difficult to find sufficient outlets for the concentrated sewage sludge generated. Another challenge is sewage sludge disposal, which includes a separate treatment facility (Liu et al. 2012). Contrary to this, vermifiltration technology encompasses all forms of treatment, that is, primary (removal of grit, silt, etc.), secondary (biological degradation and nutrients removal), and tertiary (removal of pathogens) treatment technology into one unit. The performance of a VF for wastewater treatment is dependent on certain crucial factors. These factors affect the design of the VF, which includes hydraulic loading rate (HLR), hydraulic retention time (HRT), type of earthworms species used, stocking density (SD) of earthworms, filter medium (filterbed or bedding) for the earthworms, and suitable environmental conditions (temperature, pH, and moisture) for earthworm's growth and survival. The important design parameters are listed in Table 23.1 along with the major roles played by them during the filtration process. Together these parameters help in the efficient removal of solids, organics, and pathogens from the wastewater. The reclaimed water can then be reused in agricultural fields or for recreational household purposes as represented in Fig. 23.2.

23.3 Mechanistic Insights: Earthworm–Microorganisms Interactions

Vermifiltration is an organic degradation process involving earthworms–microorganisms interactions. The microorganisms are responsible for the biochemical degradation of the organics, and earthworms further enhance the process by

Table 23.1 Important parameters involved in the vermifiltration process and their role in the treatment

Parameters	Role in the treatment	Optimum conditions/ types	References
Hydraulic retention time (HRT)	It is the time of interaction of wastewater with the VF column where the earthworms reside. An appropriate microbial community can only be established in the VF, when adequate contact time is offered by providing longer HRT	3.5–8 h[a]	Sinha et al. (2012), Singh et al. (2019)
Hydraulic loading rate (HLR)	It is the rate of application of wastewater to the unit area of filterbed per unit time. Higher HLR leads to reduced HRT whereas low HLR reduces the operational benefits obtained from the system. Thus, optimum HLR is important for pathogen removal and efficient treatment performance of the VF	1.5–2.5 m^3/m^2 day[a]	Sinha et al. (2012), Kumar et al. (2014), Singh et al. (2019)
Earthworm species	Different earthworm species have different effects on the treatment processes. The epigeic worm species are a key for decomposition of biomass due to their preference for organic-rich substrates compared to the other species	*Eisenia fetida, Eisenia andrei, Perionyx sansibaricus*, *Perionyx excavates, Lumbricus rubellus*, *Eudrilus eugeniae,* and *Eisenia hortensis*	Gajalakshmi et al. (2001), Rajpal et al. (2014), Kumar et al. (2016)
Stocking density	It is the density of earthworms per unit area of filterbed. To achieve higher treatment efficiencies, higher earthworms population should be applied in the optimum nutrient enriched media	15,000–20,000 worms/ m^3	Sinha et al. (2012), Samal et al. (2018)
Filter media	Characteristics of VF bedding materials influence the establishment of microbial biofilms and the microbial community structure within the vermin-ecosystem. It also affects the activity of earthworms by offering different humic substances and different retention time for processing and decomposition of the organics loaded into it	Riverbed material, gravels, garden soil, vermicompost, wood chips, bark, cocopeat, straw	Arora et al. (2014), Kumar et al. (2015), Lourenço and Nunes (2017)

(continued)

Table 23.1 (continued)

Parameters	Role in the treatment	Optimum conditions/ types	References
Environmental conditions (pH, temperature, etc.)	Earthworms are sensitive organisms and their survival depends on the environmental conditions of their habitat such as pH, temperature, soil salinity, moisture content, and nutrient profile of the soil	pH 5.5–8.5 Temperature 25–30 °C Moisture content 50–90%	Arora and Kazmi (2015), Samal et al. (2018)

[a]Values based on vermifilter volume and configuration

proliferating the growth of aerobic microflora through their burrowing activity. Earthworms can modify the microflora of a vermifilter (VF) directly and indirectly by three main mechanisms: (1) comminution: burrowing and casting; (2) grazing; and (3) dispersal. These activities help in changing the physicochemical and biological status of the effluent as well as the filterbed and cause drastic shifts in the density, diversity, compositions, and activities of microbial communities present in the VF (Liu et al. 2012). The indigenous microbial community also forms a symbiotic association with the earthworm-associated microbes. This consortium between the microbial communities leads to the formation of a biofilm, which is responsible for the degradation activity observed in the VF against wastewater components and pathogens (Arora et al. 2020a). The treatment performance of vermifiltration is highly dependent upon the microorganisms present in this biofilm, and the degradation activity has been elucidated in various previous studies, in the form of antimicrobial activity (Arora et al. 2016) and enzymatic activity (Arora et al. 2014). In vermifiltration, microbes are responsible for biochemical degradation of organic matter and earthworms present in the VF also help in the removal of harmful pathogens by engulfing them and by the discharge of antibacterial coelomic fluid pertaining antimicrobial properties. Antimicrobial activity of the microorganisms inhibits the growth of pathogens during the treatment. Other attributed factors affecting pathogen removal may include bacterial adhesion, that is, the property of filter media to retain pathogens during filtration, unsuitable physicochemical environment for pathogen survival and predation of pathogens to regenerate bed for further adhesion. Enzymatic activity of the microorganisms helps in biodegradation of the organic matter. Microorganism releases certain enzymes such as cellulase, amylase, and protease that cause degradation of cellulose, amylase, and protein components of organic matter into smaller and simpler units. This also contributes to the decontamination and nutrient enrichment of filterbed layer, which in turn can be reused as manure. Therefore, vermifiltration is a wholesome, decentralized eco-innovation that provides nutrient-enriched and safe end products fit for reuse (Fig. 23.3).

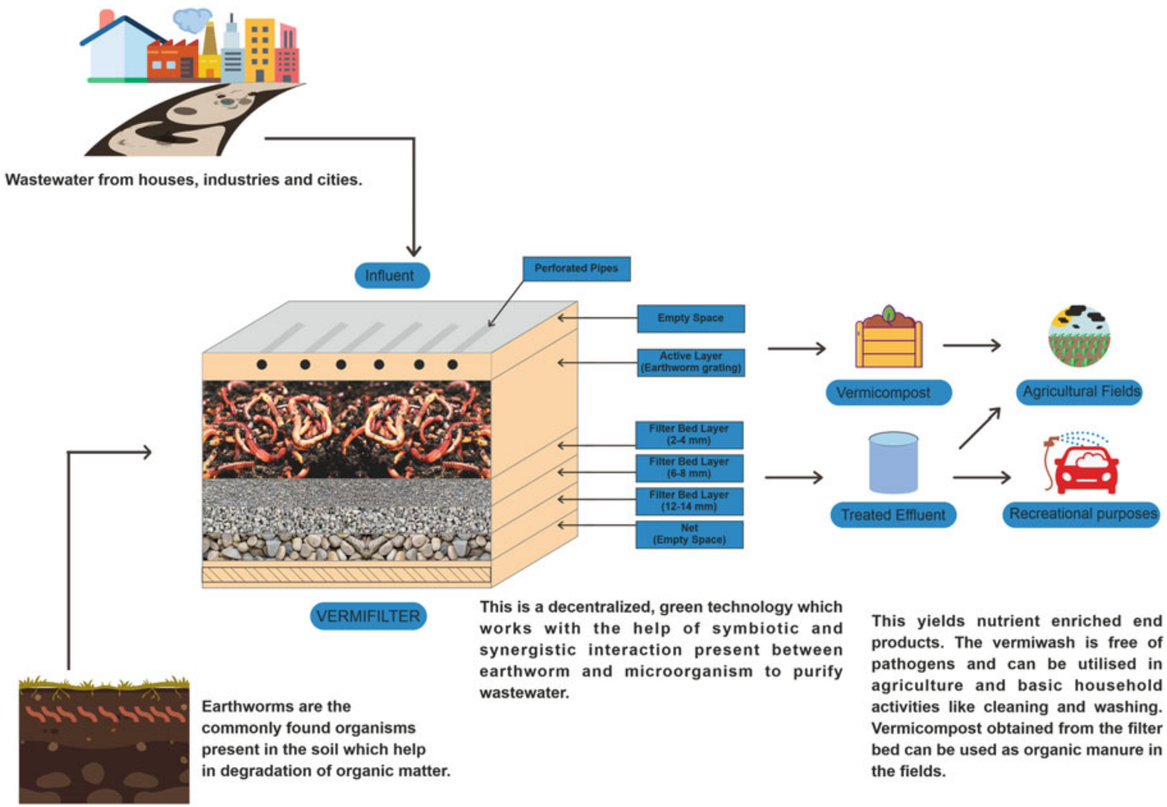

Fig. 23.2 Schematic diagram of vermifiltration technology for wastewater treatment

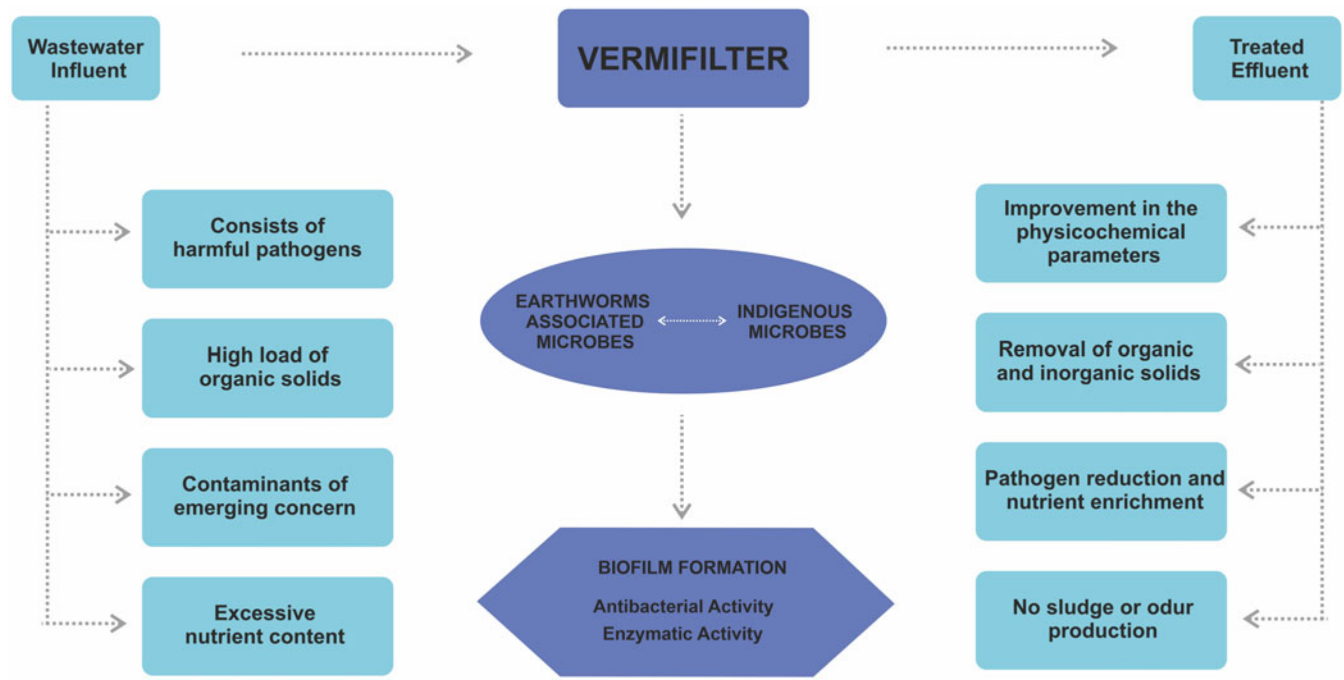

Fig. 23.3 Mechanism elucidating vermifiltration process for wastewater treatment

23.4 Applications of Vermifiltration Process

In the recent years, vermifiltration technology is applied for the treatment of domestic wastewater and effluents of various industries like dairy industry (Samal et al. 2018), gelatine industry (Ghatnekar et al. 2010), palm oil industry (Azuar et al. 2015), herbal pharmaceutical industry (Dhadse et al. 2010), etc. It is also a recommended solution for wastewater treatment in developing countries as represented in Table 23.2. Vermifiltration of wastewater results in treated water, which can be used for irrigation purposes and a biofertilizer—vermicompost instead of unwanted sewage sludge is also obtained. Another big advantage of great economic and environmental significance is that production of vermicompost is at least 75% cheaper than the chemical fertilizers and over successive years of application, vermicompost build-up the soils natural fertility improving its total physical, chemical, and biological properties (Sinha et al. 2012).

23.4.1 Booming Vermitechnology in India

Bihar, Tamil Nadu, Gujarat, Maharashtra, and Karnataka are the leading states in vermitechnology. A household pour flush toilet combined with primary vermifiltration and direct effluent soakage to soil, called the "Tiger Toilet," has been tested by Bear Valley Ventures and Primove Infrastructure Development Consultants in rural India. It was found that there was virtually no accumulation of fecal material over a 1-year period. In the effluent, there was a 99% reduction in fecal coliforms (Furlong et al. 2015). This system is now being marketed commercially in India where over 2000 of these toilets and treatment systems had been sold and installed by May 2017.

23.4.2 Vermifiltration Associated with Plants

Over the years, many modifications have been made in the technology especially by the addition of plants for improving the effluent quality. In 2011, Wang et al. analyzed the performance of a novel three-stage vermifiltration system planted with *Penstemon campanulatus*. The study showed that this system had higher removal efficacy for all the pollutants and novel microorganisms were isolated from the filter media. Samal, Dash and Bhunia, in 2018 designed and developed a hybrid macrophyte (*Canna indica*)-assisted vermifilter for the treatment of dairy wastewater. This system worked well for effective reduction of pollutants in dairy wastewater. Kumar and Ghosh, in 2019, evaluated the effects of vermifiltered water on *Allium cepa* (onion). The treated wastewater inhibited chromosomal abnormalities and helped in normal root growth and root germination. This study proved the worth of vermifiltered wastewater for agricultural purposes. Time scales of these modifications are mentioned in Fig. 23.4.

Table 23.2 Summary of the recent studies for wastewater treatment in developing countries by vermifiltration technology

References	Country	Vermifilter design	Important findings
Domestic wastewater			
Wang et al. (2011)	China	A novel three-stage VF system made out of PVC using *Eiseniafetida* was established and planted with *P. campanulatus* at a density of 20 plants/m^2. The filterbeds of four layers of soil, silver sand, fine detritus, and cobblestones	Average removal efficiencies were as follows: COD 81.3%; NH_4 98%; TN 60.2%; TP 98.4%. Three-sectional design with increasing oxygen demand concentration in the effluents, and the distribution of certain oxides in the padding proved beneficial for ammonium and phosphorus removal. Introduction of *P. campanulatus* improved the plant uptake of nutrients and aesthetics. Changes in microbial community diversity in different paddings and stages were observed and sequences retrieved mainly belonged to *bacilli of Firmicutes*
Manyuchi and Phiri (2013)	Zimbabwe	VF bed was made up of gravel of different sizes, sand, garden soil, and fibrous plastic filter. 500 *Eiseniafetida* earthworms were used based on a 5000–10,000 worms/m^2 calculation	Neutralization of pH was observed. Decrement in BOD_5, COD, TDSS, and turbidity was reported by 98%, 70%, 95%, and 98%, respectively
Arora et al. (2014a)	India	Different VFs made up of polypropylene material were set up with a cross-sectional area of 250 × 200 × 250 mm. Top layer consisted of vermicompost followed by second layer of different filter media: Riverbed gravel, mud balls, glass balls, and coal. Third and fourth layers comprised gravel of size 1–2 mm and 10–12.5 mm each. It was inoculated with 100 worms (*Eiseniafetida*) to achieve a SD of 10,000 worms/m^3 and were fed at HLR of 1.0 m^3/m^2 day	Application of different filter media was analyzed. Riverbed and mud balls media proved to be promising bed materials. VF with riverbed material showed maximum BOD and COD removal of 76% and 67%, respectively, and VF with mud balls showed maximum log removal of TC (2.8), FC (2.7), FS (2.2), *salmonella* (2.1), and *E. coli* (2.1)
Arora et al. (2014b)	India	VF and geofilter (GF) chamber of dimension 80 × 40 × 80 cm were set up. Filterbed consisted of vermigratings, sand, small and large gravels. HLR of 1.0 m^3/m^2 day and HRT of 7.8 h were setup. The VF was inoculated with *E. fetida* at SD of 10,000 worms/m^3	This study explored symbiotic interrelationship between earthworms and microorganisms for elucidating the mechanism of organic matter biodegradation and removal of pathogens. The removal efficiency of BOD and COD with VF were 92% and 74% while in geofilter it was observed 74% and 68%, respectively. Antibacterial activity

			of the microorganisms inhibited the growth of pathogens during the treatment. The concentration of pathogens (FC and FS) was reduced considerably. Cellulase, amylase, and protease activity were responsible for biodegradation and stabilization of organic matter
Arora and Kazmi (2015)	India	A PVC VF having dimensions 25 × 20 × 30 cm was setup. The filterbed comprised gravels of varying size, sand particles, and vermicompost. *Eiseniafetida* were inoculated with SD of 10,000 worms/m^3 and HLR of 1.0 m^3/m^2 day	The study explored the effects of seasonal temperature on the treatment efficiency of VF. Higher BOD and COD removal was accomplished during spring and autumn. However, during summer, the indicator bacteria removal was maximum by 99.9%, *salmonella* reduction by 96.9%, and *E. coli* by 99.3%. The pathogen removal efficacy of VF increases with the increase in temperature. All the performance parameters were positively correlated with temperature, except TSS removal and fungi population
Kumar et al. (2016)	India	*E. Fetida* (VF1) and *E. eugeniae* (VF2) were cultured in individual reactors of dimension 250 × 200 × 300 mm. The HLR of 2.5 m^3/m^2 day was set. Filter media consisted of vermicompost and river bed material of varying sizes	Results revealed a significant removal of BOD (88%), TSS (78%), and TDS (75%) in the treated wastewater from VF1, while in VF2, it was observed to be 70, 67, and 66%, respectively. Significant reduction of TC (3.1 log) was observed in VF1 along with increase in earthworm biomass as compared with 0.98 log reduction in VF2 where mortality was observed. Overall, in VF1, the effluent was rich in nitrate, phosphate, and showed the potential of *E. fetida* over *E. eugeniae*
Lourenço and Nunes (2017)	Portugal	4 VF was constructed from PVC container containing vermicompost and sawdust with (VE and SE) and without *Eisenia fetida*. Filterbed consisted of vermicompost, gravels, sawdust, and quartz sand. SD was of 20 g/L, HRT of 6 h, HLR of 0.89 m^3/m^2 day, and OLR of 7.38 g BOD_5/day	Treatment efficiencies were 91.3% for BOD_5, 87.6% for COD, 98.4% for TSS, and 76.5% for $NH4^+$ in VE, and 90.5% for BOD5, 79.7% for COD, 98.4% for TSS, and 63.4% for $NH4^+$ in SE. Earthworms contributed to reduce $NH4^+$ and TN removal and to increase NO_3-concentration. No treatment was able to eliminate FC down to guidelines values but helminth eggs were completely eliminated

(continued)

Table 23.2 (continued)

References	Country	Vermifilter design	Important findings
Wang et al. (2017)	China	Perspex cylindrical filters were inoculated with *Eisenia fetida* with initial SD of 32 g/L. The HLR and the organic loading were kept at 2 $m^3/(m^2$ day) and around 1.0 kg COD/m^3 day	Earthworm biomass decreased slightly from 32.0 to 24.2 g/L, while their average weight increased. Notably, the earthworms showed a higher Shannon–weaver index (from $H = 2.76$–3.06) than the BF and upregulated some proteins to cope with the negative effects from the VF. V-ATPase, actin, and tubulin were the main upregulated proteins that play a variety of crucial roles in many cellular processes
Adugna et al. (2019)	Ethiopia	Filter media consisted of medium-size gravel, coarse gravel, and sand varying with the topmost layers in VF1 and VF2 as fine sawdust and VF3 as cow dung. It was inoculated with 200 individuals of *Eudrilus eugeniae* at HLR of 16 L/m^2 day	The VFs performed better for BOD_5, tCOD, dCOD, TSS, and NH^{4+} removal, and the control unit was slightly better for NO^{3-}, NO^{2-}, and PO_4^{3-} removal. VF2 and VF3 had 5–7% higher removal efficiencies than the control, except for TSS. Most common microbial communities working with earthworms, i.e., fungi, bacteria, and actinomycetes were identified in the sample of filter materials at different depths. More bacteria were observed in VF2 and VF3. VF2 performed slightly better than VF3
Arora et al. (2020a)	India	A concrete VF having dimensions 1 m^3 was setup. The filterbed comprised river gravels of varying sizes. *Eisenia fetida* were inoculated with SD of 10,000 worms/m^3 with HLR of 1.0 m^3/m^2 day and HRT of 7–8 h	The average removal efficiencies of BOD_5 and COD were found to be 98% and 92%. The major focus of the study was to elucidate the mechanism involved in vermifiltration by investigating antimicrobial and enzymatic activity of isolates from influent, effluent, earthworm gut and filter media, and protein profiling assay of earthworm coelomic fluid
Ghasemi et al. (2020)	Iran	Metal cube VF of dimension 1 × 1 × 1.5 m was bedded with coarse gravel, fine gravel, windmill, and vermicompost. 5000–6000 *Eisenia fetida* were inoculated per cubic meter of sewage and the HLR of 2 m^3/m^2 day was setup	The results demonstrated that the VF system was able to reduce the EC, COD, nitrate, and turbidity with removal efficiencies of 80%, 80%, 60%, and 90%, respectively. But the average amount of 19 ppm nitrate with 54% reduction efficiency in treated water specifies that the system has been working weak to reduce the nitrate

Ndiaye et al. (2020)	Burkina Faso	The VF system was made of gravel, sand and sawdust, on which 200 earthworms—*Eudrilus Eugeniae* were added	BOD_5, COD, TSS, and *E. coli* removal efficiencies ranged from 93 to 98%, 68 to 93%, 88 to 96%, and 1.4 to 3 ULog, respectively. High proportion of surfactants (95–99%) and oil (84–89%) were removed but sodium was not removed
Industrial wastewater			
Ghatnekar et al. (2010)	India	Three-tier vermiculture biotechnology coupled with VF system and inoculated with *Lumbricusrubellus*. Filterbed consisted of rammed clay, semi-crushed bricks, gravel, and fine sand	The treated water exhibited a significant decrease in COD by 90.08 ± 0.176% and BOD by 89.24 ± 0.544%
Sinha et al. (2012)	Australia	About 1000 earthworms (*Eisenia fetida*) were released into the VF bed, which consisted of gravels, aggregates, and garden soil. HRT was kept between 1 and 2 h and SD of 1000 worms/m^2 was maintained	Removal of BOD_5 by 90%, COD by 60–80%, TDSS by 90–95%, and toxic chemicals and pathogens from wastewater was observed. The hydrocarbons C10–C14 were reduced by 99.9%, the C15–C28 by 99.8%, and the C29–C36 by 99.7% by earthworms
Ghobadi et al. (2016)	Iran	Pyrex glass reactor of dimension 40 × 40 × 120 cm was inoculated with *Eisenia fetida* with HLR of 1 m^3/m^2 day. Filter media consisted of garden soil-earthworm, sand, detritus, and cobble stones	Treatment caused a significant decrease in the levels of COD (75%), BOD_5 (93%), and TSS (89%) as well as neutralized pH of the wastewater
Manyuchi et al. (2018)	Zimbabwe	10 kg of *Eisenia fetida* earthworms were used as the VF media in a 0.5 × 0.5 × 0.3 m VF bed over a 40 h period cycle. Filter media consisted of earthworms and gravels mixed with sand	The effluent pH changed from acidic to neutral whilst a decrease of 94.9% was observed for TKN, 91.1% for BOD, 91.9% for TDS, 92.4% for TSS, and 89.4% for COD. The vermicompost had a nitrogen, phosphorous, and potassium composition of 1.87%, 0.87%, and 0.66%, respectively
Samal et al. (2018)	India	Two VF having earthworms *Eisenia fetida* (SD of 10,000 worms/m^2) were set up in which one reactor was planted with *Canna indica* (MAVF). HLR of 0.6 m^3/m^2 day was setup. Filter media consisted of a mixture of vermicompost and garden soil in the ratio of 1:3, washed sand, laterite stone, and coarse gravel	The average removal efficiencies of BOD_5 and COD in MAVF were found to be 88.4% and 80.7%, respectively, while for VF, it was 78.3% and 69.1%. TN and TP removal of 61.7% and 51.3% and 77.8% and 73.4% was observed in MAVF and VF, respectively. The efficiency of MAVF was more than VF

(continued)

Table 23.2 (continued)

References	Country	Vermifilter design	Important findings
Singh et al. (2018)	India	Plexiglass reactor of dimension 80 × 20 × 20 cm was inoculated with *Eiseniafetida* at SD of 0–10,000 worms/m^2. HLR of 1.8–4.5 m^3/m^2 day was setup. Dolochar is chosen as an adsorptive layer along with the filterbed media	The optimal conditions for achieving maximum COD removal were 3542.22 mg/L, SD of 9661.33 earthworms/m^3, and HLR of 1.84 m^3/m^2 day. At the optimum conditions, COD removal of 94.99% was obtained. ANOVA analysis stated that HLR has significant negative impacts on the COD removals whereas SD impacts positively on the COD removals
Kumar and Ghosh (2019)	India	Filter media consisted of vermicompost, sawdust, sand, small-, medium-, and large-sized stone chips	Properties of vermiaqua and wastewater on root germination of *Allium cepa* at different concentrations (10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}) were investigated to study the impacts on morphological and cytological characteristics. BOD_5 loads were also reduced by 91%. Vermiaqua treated root germinations were "accelerated," with the highest number of germinated roots at 10^{-5} concentration. Only a single type of chromosomal abnormality was observed at anaphase stage of vermiaqua-treated roots
Manyuchi et al. (2019)	Zimbabwe	A 3-stage VF that comprised three 20 L/day vermifiltration beds arranged in series comprising 40% garden soil and earthworms (*Eiseniafetida*), 30% sand and 15% 10 and 30 mm quartz stones was established	The study observed a significant decrement in COD, BOD_5, TSS, TDS, and EC by 99.2%, 99.4%, 99.9%, 80.2%, and 86.9%, respectively. DO values increased by >345.5% and earthworms and vermicompost were produced as by-products during the vermifiltration process
Singh et al. (2019)	India	Plexiglass reactor of 80 × 20 × 20 cm was setup with *Eiseniafetida*. The HLR was ranged between 1.80 and 4.50 m^3/m^2-day. the flow in the filters was varied between 0.0144 and 0.0360 m^3/day with the SD ranged between 0 and 10,000 worms/m^3 and HRT of 10.67 and 26.67 h. filter media consisted of compost, soil mix, and dolochar	A statistical experiment was conducted with the objective of optimization of the role of earthworms in alleviating the bioclogging of a horizontal subsurface flow vermifilter (HSSFVF). SD of 9475 earthworms/m^3, HLR of 1.84 m^3/m^2-day, and influent COD of 3701 mg/L have been observed as the optimized values for the minimum bioclogging in the VF

Arora et al. (2020b)	India	A concrete VF having dimensions 1 m^3 was setup. The filterbed comprised river gravels of varying sizes. *Eiseniafetida* were inoculated with SD of 10,000 worms/m^3 with HLR of 1.0 m^3/m^2 day and HRT of 7–8 h	The results showed that earthworms and VF associated microbial community had a significant effect on BOD and COD reduction (78–85%), pathogen removal (>99.9%), and caused a significant shift in the prevalence pattern of ARB. Molecular profiling of ESBL (bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$), MRSA (*mec-A*), and Colistin (*mcr-1*) gene confirmed the probable mechanisms behind the resistance pattern. The microbial community diversity assists in the formation of biofilm, which helps in the removal of pathogens and results in a paradigm shift in the resistance profile of ARB and ARG
Rustum et al. (2020)	UAE	A four layer vermi biofilter made using a plastic bucket of 20 L capacity contained earthworms—*Eisiena fetida* and *Eudrilos eugeniae*. Filter media consisted of different sizes of aggregates topped with garden soil and earthworm	The percentage changes in pH, TDS, TSS, COD, oil and grease, and DO after 60 h of treatment were −18, 79, 75, 67, 69 and −31%, respectively
Combined treatment			
Rajpal et al. (2014)	India	Three different earthworm species—*Eisenia fetida*, *Perionyx excavatus*, and *Perionyx sansibaricus*—Were used in different polypropylene vermireactors with dimensions 25 × 20 × 40 m. filter media consisted of gravels, sand particles, and vermigratings. HLR of 1 m^3/m^2/day and earthworm biomass of 500 mg per 2 kg of organic waste was optimized	The treatment caused removal of total organic carbon (65–75%), COD_{tot} (85–86%), BOD_5 (84–87%), NH_4-N (45–59%), and coliforms (99.9%) and increment in NO_3-N (172.5–186.7%) and TP (161–201%) in treated effluent. Nutrient enhancement in solid waste samples was observed. Performance of the earthworm species was recorded in the following order *E. fetida* > *P. excavatus* > *P. sansibaricus*. SEM revealed that vermicompost exhibited a distinct physical appearance than initial solid waste samples characterized by predominantly spherical cell-like structure and significantly lower number of filamentous bacteria

(continued)

Table 23.2 (continued)

References	Country	Vermifilter design	Important findings
Arora et al. (2016)	India	A pilot-scale VF having dimensions 80 × 40 × 100 cm was setup. Filter media comprised vermigratings, sand, and small and large gravels topped with OFMSW. *Eiseniafetida* were inoculated at a SD of 10,000 worms/m^2. HLR of 1.0 m^3/m^2 day was setup	The study focused on selecting the potential bacterial strains from VF that possess antimicrobial activity and release antimicrobial substances significant for pathogen removal. 16S rRNA gene sequencing showed that majority of bacterial isolates belong to phylum *Firmicutes* (50% of all strains) and *Proteobacteria* (50%), with representatives of class γ*proteobacteri*. SEM analysis showed diverse microbial colonies on the surface and confirmed degradation of OFMSW

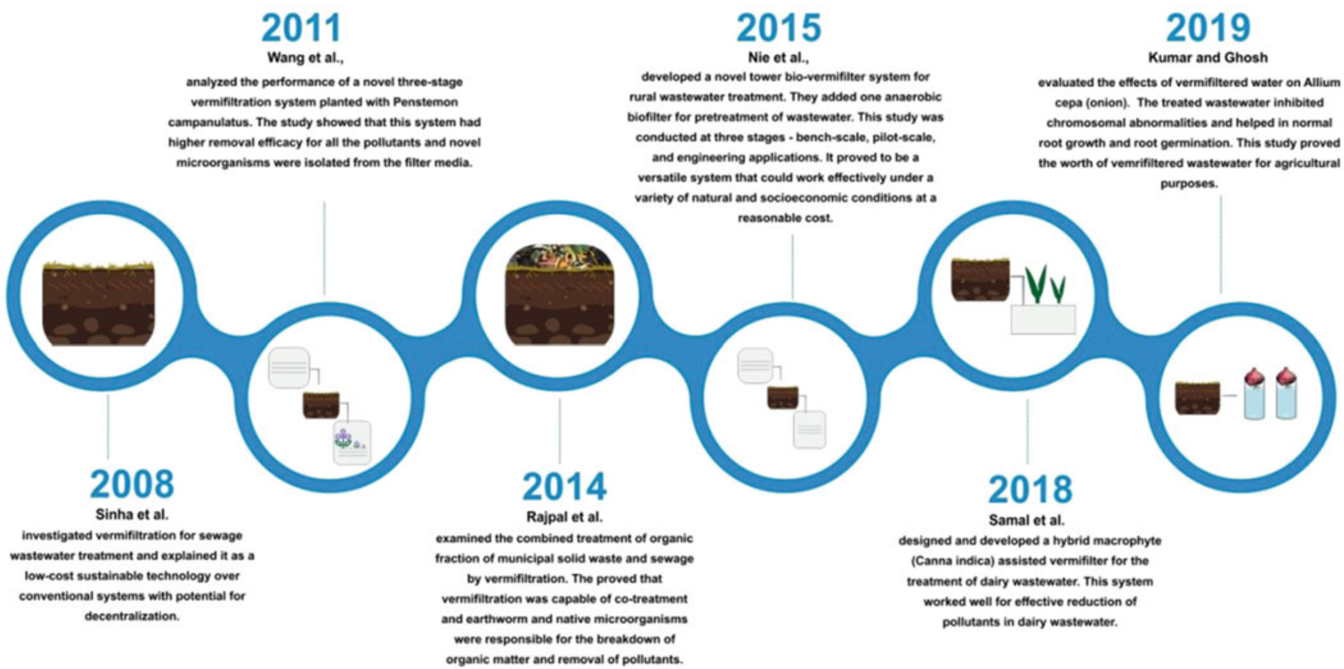

Fig. 23.4 Modifications and improvement in vermifiltration technology over the last decade

23.5 Conclusions

Vermifiltration has proven to be a capable technology for the treatment of different kinds of wastewater over the past decade. It is an ecofriendly wastewater management technology, which takes the assistance of both earthworms and the associated microorganisms to eliminate pathogens and make the effluent suitable for reuse and recreational purposes. The key factor behind effective treatment is the antimicrobial activity and enzymatic activity of earthworms and associated microorganisms. It is a cost-effective process, which is completely sustainable and profitable for wastewater treatment with efficiency, convenience, and potential for decentralized wastewater treatment and recently has also been applied at various field scale levels, majorly in the developing countries for the benefit of the community.

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Vermifiltration: A Novel Sustainable and Innovative Technology for Wastewater Treatment

24

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Abstract

Vermifiltration is a viable technology used to treat wastewater, which is formulated using earthworms by their potential to enhance the permeability of the soil and increase the rate of organic matter decomposition. It is an eco-friendly and low-cost technology for wastewater treatment affordable to the rural environment. Pollution originated from nonpoint sources causing negative impact on environment and human health. Many techniques deployed to treat wastewater by proving its efficiency in significant manner. But it is essential to find out the treatment method to low cost, easily affordable, available in around our premises, consume less energy and man power, and meet the standard of effluent discharge from the treatment unit. Wastewater treatment is performed by vermifiltration technique, an environmental-friendly approach to protect our mother earth through the sustainable and low-cost technology. Wastewater comprises many kinds of contaminants originated from various organic and inorganic sources. Pathogens and dissolved solids also reported in wastewater. Vermicast

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discharged by the earthworms provides proliferative microbial load and related enzyme activity to act upon the organic contaminants in the wastewater. In overview, vermifiltration technology is a prominent and feasible method to solve the problems raised due to the wastewater in terms of chemical contaminants and pathogenic organisms.

Keywords

Earthworm activity · Nutrient dynamics · Pathogen removal · Sustainability · Vermifiltration technology

24.1 Introduction

Availability of freshwater is the essential resource; it represents less than one percentage of water available to the consumption of human beings; 97.5% existed in the ocean and two percentage of water in the frozen state. Water greatly influences food security, public health, and socioeconomic development. A severe water crisis is expected in the near future, and in a few decades, 50% of the global population will face acute water scarcity. The contaminants available in wastewater cause severe risk to the health of human and environment (WHO-FAO-UNEP 2006). According to the National Water Policy (2012), the allocation priorities of water in planning and operation include (1) drinking water, (2) irrigation, (3) hydropower, (4) ecology, (5) agro-industries, and (6) navigation. Water and energy are two important factors essential for the global agriculture and sustainability to meet the food crisis and acute shortage of resources for human beings (Mohan et al. 2017). This is the right time to find out alternative robust method to solve the wastewater-related problems. Vermifiltration is a prospective approach by using total efficiency of earthworms to treat wastewater in a lucid manner without disturbing the environment and using less energy with maximum benefits. Earthworms are known to involve waste management over the past 600 million years. Charles Darwin pointed this as “unheralded soldiers of mankind,” and Aristotle represented them as the “intestine of earth” (Darwin et al. 1903; Martin 1976). Tamil scholar, Philosopher Manonmaniam Sundaram Pillai called the earthworm as “Nangoolpuzhu” and highlighted the importance of earthworms to improve soil by ingesting and digesting the soil contents through chemical conversion to support farmers (Sundaram 1891). The main intention of this chapter is to elucidate the role of humble creature earthworm and its marvelous attempt around million years to shape and structure the soil and nourish the inhabiting plants. Tapping of this potential to treat the wastewater in eco-friendly manner with low or no cost embedded with our agro-biodiversity, and it makes right direction to know the simple concept to support the sustainable development.

24.2 Vermifiltration Process

Earthworms are considered to be an effective organism used to disinfect, detoxify, and neutralize the organic contents present in their habitat (Sinha et al. 2012). Bioaccumulation, biodegradation, and biotransformation of toxic materials like heavy metals, pesticides, and many organic pollutants by the earthworms are reported by many researchers (Jiang et al. 2016). The cast or excreta of earthworms extend their bioactive sites for absorbing heavy metals and pollutants existed in the wastewater. The physicochemical properties of vermifiltration are given in Table 24.1.

Soil comprises decomposing organisms in which earthworms exhibit its vermifiltration capacity. The microorganisms and soil fauna interacted with earthworm to make the vermifiltration process as bio-oxidative in nature strongly disintegrate lignin contents. Consumption of organic solids by earthworms accelerating assimilation resulted through mechanical disintegration and enzymatic digestion of detritus observed in wastewater. Earthworms have high reproductive rate and possess maximum tolerance capacity to the environmental pollutants (Sinha et al. 2008). The organic matter engulfed by earthworms finely fraction the size by its muscular movements. Figure 24.1 shows the pathway of earthworm as a bioreactor convert the waste into wealth by means of the production of vermiwash, a liquid fertilizer consist the potential pesticide value, and vermicompost, enriched compost, making the natural manure to nourish the plants. Aerobic environment provided to the vermifilter system by the burrowing nature of the earthworms, which in turn increase the surface area to enable the microbial action on the debris loaded in the wastewater. The mucus along with minerals present in the gut encompasses enzymatic potential mixed with the organic matter ingested by the earthworms. The mixture exhibits neutral pH that proliferates the microbial community in the gut of earthworms.

Vermifilter-based treatment of wastewater resulted greater reduction in biological oxygen demand (BOD) for more than 90%, chemical oxygen demand (COD) from 80% to 90%, total dissolved solids (TDS) up to 92%, and total suspended solids (TSS) maximum of 95% (Sinha et al. 2008). Earthworms efficiently increase the

Table 24.1 Physicochemical properties of vermiaqua (vermifiltered water) (Kumar and Ghosh 2019)

Parameter	Property
Odor	Odorless
Color	Pale yellow
Turbidity (NTU)	5
pH	7.5
Nitrate (mg/lit)	10
Ammonia (mg/l)	3
Iron (mg/l)	0.3
Phosphorus (mg/l)	Below detectable limit
Biological oxygen demand (BOD) (mg/l)	19

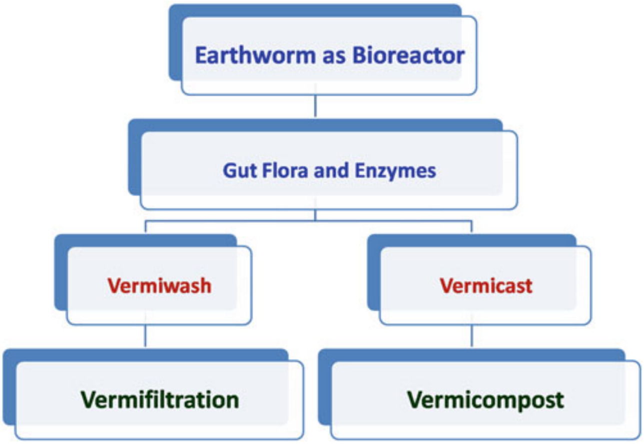

Fig. 24.1 Vermitechnology

decomposition of carbon, hydrogen, and nitrogen containing group resulted the formation of humus-rich organic material (Xing et al. 2010). The insoluble material is converted into soluble material by the action of earthworms to improve its rate of decomposition by the microbial and enzymatic reaction. Earthworms are depending upon organic matter constituting microbial communities for their nourishment. The cast of earthworm bears different kinds of macro- and micro-nutrients, and it has enzymatic activity due to the action of aerobic microorganisms. More number of enzymes like cellulase, protease, chitinase, lignase, lipase, phosphatase, and many other kinds of enzymes observed in the gut of earthworms. These enzymes involved in the digestion of organic matter comprise cellulose, lignose, pectin, etc. The range of pH observed in the earthworm's intestine is from 6.3 to 7.3 and it is maintained by the inherent buffering capacity observed in the intestine. The foregut and midgut of earthworms possess strong enzymatic activities (Brown et al. 2000).

24.2.1 Role of Earthworm Gut Microflora and Enzymes in Vermifiltration

Earthworms potentially disintegrate a variety of organic compounds in the presence of rich microbial biomass available in the mucus and intestine of the worms. Earthworms act as ecosystem engineer, efficiently involved in the process of cycling the nutrients. The cycling process by the influence of soil microorganisms is combined with the action of earthworms (Blouin et al. 2013). Earthworms play multiple roles in the natural environment transformed to treatment plant in turn create favorable habitat for the aerobic microbiota. Activity of the earthworms is depicted and highlighted in Fig. 24.2.

The vermicast of *Eisenia foetida* reported to increase the microbial load at about 2.7 times and the growth of microbes increased 124 times greater in the

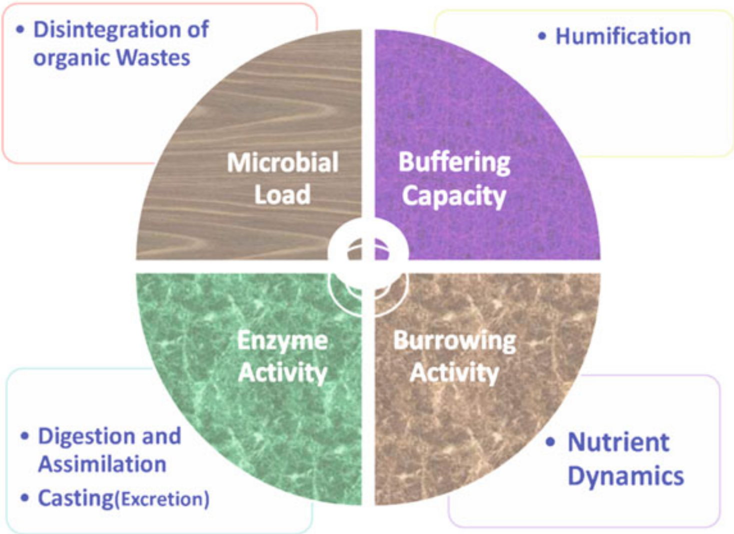

Fig. 24.2 Role of earthworms in vermifiltration unit

vermicompost when compared with the compost and both composts prepared in 9 h period (Yakushev et al. 2009). The increase in bacterial count of 3.2×10^4 CFU g^{-1} in farm yard manure compost to 1.3×10^7 CFU g^{-1} is recorded in vermicompost by the action of *Eisenia foetida* (Toyota and Kimura 2000). COD removal by the earthworms is possible by the presence of microbes and gut enzymes that decompose the organic matter present in the wastewater (Sinha et al. 2007).

24.2.2 Nutrient Dynamics Observed in the Vermifiltration Process

The nitrogen transformation happened through immobilization, nitrification, and denitrification process mediated by numerous microbes involved in the nitrogen metabolism in the gut of earthworms. The burrowing activity enables the aerobic condition in the vermin bed that creates ample environment for nitrogen stabilizing bacteria. The conversion of toxic ammonia and other forms of nitrogen into nitrate is effectively activated by the influence of earthworms. Biological nitrification in the wastewater increases the nitrate content activated by the earthworm activity. The results of in situ studies using 35–40 cm thickness earthworm bed for vermifiltration actively removed NH_3-N and total nitrogen from the wastewater (Yang et al. 2015). The earthworms are involved in the conversion of organic nitrogen and it acts as major determinant of nitrogen in the soil (Amosse et al. 2013). Nitrogen removal from dairy wastewater during vermifiltration processes performed by denitrifying microbial communities consist of beta-*proteobacteria* family (Lai et al. 2018).

After the mechanical degradation of organic matter by the earthworms along with its mucus substance secreted in the gut, it is excreted as worm casts that constitute

Table 24.2 Organics removal during the wastewater treatment with vermifiltration

S. No.	Wastewater type	Earthworm species used	BOD (%)	COD (%)	TDS (%)	References
1	Distillery effluent	*Eisenia foetida*	91.1	89.4	91.19	Manyuchi et al. (2018)
2	Sewage	*Eisenia foetida*	59.8–72.3	70.9–81.2	–	Kumar and Pruthi (2015)
3	Urban wastewater	*Eisenia foetida*	97.5	74.3	–	Lourenco and Nunes (2017)
4	Sewage	*Eisenia foetida*	98	70	95	Manyuchi and Phiri (2013)
5	Sewage	–	90	80–90	90–92	Sinha et al. (2008)
6	Herbal pharmaceutical wastewater	*Eudrilus eugeniae*	89.77–96.26	85.44–94.48	–	Dhadse et al. (2010)
7	Domestic wastewater	–	80	90	85	Priyanka et al. (2017)
8	Rural domestic sewage	*Eisenia foetida*	78	67.6	–	Liu et al. (2013)
9	Synthetic domestic wastewater	*Eisenia foetida*	81.2	72.3	–	Kumar et al. (2015)
10	Domestic sewage	*Eudrilus eugeniae*	85	82.2	–	Kumar et al. (2016)

enriched organic matter, nitrogen and phosphorus. These nutrients are readily absorbed by the microorganisms (Pathma and Sakthivel 2012). The vermicompost derived from the vermifiltration process reported that the compost bears the NPK content of 1.87%, 0.87% and 0.66% respectively (Manyuchi et al. 2018). Removal efficiency of COD, nitrogen, and phosphorous reported to be more than 90% in the integrated system using *Eisenia foetida* and *Phragmites australis* (Wang et al. 2010). Highest removal of BOD and COD of 94.5% and 91.5%, respectively, resulted in the treatment of herbal pharmaceutical wastewater (Dhadse et al. 2010). The potential of earthworms in organic removal in terms of BOD, COD, and TDS is listed in Table 24.2. In the vermifiltration process, organic matter and minerals are restored, and it is observed that the decrease of 40% dry matter, 50% of organic matter, 50% of ammonia nitrogen, 60% of total nitrogen, 30% phosphorous, organic carbon of 30%, inorganic carbon of 60%, and the concentration of potassium was found to be unchanged (Li et al. 2008).

24.3 Factors Affecting Vermifiltration Process

There are many factors influencing the rate and quality of vermifiltration process, viz., hydraulic loading rate (HLR), hydraulic retention time (HRT), earthworm culture and their density, culture media, C/N ratio, pH, temperature, and aeration (Fig. 24.3).

Hydraulic loading rate (HLR) is measured by taking the volume of wastewater in the vermifiltration unit, and it can be determined by using the following formula:

$$\text{HLR} = V/(A \times T)$$

where,

V = Volume of wastewater flow through the unit.
A = Area of the processing unit.
T = Time taken by the wastewater flow through the unit.

HLR mainly depends upon the available reproductive stage earthworms actively feeding per unit area of the vermifilter unit. The number, size, and healthy worms are considered to be a determination factors. HLR significantly vary with the type of medium, its porosity, bulk density, and structure. A study on HLR of wastewater used in vermifilter resulted the HLR with range of 1.5–3.0 m/day. The optimum HLR reported is 2.5 m/day, and it gives BOD and TDS removal efficiency by 96% and 82%, respectively (Kumar et al. 2014).

Hydraulic retention time (HRT) is calculated by considering the time taken by the wastewater to flow through the vermifilter unit in the tenure of wastewater treatment. The retention of wastewater in the vermifilter unit is essential to enable the earthworm action on the wastewater. HRT depends upon the rate of flow of wastewater through the vermifiltration unit, volume of stocking material, and its quality. During

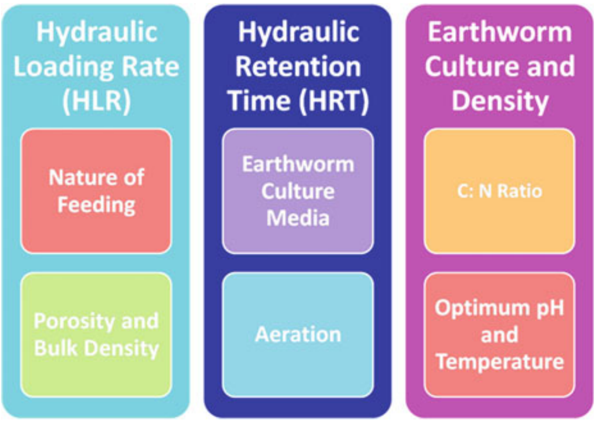

Fig. 24.3 Factors affecting vermifiltration unit

the retention time, earthworms perform physicochemical and microbial enzymatic processes. They can remove the nutrients and greatly reducing COD, BOD, and TDS. The length of retention time determines the vermiprocessing by earthworms. HRT of vermifiltration unit can be calculated by using the following formula:

$$\mathrm{HRT} = (p \times \mathrm{Vs}/Q)$$

where,

Vs = Volume of the vermifilter bed in which the wastewater flows.
p = porosity of the medium.
Q = Rate of flowing the wastewater through the vermifilter unit.

The reduction of HRT increases the HLR, and it reduces the contact time within wastewater, earthworms, and microorganisms (Kumar et al. 2014).

There are many kinds of feeding strategies undertaken, which comprise batch mode, continuous mode, and intermittent mode of feeding processes. The continuous mode of vermifilter process removed the COD and BOD up to 95% (Sinha et al. 2008). Intermittent mode of feeding procedure maintained regular flow and drains in the vermifilter unit and increased the aeration of the subsurface (Knowles et al. 2011). C/N ratio influences the removal of nutrients and greenhouse gases (Zhao et al. 2014).

Earthworm population is the important criteria in the vermifiltration process that strongly influence the rate of feeding and reproduction capacity. Right population density maintenance evidenced to restore earthworms by reducing mortality rate and increasing the rate of growth and cocoon production by an individual earthworm. It is reported that the density of earthworms at 1000 number of worms per cubic meter greatly remove the organics and pathogens (Arora et al. 2014).

24.4 Pathogen Removal during Vermifiltration Process

The significant removal of total coliforms, fecal coliforms, fecal, *Streptococci* and *Escherichia coli* and the reduction of BOD and COD at the rate of 84.8% and 73.9%, respectively, are observed during the vermifiltration treatment of wastewater (Arora et al. 2014). The temperature observed during spring and autumn season of 25–27 °C reported to be effective on the reduction of COD, BOD, and removal of pathogens like bacteria by 99.9%, *Salmonella* by 96.9%, and *Escherichia coli* by 99.3% and indicator species by using the earthworm *Eisenia foetida* (Arora and Kazmi 2015). Sewage mainly contains pathogens, and it is known to be left out by many researchers due to its complexity. Vermifilter unit possesses earthworms drastically remove the pathogens absorbed by its filter media (Kadam et al. 2008). The release of antibacterial material by earthworms reduces the coliforms and pathogenic organisms (Sinha et al. 2008). Vermifiltration process of domestic wastewater treatment converts waste into soil enriching compounds in a sustainable manner, and this method removes the pathogens from wastewater (Bajsa et al. 2003). A vermifiltration study consisting of different natural media from riverbed material, wood coal, mud balls, glass balls were carried out and the maximum removal of total coliforms, fecal coliforms, and *Escherichia coli* resulted with the river bed material as a feeding substance (Kumar and Pruthi 2015).

24.5 Sustainability by Vermifiltration Technology

Sustainability in wastewater management is to find out the appropriate disinfection method, membranes, wet land construction, and the reuse strategy of treated wastewater. United Nations suggested its 17 sustainable development goals, which includes ethical, social, and economics related aspects and giving importance to gender equality and eradication of poverty. The sixth goal listed in the 17 sustainability goals insisted the availability of sustainable management of water and sanitation for all by implementing water harvesting, desalination of salt water, efficiency of water and wastewater treatment, recycling, and reuse of technologies (UN 2015).

24.5.1 LEISA: Low External Input for Sustainable Agriculture

The indicators of environmental quality are indirectly measured by the utilization of resource, use of effective technology and its performance to remove the constituents of wastewater like BOD, NH_3-N, phosphorous, and pathogenic organisms. The sustainability in wastewater management is achieved through environmental indicators and societal indicators like cultural acceptance of these technologies, active participation by the communities, and by the way increasing job opportunities and better education (Muga and Mihelcic 2008). The achievement of sustainability by vermifiltration technology is illustrated in Fig. 24.4 and possibly highlighted many ways to make the sustainable environment to help the poor farmers to achieve the one among the 17 goals of sustainability.

Vermifiltration alone or coupled with macrophytes is considered to be a sustainable and eco-friendly method for treatment and reuse of wastewater (Singh et al. 2019). Bioaugmentation is achieved by the advantages of the following techniques: microbial ecology, immobilization techniques, and bioreactor design to meet the standard and quality of the wastewater treatment process (Herreno and Stuckey 2014).

Fig. 24.4 Achievement of sustainability in vermifiltration technology

24.6 Vermifiltration as an Innovative Technique for Environmental Protection

The body of earthworm acts as a bioreactor, and its coelomic fluid comprises plenty of microorganisms actively participating the decomposition and neutralization of waste materials in natural manner harmonic to the environment. Vermifiltration process is a newly conceived method for treating wastewater, which is effective as the conventional method of filtration systems. The biofiltering capacity of earthworms dynamically reduces the BOD, COD, and TDS. The ingestion of waste during the filtration process by earthworms is carrying out the biodegradation of organic waste materials, solid substances, and heavy metals. The reduction of heavy metals resulted due to uptake by the earthworms (Sinha et al. 2005).

Random sequencing of vermicompost observed the abundance of bacterial strains consisting *Proteobacteria*, *Chloroflexi*, *Actinobacteria*, *Bacteroides*, *Acidobacteria*, and *Saccharibacteria* and fungal strains like *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Cryptomycota*, and *Zygomycota* (Cai et al. 2018). The next-generation sequencing (NGS) recently emerged innovative technology based on the target of hyper variable regions recorded in the small subunit of rRNA for the identification of microbial varieties that known to be uncultured and capable of degrading toxins and contaminants blended in the wastewater (Senthilkumar et al. 2018). The survey of microbiota in dairy unit wastewater during vermifiltration process using NGS process found to be the abundance of appearance of nitrification genes, and it exhibited the potential of vermifiltration that reduces the ammonia content and effective nitrogen load reduction by microbial conversion (Lai et al. 2018).

24.7 Conclusion

Vermifiltration is an appropriate technology employed to treat wastewater for attaining environmental sustainability through transforming waste into cost-effective useful products. This process increases overall reaction to wastewater treatment consists of aerobic microorganisms effectively convert the organic materials into eco-friendly products. Organic nitrogen is converted into atmospheric nitrogen, removal of ammonia form of nitrogen without gaseous emissions to combat climatic change. There are some organisms actively participate the removal of many kind of organic debris in the wastewater is still need to explore for better understanding of the process. Metagenomic analysis, gene sequencing, protein profiling of the mucosal secretions of the earthworm, and vermifiltrate are essential to improve the potential of vermifiltration process to enhance the quality treatment process for improving the total quality of our environment.

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Small-Scale PVA Gel-Based Innovative Solution for Wastewater Treatment

25

Ankur Rajpal, Nilesh Tomar, Akansha Bhatia, and A. A. Kazmi

Abstract

Incomplete removal of organic matter and nutrients in the effluent is the major problem in the wastewater treatment system. In developing countries like India, different treatment technologies are widely used, and moving bed biofilm reactor (MBBR) has shown greater efficiency and stability compared to the conventional process. The majority utilize nonporous media of low specific surface area (~500 m^2/m^3), leading to slow start-up and easy detachment of biofilm due to the smooth surface. But, the porous carriers can escape from those drawbacks due to the excellent structure for adequate biomass growth. Several researchers have recently explored adding polyvinyl alcohol (PVA) gel beads as a biomass carrier in moving bed biofilm reactor, which not only traps and intercept the biomass efficiently, shorten the start-up period, but also promote biofilm accumulation by providing a large surface area. A study on a small-scale 50 m^3/day sewage treatment plant (STP) in a small locality of Kirtinagar on the bank of Alaknanda River was conducted. Process configuration is included in aeration (with 10% PVA gel beads) followed by anoxic then aeration tanks. Considerable attention is given to its performance under variables such as seasonal variation, simultaneous nitrification, denitrification (SND), and carbon and nitrogen removal. The effluent averages of BOD, COD, TSS, and TN concentration were <10 mg/L, <50 mg/L, <10 mg/L and <10 mg/L, respectively. Overall, it was observed that STP operated in a continuously fed regime at 6 h system HRT was able to bring the effluent quality to new effluent standards level along with minimal sludge production during the study.

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Keywords

Attached biomass · Carbon and nitrogen removal · PVA gel beads

Nomenclature

BOD	Biochemical oxygen demand
CCT	Chlorine contact tank
CFU	Colony forming unit
COD	Chemical oxygen demand
DO	Dissolved oxygen
F.B.	Free board
FC	Fecal coliform
HRT	Hydraulic retention time
KLD	Kilo-liter per day
MBBR	Moving bed biofilm reactor
MLSS	Mixed liquor suspended solid
MLVSS	Mixed liquor volatile suspended solid
MPN	Most probable number
N	Nitrogen
NH_4^+-N	Ammonia-N
NO_3^--N	NitrateORP
	Oxidation reduction potential
P	Phosphorous
PVA	Polyvinyl alcohol
Q	Discharge
SCOD	Soluble chemical oxygen demand
SND	Simultaneous nitrification and denitrification
STP	Sewage treatment plant
SVI	Sludge volume index
TC	Total coliform
TN	Total nitrogen
TSS	Total suspended solid
VSS	Volatile suspended solid

25.1 Introduction and Technical Details

MBBR system required low construction cost, minimal space requirement, low sludge production, high biomass concentration, lower head loss, and no clogging issues (Andreottola et al. 2000; Odegaard 2006; Singh et al. and Rajpal et al. 2020). Therefore, due to inadequate construction cost and effective nutrient removal efficiency, this MBBR using PVA gel having a high surface area–based sewage treatment plant (STP) was installed. This STP consists of a receiving chamber, manual screen, grit chamber, equalization tank, and followed by three biological reactors: (1) MBBR with PVA gel as biofilm reactor, (2) anoxic reactor, and (3) aeration reactor and tube settler clarifier. Clarified water from tube settler flows to chlorine contact tank (CCT) for disinfection and a bag filter for polishing treatment. The sludge from the bottom of the tube settler is recirculated to an anoxic tank, while the excess sludge is transferred to the sludge holding tank. For dewatering, the sludge is pumped from the sludge holding tank to the basket centrifuge. The dewatered sludge is dried and utilized in agricultural fields. The typical process flow sheet and schematic diagram and the pictorial view are shown in Figs. 25.1 and 25.2.

25.2 Materials and Methods

25.2.1 On-Site Parameter Analysis

On-site parameters such as temperature, pH, DO, and ORP were measured by Hach 110 Q multimeter and HQ11d ORP, respectively.

25.2.2 Physicochemical Analysis

Reactor performance was monitored by analyzing water quality of influent, PVA gel tank effluent, anoxic tank effluent, clarified effluent, and finally disinfected and filtered effluent. These samples are collected weekly from May 2018 to March 2019 and analyzed for BOD, COD, TSS, VSS ammonia (NH_4-N), nitrate (NO_3-N), total nitrogen (T-N), and orthophosphorus (O-P). For the determination of soluble components, samples were passed through Whatman No. 42 (0.45 μm) filter paper before analyses. Sludge from PVA gel, anoxic, and aeration tanks was also characterized by mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). Sludge settling tests (SVI) were conducted from the final aeration tank sludge. All analyses of collected samples were conducted as per Standard Methods (APHA 2005).

25.2.3 Bacteriological Analysis

Microbial enumeration was performed in the raw sewage, tube settler effluent, and chlorinated samples to analyze the pathogen removal efficacy of the plant (Bhatia et al. 2012). Coliforms analysis was performed by MPN tube method. The cell count for heterotrophic plate count, *Escherichia coli* sp., *Staphylococci* species, *Pseudomonas* species, and *Shigella and Salmonella* species was performed by the culture plate method.

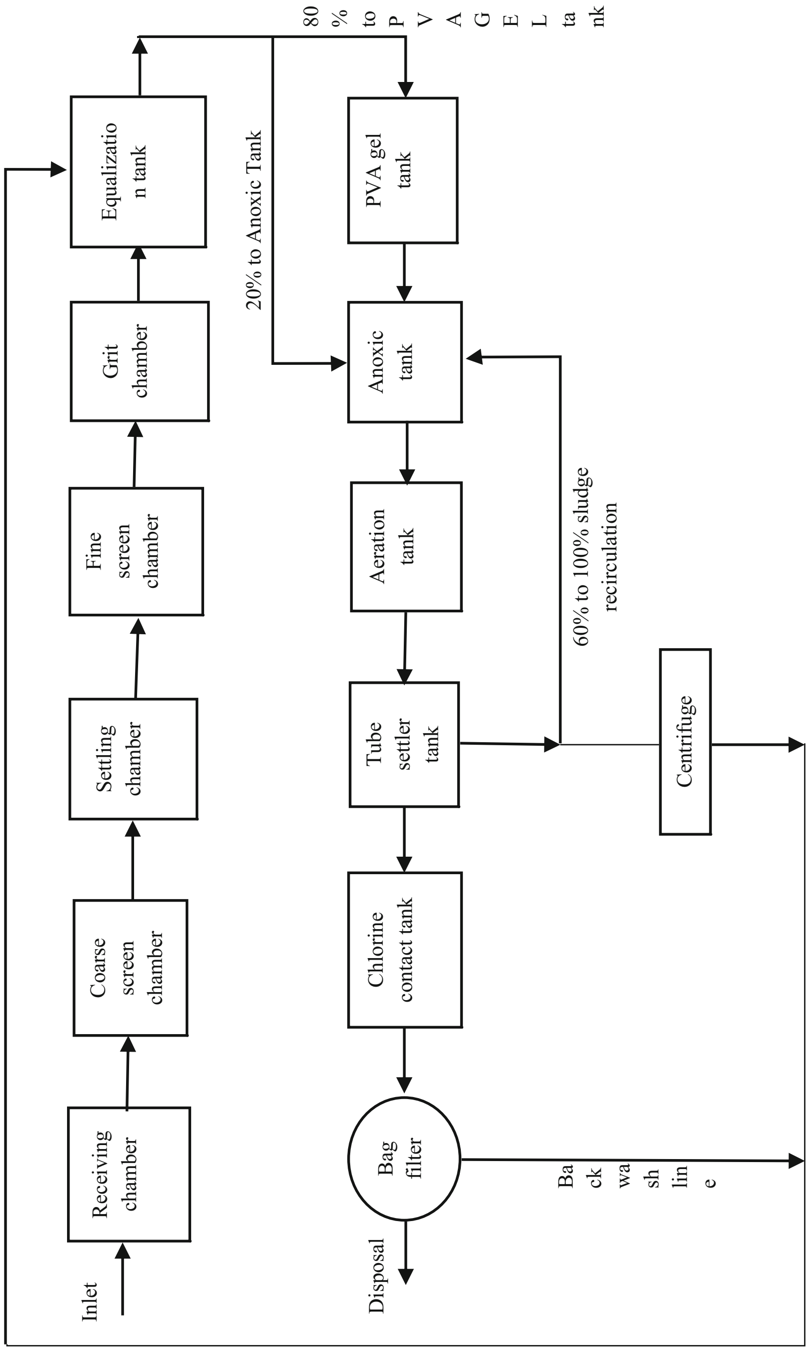


Fig. 25.1 Process flowsheet of 50 KLD STP

Fig. 25.2 A view of the 50 KLD STP at the site

25.2.4 16S rRNA Analysis of PVA Gel Beads

25.2.4.1 DNA Extraction

Microbial diversity of the biomass inside the PVA gel beads was identified using the 16S rRNA analysis (Bhatia et al. 2013). PVA gel beads were microtomed (no. 10) DNA extraction was done using the commercially available kits such as QIAGEN, ZYMO RESEARCH, and ThermoFisher, as per the manufacturer's recommendation.

25.2.4.2 PCR Amplification and Cloning

Forty nanogram of extracted DNA was used for amplification along with 10 pM of each primer. Initial denaturation was carried out at 95 °C. The aforementioned condition, denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s, elongation at 72 °C for 2 min, final extension at 72 °C for 10 min, and hold at 4 °C was repeated for 25 cycles. Universal bacterial primers, 16F-5′ AGAGTTTGATCMTGGCTCAG 3′ 16R-5′ TACGGYTACCTTGTTACGACTT 3′, were used for the study.

25.2.4.3 Sequencing and Analysis

Nanopore sequencing was performed by using 1 μg of DNA template. Alpha and beta diversity was analyzed for the tested sequences. OTUs obtained were tested based on similarity and consistency.

25.2.4.4 Abundance

The abundance percentage of the prevalent bacterial species and their metabolic pathways was studied.

25.2.5 Microfauna Analysis

Microscopic observations of protozoan species were carried out in the following samples:

1. Biomass in PVA gel tank.
2. MLSS in the final aeration tank.

The microbiota identification and enumeration: 25 μL subsamples of sludge were examined under phase contrast illumination (×100 magnifications). Four replicates of the final volume 1 mL were tested.

25.3 Results and Discussion

25.3.1 Monthly Performance of STP

25.3.1.1 pH, DO, ORP Observations

Temperature, pH, ORP, and DO variations are shown in Table 25.1, the raw sewage temperature (25–31 °C) from May, 2018 (start-up phase) to November 2018. The temperature reduced suddenly in November, and a gradual reduction was observed until January 2019 (19–8 °C), and further increment was observed (14–24 °C) up to March 2019.The average pH of the influent and effluent was varied from 6.8 to 7.8, respectively. ORP values varied from ~+38 to +130 mV in PVA gel tank, ~−60 to −162 mV in anoxic tank, and ~+39 to +92 mV in final aeration tank, respectively. DO concentration fluctuates ~3.3–7.5 mg/L, 0.1–0.2 mg/L, and ~2.6–5.5 mg/L in PVA gel tank, anoxic tank, and aeration tank, respectively.

25.3.1.2 Operational Sludge Parameters

- **MLSS and MLVSS.**
  During the start-up phase, ~10% seed activated sludge was added to all three tanks for acclimatization. After achieving steady-state condition, average MLSS and MLVSS concentrations were 1450 ± 134 and 695 ± 113 mg/L, respectively, in PVA gel tank. In anoxic tank and aeration tanks, MLSS and MLVSS were maintained at 4108 ± 1310 and 2456 ± 884 mg/L and 4209 ± 1406 and 2587 ± 775, respectively (Figs. 25.3 and 25.4).

25.3.1.3 COD, BOD, and TSS Removal

Figures 25.5, 25.6, and 25.7 depict BOD, COD, and TSS removal efficiencies, respectively, based on monthly sampling from May 2018 to March 2019. The

Table 25.1 Temperature, pH, DO, and ORP variations in PVA gel, anoxic and aeration tanks

Parameters	Point sources	Months										
		May-18	Jun-18	Jul-18	Aug-18	Sep-18	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19
Temp (°C)	Sewage	30	30	29	28	26.5	25	16.8	13.4	8	16	24
pH	Inlet	7.2	7.3	7.3	7.2	7.2	7.3	6.8	7.7	7.4	7.3	7.4
	Gel tank	7.5	7.8	7.5	7.3	7.4	7.4	7.0	7.8	7.7	7.4	7.5
	Anoxic tank	7.4	7.4	7.6	7.5	7.2	7.3	6.9	7.9	7.8	7.5	7.7
	Aeration tank	7.5	7.4	7.7	7.4	7.3	7.2	7.0	7.8	7.9	7.6	7.6
	Outlet	7.4	7.4	7.7	7.3	7.2	7.2	7.0	7.8	7.5	7.5	7.5
DO (mg/L)	Inlet	–	–	–	–	–	–	–	–	–	–	–
	Gel tank	5.24	6.7	3.3	3.9	5	7.5	4.5	5.2	6	5	5
	Anoxic tank	0.45	0.3	0.2	0.18	0.2	0.1	0.2	0.1	0	0	0
	Aeration tank	5.4	5.5	4.2	3.9	4.8	2.6	4.4	4.6	5	4	4
	Outlet	–	–	–	–	–	–	–	–	–	–	–
ORP (mV)	Inlet	–	–	–	–	–	–	–	–	–	–	–
	Gel tank	–	–	–	+21.4	+55.5	+38.5	+69.0	+130.5	+82.6	+19.7	+31.3
	Anoxic tank	−60	−141	−183	−55.3	−91.4	−162.0	−59.7	−97.0	−48.3	−84.5	−80.3
	Aeration tank	–	–	–	+35.1	+92.2	+62.1	+54.2	+79.5	+82.1	+39.6	+48.7
	Outlet	–	–	–	–	–	–	–	–	–	–	–

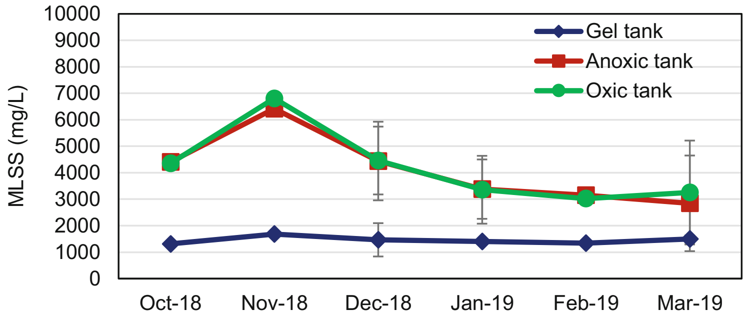


Fig. 25.3 Variations in MLSS in PVA gel, anoxic, and aeration tank

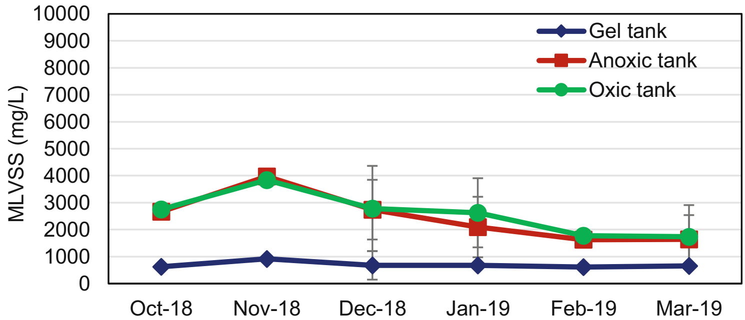


Fig. 25.4 Variations in MLVSS PVA gel, anoxic, and aeration tank

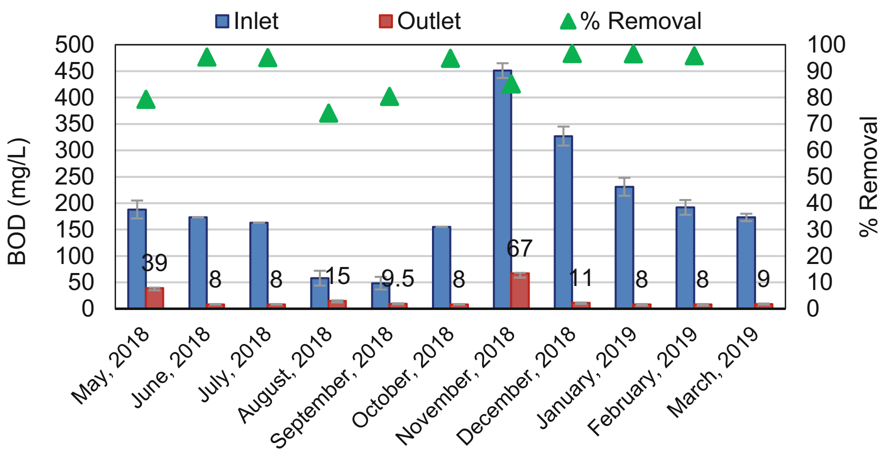


Fig. 25.5 BOD removal in sewage treatment plant

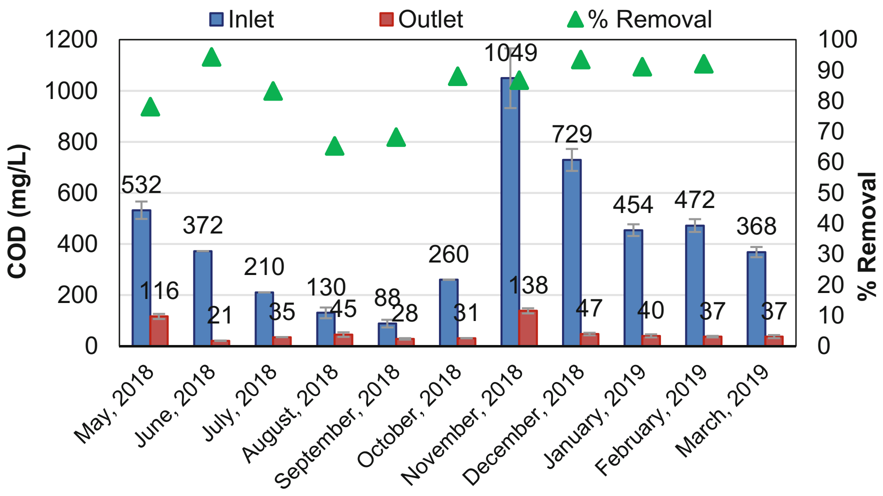


Fig. 25.6 COD removal in sewage treatment plant

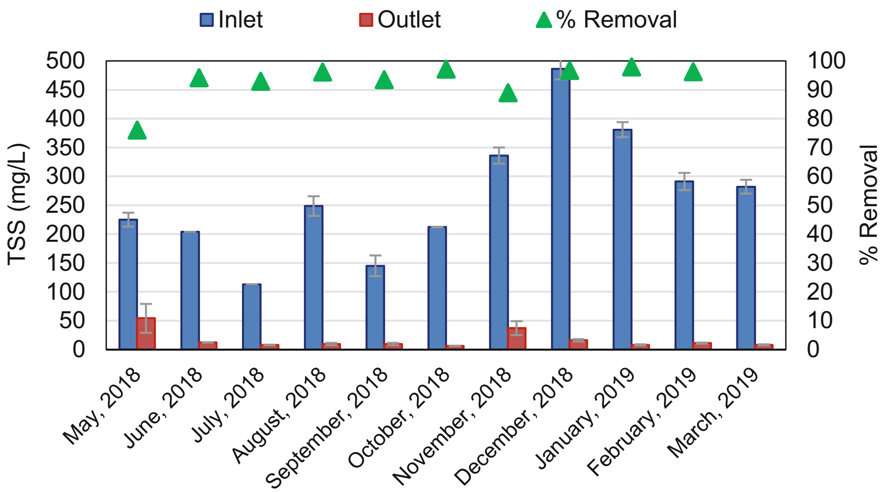


Fig. 25.7 TSS removal in sewage treatment plant

average values of BOD, COD, and TSS in the influent varied from ~58 ± 14 to ~451 ± 14 mg/L, ~88 ± 15 to ~1049 ± 117 mg/L, and ~145 ± 18 to ~486 ± 18 mg/L, respectively, from May 2018 to March 2019. After start-up period of 1 month, the process achieved a steady state, and the value of BOD, COD, and TSS in the effluent was about ~10, ~40, and ~10, respectively, most of the time during the process except in November. During the Diwali festival in November, people residing in this locality prepared sweets from milk and its products and discharges high BOD and COD wastewater. Very high influent values were observed in November, i.e., BOD

(1136 mg/L), COD (2835 mg/L), and TSS (629 mg/L), respectively. The continuous input of high BOD and COD wastewater deteriorates the effluent quality. During this period, effluent BOD (~67 ± 8), COD (~138 ± 10 mg/L), and TSS (~37 ± 12 mg/L) values were observed in the effluent.

25.3.1.4 Ammonia-N and Total Nitrogen (TN) Removal Efficiency

In the initial phase of start-up, sewage temperature was nearly around 30–35 °C. This temperature range was suitable for nitrification and good sludge settle ability. In addition, process was optimized by maintaining suitable operational conditions, i.e., DO, ORP, and biomass in each tank. As shown in Figs. 25.8 and 25.9, the average

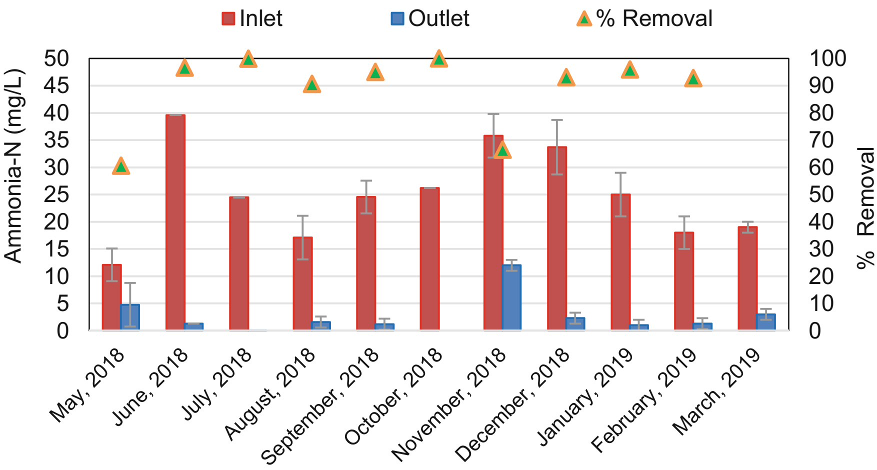


Fig. 25.8 Ammonia removal in sewage treatment plant

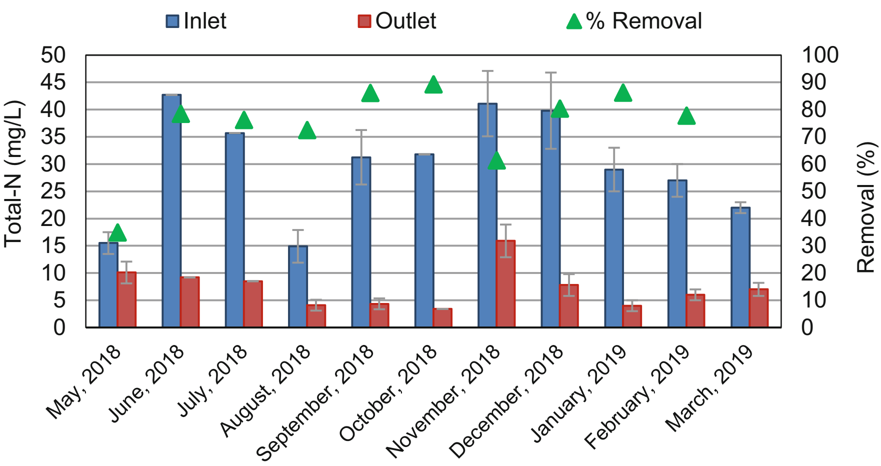


Fig. 25.9 Total nitrogen removal in sewage treatment plant

values of NH_4-N and T-N in the influent varied from ~12.1 ± 3 to ~39.6 ± 14 mg/L and ~14.9 ± 3 to ~39.8 ± 7 mg/L, respectively, from May 2018 to March 2019. Within 1 month of start-up phase, NH_4-N, and T-Nin, the final effluents were reduced to <2 and <10 mg/L. The effluent quality is disturbed only in November 2019. During the month of November, very high organic shock load disturbs the process of nitrification and denitrification, which leads to elevated values of *n* NH_4-N and T-N during entire month of November.

25.3.1.5 Chemical Phosphorus Removal

Figure 25.10 shows the chemical phosphorus removal during the study period. About 30 mg/L alum dosed keep the PO_4-P concentration below 1 mg/L.

25.3.1.6 Pathogen Removal

Pathogen removal efficiency was examined on the basis of culture plate technique for indicator organisms (fecal coliforms and *E. coli*) and *Salmonella*, *Shigella*, and *Pseudomonas* species (Table 25.2). It is also observed that very low chlorine dosing (1.5 mg/L) doesn't remove residual pathogens effectively. Hence, to achieve the tender effluent value of 100 MPN/100 mL fecal coliforms, chlorine dose needs to be increased to 3–5 mg/L.

Further, it was observed that PVA gel–based process enhanced the pathogen removal efficacy attributing to the bacterial competition within the system and predation by the protozoans (Singh et al. 2016; Bhatia et al. 2017 and Wang et al. 2018). These values indicated that higher pathogen removal efficiency (>90%) is observed in the biological process. The effluent fecal coliforms always satisfy World Health Organization (WHO) effluent standard of 1000 MPN/100 mL. The higher

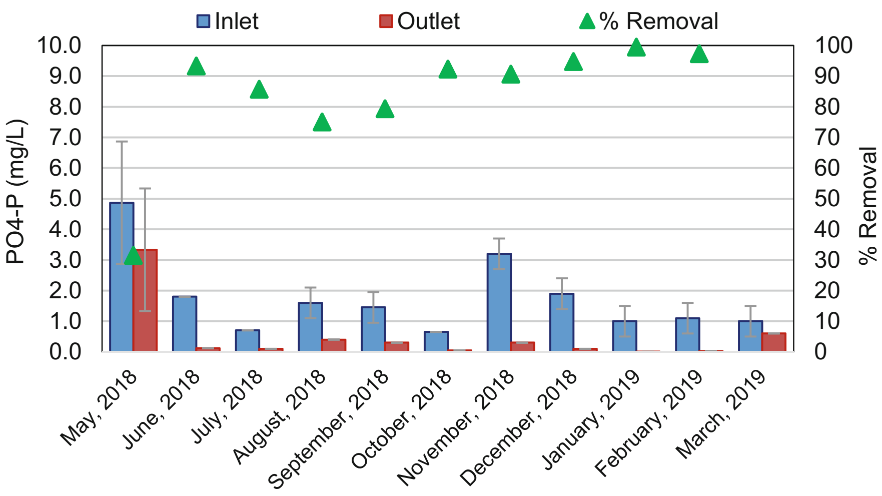


Fig. 25.10 Chemical phosphorus removal in sewage treatment plant

Table 25.2 Pathogen removal in sewage treatment plant

	Influent					Effluent (with chlorination)				
Microbes	Nov'18	Dec'18	Jan'19	Feb'19	Mar'19	Nov'18	Dec'18	Jan'19	Feb'19	Mar'19
FC (MPN/100 mL)	23,000	21,000	24,000	21,000	24,000	270	220	210	190	170
Escherichia coli (CFU/100 mL)	21,000	19,000	11,000	12,000	13,000	190	170	160	150	170
Salmonella species (4MPN/100 mL)	230	210	120	160	170	48	43	40	39	37
Shigella species (CFU/100 mL)	270	134	126	176	124	37	39	40	45	42
Pseudomonas species (CFU/100 mL)	210	156	168	135	178	67	45	38	32	36
Staphylococci species (CFU/100 mL)	260	122	167	156	178	70	67	46	58	69

Fecal coliforms were reduced from 21,000–24,000 MPN/100 mL (influent) to 290–340 MPN/100 mL (clarified effluent). These numbers were further reduced to 170–270 MPN/100 mL by chlorine addition

Escherichia coli reduced from 19,000–21,000 CFU/100 mL in inlet to 120–210 CFU/100 mL (clarified effluent). These numbers were further reduced to 150–190 CFU/100 mL by chlorination

Salmonella species were decreased in clarified effluent from 120–230 (4MPN/100 mL) to 57–92 (4MPN/100 mL). These numbers were further reduced to 37–48 (4MPN/100 mL) by chlorination

Shigella species were finally reduced in clarified effluent from 124–270 CFU/100 mL to 69–79 CFU/100 mL in the biological process. These numbers were further reduced to 37–45 CFU/100 mL by chlorination

Pseudomonas species were reduced from 135–210 CFU/100 mL to 56–89 CFU/100 mL biologically. These numbers are further reduced to 32–45 CFU/100 mL by chlorination removal efficiency in the process configuration is attributed to the autolysis within the PVA gel beads and the predation by protozoans in the final aeration tank.

25.4 Microbial Diversity in PVA Gel Beads

25.4.1 Phylogenetic Analysis of Biomass in the PVA Gel Beads

Knowledge of the *microbial* community structure and their relation to the changing environmental conditions is therefore crucial for the development and optimization of biological systems. The quality of effluent is dependent on the bioreactor's microbial community structure and dynamics. The diversity of microorganisms in wastewater performs different roles and has other operational conditions for optimal activity and growth. A comprehensive understanding of adequate and active microbial populations and their biological role is essential to study the efficacy of the wastewater treatment process. The diversity of the microbial community and their role in PVA gel–based treatment process was tested in the present study.

25.4.1.1 Microbial Diversity and Their Functional Roles

Microbial community composition and diversity were studied using OTU-based analysis.

25.4.1.2 Organic Removal Species

- Alpha proteobacteria play a vital role in the degradation of organic compounds and nitrogen in wastewater.

25.4.1.3 Nitrification Species

- *Nitrospira* species are the dominating nitrite oxidizers observed in the studied plant.
- *Nitrosomonas* spp. found is responsible for oxidizing ammonia to nitrite, supporting the nitrification process.

25.4.1.4 Denitrifiers

- The presence of *Flavobacteriales* relates to the denitrification process, i.e., nitrate is converted to atmospheric nitrogen. Denitrifies includes the species of *Clostridium*, *Pseudomonas*, and *Flavobacterium*. Phylum *Firmicutes* were identified previously as denitrifying bacteria.

25.4.2 Abundance of Microbes in Relation to Metabolic Pathways

The abundance percentage of microbial species was calculated based on the 16SrRNA sequences. These bacterial groups were tested for their metabolic functions to treat the wastewater.

- Dominant groups were nitrite reducer (17.4%) and denitrifiers (15.2%), sulfate reducing bacteria (SRB) (15.2%), and ammonia oxidizer (15.1%).

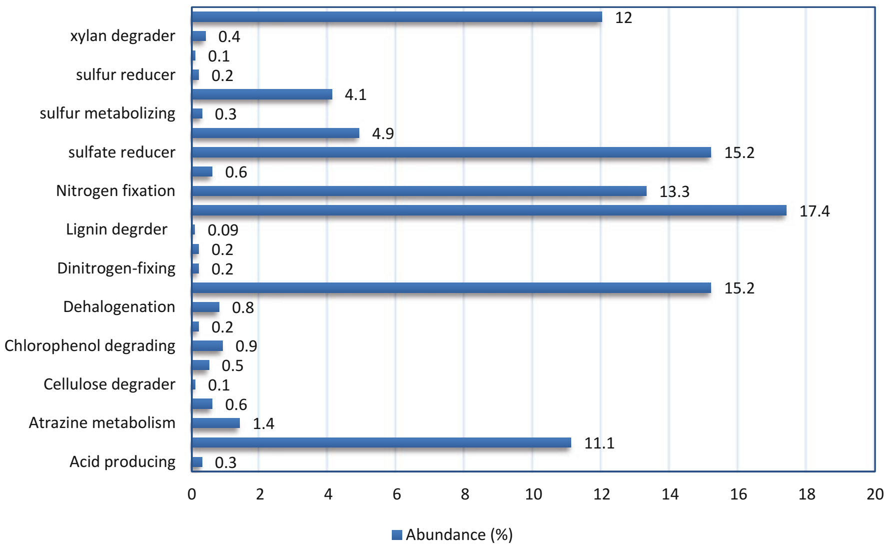


Fig. 25.11 Abundance (%) of microbes prevailing in the PVA gel beads

- Other relevant groups supporting the metabolic pathways for the degradation of organic matter prevailing in the wastewater systems are shown in Fig. 25.11.

The molecular analysis of PVA gel beads is depicted in Fig. 25.11, the dominance of ammonia oxidizers and nitrite reducers that are responsible for the high level of nitrification and denitrification in the PVA gel tank.

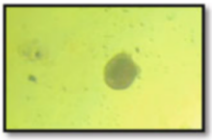
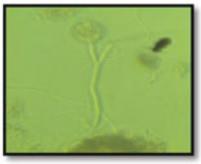
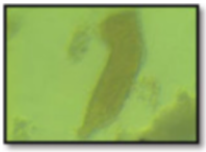
25.4.3 Microscopic Analysis and Sludge Settleability

Protozoans are responsible for the flocculation process, which results in the biosorption phenomenon of organic particles. These processes are significant in the treatment of conventional pollutants and micro-contaminant degradation.

Qualitative microscopic observations were carried out in mixed liquor samples of PVA gel and the final aeration tank.

- A higher number of stalked ciliated protozoans such as *Peranema*, *Opercularia*, *Epistylis*, *Litonotus*, *Aspidisca*, *Vorticella*, and *Arcella* were observed. Their presence relates to good settling conditions due to an adequate amount of aeration and significant nitrification activity.
- Detailed identification and quantification of filamentous bacteria such as *Nostocoida limicola*, *Microthrix parvicella*, Type 1701, and *Nocardia* both the tanks relate to the optimum conditions for better sludge settling (Table 25.3).
- Floc morphology shows the sludge dissolution stage depicting the satisfactory treatment process.

Table 25.3 Microbiota profile and the sludge conditions in PVA gel and aeration tanks

S. no.	Protozoan species	Enumeration		Conditions	Pictures taken from phase contract microscope at ×100
		PVA tank	Aeration tank		
1	*Arcella.*	+	++	• Nitrification activity	
2	*Litonotus*	++	++	• Organics are being removed	
3	*Aspidisca*	++	+	• Water quality is satisfactory	
4	*Vorticella*	+	++	• Well-formed flocs • Dark brown color of sludge indicates maturation of sludge	
5	*Aeolosoma*	+	+	• Feeds on the waste matter • Good settling	
6	Filamentous bacteria	+	+	• Sludge bulking conditions when found in large number	

Where (+) = 5–10 protozoans/mL, (++) = 10–100 protozoans/mL, (+++) = 100–1000 protozoans/mL, and (++++) ≥ 1000 protozoans/mL

25.4.4 Settling Characteristics

In aeration tank, the value of sludge volume index varies (SVI) between 66 mL/g and 115 mL/g as shown in Fig. 12. SVI values ranging between 70 mL/g and 120 mL/g relates to the good settling conditions of sludge (Fig. 25.12).

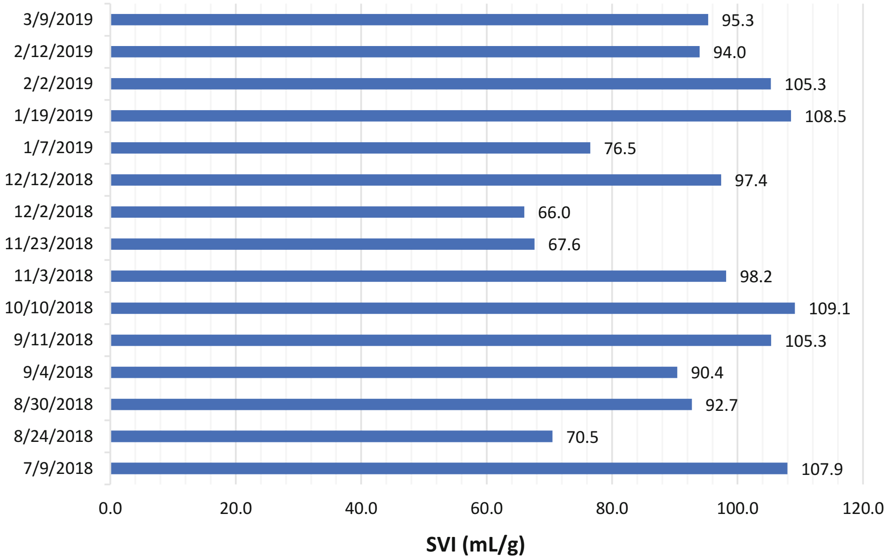


Fig. 25.12 SVI in aeration tank sludge

Table 25.4 Physicochemical characteristics of the dewatered sludge

Parameters	Units	Mean	Organic compost (FCO 2009)
pH	–	7.2±	6.5–7.5
EC	(μS/cm)	4.9	–
Moisture content	(%)	81	15–25
Organic matter	(%)	42.5	–
Total organic carbon, percent by weight	(%)	33.0	12
Total nitrogen (as N), percent by weight	(%)	4.5	0.8
Total phosphate (as P_2O_5), percent by weight	(%)	1.9	0.4
Total potassium (K_2O), percent by weight	(%)	1.1	0.4
Carbon nitrogen ratio (C/N)	–	7.3	<20

25.5 Characteristics of the Dewatered Sludge

It was observed that basket centrifuge produced 10–15 kg dewatered sludge per day. Usually, plant operator stored sludge in bags for several days; after that, it is dispose of in agricultural field. The dewatered sludge samples were taken from basket centrifuge and are analyzed for important parameters for land application. The data is summarized in Table 25.4.

Table 25.5 Heavy metals in dewatered sludge

Heavy metals	Unit	Average	Organic compost (FCO 2009)
Chromium (Cr)	mg/kg	40.5 ± 14	50
Lead (Pb)	mg/kg	60.8 ± 11	100
Cobalt (Co)	mg/kg	4.4 ± 1	–
Copper (Cu)	mg/kg	68.3 ± 22	300
Manganese (Mn)	mg/kg	147.3 ± 47	–
Nickel (Ni)	mg/kg	30.7 ± 10	50
Zinc (Zn)	mg/kg	651.8 ± 194	1000
Cadmium (Cd)	mg/kg	4.2 ± 1	5

It is observed that the fertilizer value of sludge in terms of nitrogen, phosphorus, and potassium is high. It can be used effectively for agriculture purposes after sun drying and/or composting. Processes can further reduce the moisture content and carbon for effective application on land. Heavy metal analysis of dewatered sludge was also conducted. All heavy metals were found in the desired limits of the organic compost (FCO 2009), as shown in Table 25.5.

25.6 Conclusions

All over the study period, STP shows good organics and nutrient removal efficiency. After achieving the steady-state, the BOD, COD, TSS, and TN removal were more than 91%, 90%, 93%, and 84%, respectively. In PVA gel tank, % SND rate was about 85%, which resulted in more than 60% total nitrogen removal. The effluent fecal coliforms always satisfy WHO effluent standard of 1000 MPN/100 mL. Ammonia, total nitrogen, and BOD removal do not affect seasonal temperature variation. The effluent BOD, COD, TSS, and TN concentrations were <10, <50, <10, and <8 mg/L, respectively, except in November, which satisfies Central Public Health and Environmental Engineering Organization (CPHEEO) standards for reuse of water. Therefore, present study concluded that PVA gel–based process can be implemented for the treatment of real wastewater in large scale for the removal of carbon and nitrogen.

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Cyclic Technology–Based Sequencing Batch Reactors (SBR) Treating Municipal Wastewater: Full-Scale Experience

26

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Abstract

Cyclic technology–based sequencing batch reactors (SBR) treating municipal wastewater were audited for their performance under full hydraulic load design conditions. The performance data showed that the WWTPs produced an effluent having a very low concentration of chemical oxygen demand (COD, 10 mg/L), biochemical oxygen demand (BOD, <10 mg/L), and SS (<10 mg/L). Total nitrogen and total phosphorus concentrations were reduced significantly, i.e., <5 mg/L and <0.5 mg/L, respectively, which is very impressive in biological nutrient removal (BNR) systems. It was found that more than two log removal of fecal coliforms takes place in the SBR reactor itself. Also, 2–3 mg/L chlorine dosage in the chlorination tank was found sufficient to reduce the fecal coliform numbers well below the WHO standard (1000 MPN/100 mL) set for the reuse of the effluent for unrestricted irrigation. The dewatered sludge has high nitrogen,

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phosphorus, and potassium (NPK) value; hence, it can be used very effectively as an organic fertilizer after drying/composting. Furthermore, the bio-aerosols concentration in ambient air near the SBR was observed safe for plant workers concerning the health risks derived from exposure to bio-aerosols. All the WWTPs were found to be efficient in producing the excellent effluent quality that not only completely fulfills the Indian effluent discharge standards but also almost fulfills US Environmental Protection Agency (EPA) requirement for non-potable reuse standards. Merely increasing the chlorine dosage can satisfy the US EPA requirement for non-potable reuse such as recreational impoundments, cooling water, horticulture, and toilet flushing.

Graphical Abstract

Keywords

Municipal wastewater treatment · Cyclic technology · Sequencing batch reactors · Biological nutrient removal

26.1 SBR Process Description

The wastewater treatment plant comprises of coarse and fine screens, screenings handling equipment, grit chambers with classifiers, SBR basins, air blowers, sludge holding tank, centrifugal dewatering, and chlorine disinfection system. The technical details of the WWTPs are presented in Table 26.1.The main reaction basin is the SBR, which is an improved version of conventional SBR. It incorporates an initial multicell selector zone within the tank to effectively control filamentous sludge bulking overall as received loading conditions. Each SBR basin is divided by baffle walls into two sections (Zone 1: Selector and Zone 2: Main Aeration). Sludge biomass is transferred from Zone 2 to the Zone 1 selector to remove the readily degradable soluble organics from the sewage and favor the growth of the floc-forming microorganisms and a well-settling biomass of low sludge volume index.

System design is such that the sludge return rate causes an approximate daily cycling of the total biomass through the selector zone. The mechanisms in Zone 1 and the internal sludge transfer eliminate the requirement for separate fill-ratio selectivity and anoxic and anaerobic mixing periods that are absolutely necessary in the older generic SBR configurations. The selector is self-regulating for all loadings and operates under anoxic conditions during aerobic sequencing and anaerobic reaction conditions during non-aerated periods. Polishing denitrification and enzymatic transfer of available substrate during enhanced biological phosphorus release/removal is also achieved in the selector zone. The complete-mix nature of the main reactor provides flow and load balancing and a tolerance to shock or toxic loading. The process prevents solids washout during peak or wet weather hydraulic surges that is not possible in conventionally designed clarifier activated sludge plants.

26.1.1 Operating Cycles in SBR Basins

The system makes use of variable volume treatment in combination with a biological selector that is operated in a fed-batch reactor mode. The complete biological operation is divided into different cycles (Fig. 26.1). A basic cycle of 3 h (total eight cycles per day) comprises 90 min fill-aeration, 45 min settling, and 45 min decanting. Three sequences constitute a cycle, which is then repeated. During the period of a cycle, the liquid volume inside the reactor increases from a set operating bottom water level. During the fill-aeration sequence mixed liquor from the aeration zone is recycled into the selector. Aeration ends at a predetermined period of the cycle to allow the biomass to flocculate and settle under absolute quiescent conditions. After a specific settling period, the treated supernatant is decanted using as electromechanically operated downward moving weir decanter. When the liquid level in the reactor is returned to the bottom water level, cycle is then repeated. The surplus sludge is wasted from the reactor during the decanting sequence. The process is capable of achieving anoxic denitrification of nitrates and phosphorus release and uptake in the biological selector zone of SBR (anoxic zone) using incoming sewage, biodegradation of organics present in the wastewater in the main aeration zone, simultaneous nitrification and denitrification (under controlled DO conditions) in the aeration zone of each SBR basin, endogenous denitrification during settling sequence, and phosphorus release and uptake in the selector and main aeration zone.

Table 26.1 Technical design details of WWTP

Wastewater treatment plants	25 MLD Kharghar, Mumbai	12.5 MLD Tonca City, Goa	45 MLD Mundhwa, Pune
			
Design flow			
• Average flow.	25 MLD	12.5 MLD	45 MLD
Influent quality			
• Bod • SS • TKN • NH_4-N • TP (as PO_4-P) • Fecal coliforms	200 mg/L 200 mg/L 35 mg/L 15 mg/L 7 mg/L 10^6 MPN/100 mL	275 mg/L 400 mg/L 45 mg/L – 7 mg/L –	250 mg/L 300 mg/L 45 mg/L 10 mg/L 5 mg/L 10^6 MPN/100 mL
Effluent quality			
• Bod • SS • TKN • NH_4-N • TP • Fecal coliforms	5 or <5 mg/L 10 or <10 mg/L 10 or <10 mg/L 2 or <2 mg/L 1 or <1 mg/L <100 MPN/100 mL	10 mg//L 10 mg/L 10 mg/L – 2 mg/L –	<10 mg/L <10 mg/L <10 mg/L <2 mg/L <2 mg/L <100 MPN/100 mL
No. of SBR basins	4	2	4
No. of basins receiving flow simultaneously	2	1	2
No. of basins aerating simultaneously	2	1	2
	1	1	1

No. of basins decanting simultaneously			
Volume of SBR basins	19,800 m^3 (total volume) 4950 m^3 (volume provided per SBR basin)	7920 m^3 (total volume) 3960 m^3 (volume provided per C-Tech basin)	31,482 m^3 (total volume) $53 \times 27 \times 5.5 = 7870.5$ m^3 (volume provided per SBR basin)
No. of cycles per day/basin			
• Filling and aeration • Settling • Decanting Total cycle time	8 90 min 45 min 45 min 3 h	8 90 min 45 min 45 min 3 h	8 90 min 45 min 45 min 3 h
Hours of aeration time/day/basin	12 h	12 h	12 h
MLSS	3200 mg/L		4300
MLVSS	2560 mg/L	–	3440
F/M	0.1		0.08
Selector (anoxic) zone			
• No. of selector compartments/basin • Retention time.	1 60 min	1 –	1 50 min
Disinfection			
• Designed chlorine dose.	2.5 mg/L	2.5 mg/L	2.5 mg/L

F/A	Fill and Aeration Phase
S	Settling phase
D	Decanting Phase

	Time, Hrs →											
	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	2.50	2.75	3.00
Basin - 1	F/A	F/A	F/A	F/A	F/A	F/A	S	S	S	D	D	D
Basin - 2	S	S	S	D	D	D	F/A	F/A	F/A	F/A	F/A	F/A
Basin - 3	F/A	F/A	F/A	S	S	S	D	D	D	F/A	F/A	F/A
Basin - 4	D	D	D	F/A	F/A	F/A	F/A	F/A	F/A	S	S	S

Fig. 26.1 Typical cycle sequences of a four Basin SBR

26.2 Monitoring, Sampling and Analysis

26.2.1 Sample Collection

Wastewater samples were collected from the various locations as follows: influent (raw sewage), effluent SBR basin, and final-treated effluent (after chlorination). Cycle-based composite sampling was carried out during the study period. Influent samples (no. 3) were collected at 30 min intervals during fill-aeration sequence of a cycle and mixed together to make the final volume of 1000 mL. Similarly with the effluent, three samples were collected over the decant sequence (first sample was collected 10 min after start of decantation and then two more samples distributed for the middle and the end of decantation). All the three samples are mixed together to make the final volume of 1000 mL. Samples for microbiological analysis were collected in sterilized 250 mL borosilicate glass bottles. Ample air space was left in the bottle (at least 2.5 cm) to facilitate mixing by shaking, before examination. Samples were preserved in an ice container at 4 °C prior to the analysis and processed within 24 h of sample collection. Samples for the analysis of MLSS, MLVSS, mixed liquor fauna (protozoans), and sludge volume index were collected from SBR basins (10 min before the end of aeration period).

26.2.2 Sample Analysis

All the wastewater samples collected from different treatment stages of WWTP were analyzed for pH, biochemical oxygen demand (BOD, total and soluble), chemical oxygen demand (COD, total and soluble), total suspended solids (TSS), turbidity, total Kjeldahl nitrogen (TKN), NH_3-N, NO_3-N, PO_4-P, and total and fecal coliforms. Sampling and analysis were also carried out for operating parameters like food to microorganisms ratio (F/M), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge settling velocity and DO drop during settling sequence, sludge volume index (SVI), specific oxygen uptake rate (SOUR), and identification of mixed liquor fauna (protozoan) within the SBR basins (biological reactors aka SBR). All the analysis were carried out as per Standards Methods (APHA, AWWA, WEF 2005).

The mixed liquor fauna was analyzed by taking the samples of mixed liquor from the SBR basins. Twenty-five microliter subsamples of mixed liquor were taken with an automatic micropipette, and a minimum of four replicates of this volume were counted under phase contrast illumination ($\times 100$ magnifications). The sludge was kept constantly homogenized and aerated for the entire duration of analysis, to keep all of the solids in suspension (Madoni 1984; Tyagi et al. 2008).

26.3 Performance Assessment of WWTP Working on Full Capacity

26.3.1 25 MLD Kharghar WWTP, Navi Mumbai

At the time of monitoring, the 25 MLD Kharghar WWTP was receiving a flow of 883 m^3/h or 21.92 MLD. However, the daily average flow from the record was found to be around 23 MLD. It was observed that the WWTP was producing the satisfactory effluent quality in terms of BOD, suspended solids, fecal coliforms, and turbidity that not only fulfills the Indian effluent discharge standards but also almost fulfills US EPA standards for non-potable reuse.

26.3.1.1 Effluent Quality

The WWTP was found to produce an effluent having low concentration of BOD (5 mg/L), TSS (10 mg/L), and turbidity (1.8 NTU). A significant removal was observed in ammoniacal nitrogen (as NH_4-N) concentration (87% removal, effluent NH_4-N: 5.3 mg/L) and phosphorus (PO_4-P) concentration (88%, effluent PO_4-P: 0.2 mg/L), which is very impressive in biological nutrient removal systems. It was found out that 98% removal of fecal coliforms takes place in the SBR reactor. In addition, 2.5 mg/L chlorine dose in chlorination tank was found sufficient to reduce the fecal coliform concentration (150 MPN/100 mL) in WWTP effluent below the WHO standard (1000 MPN/100 mL) set for reuse of the treated effluent for unrestricted irrigation and NRCD standard (FC; 1000 MPN/100 mL) to discharge the treated effluent in surface water.

26.3.1.2 Nitrogen and Phosphorus Removal

To verify the conditions for denitrification, the dissolved oxygen concentration was measured in the reactor during the start-up of settling sequence. It was observed that only after 10 min, the DO concentration reached at 0.1 mg/L into the SBR basin; thereafter, favorable condition takes place for continued denitrification in sludge blanket zone. Furthermore, favorable conditions (anoxic/anaerobic conditions) were observed in bioselector basin cells. These conditions favor the denitrification and phosphorus release in this zone. This finally leads to the significant phosphorus removal in SBR basin.

26.3.1.3 Sludge Settling and Characteristics

In order to study the reactive settling, the zone settling study was also carried out in SBR basin. After a settling period of 30 min, the sludge settled up to 2.7 m (40%) of original water depth (4.5 m) (total sludge settled: 1.8 m) (sludge settling velocity = 2.4 m/h). No sludge bulking or pin point floc was observed in the SBR basins, which resulted in excellent sludge settling and good effluent quality.

It was observed that due to high NPK [N = 66.00 g/kg (6.6%), P = 6.46 g/kg (0.646%), and K = 1.52 g/kg (0.152%)], the dewatered sludge can be used as a fertilizer after drying and/or composting. Most of the heavy metals are under limit of ceiling concentration as suggested by US EPA.

26.3.1.4 Biomass in SBR Basins

The observed SOUR value of 24 mgO_2/g VSS/h is very good, which represents to the existence of a very healthy biomass into the SBR basin and indicates the rapid oxidation of substrate.

A low food to microorganisms ratio (F/M ratio, 0.1 per day) means that the microorganisms in the SBR reactor are starved, generally leading to a more efficient wastewater treatment. The benefit of operating at the low F/M results in a reduced and mineralized (stabilized) waste sludge generation. At this sludge loading, process resistance to hydraulic and organic shock loading is very high. The hazard of low F/M filamentous bulking is eliminated by the purpose designed multicell selector.

The observed protozoan fauna of *Arcella*, *Vorticella*, *Aspidisca*, *Litonotus*, and *Stentor* in SBR basins confirms that the WWTP is performing satisfactorily, i.e., the good effluent quality, good nitrification, and existence of process favorable conditions, i.e., good floc formation, sludge settling, and DO in the SBR basins.

26.3.1.5 Remark

On the basis of the overall assessment, it can be said that the SBR technology–based WWTP is performing very well under full flow capacity conditions. The finally treated effluent can also be discharged safely in surface waters. Furthermore, merely increasing the chlorine dose to 2–3 mg/L can satisfy the US EPA requirement for non-potable reuse such as urban reuse (landscape irrigation, vehicle washing, toilet flushing, use in fire protection, and commercial air conditioners) food crop irrigation, and recreational impoundments.

26.3.2 12.5 MLD Tonca City WWTP, Goa

The plant was receiving the average flow of 12,500 m^3/day (as per the data collected by WWTP's Staff); however, on the day of monitoring and sampling, the plant received a flow of 542 m^3/h or 13,000 m^3/day (13 MLD). Despite receiving a flow more than the designed capacity (12,500 m^3/day), the WWTP was producing the satisfactory effluent quality in terms of BOD, suspended solids, fecal coliforms, and turbidity that not only fulfills the Indian effluent discharge standards but also almost fulfills US EPA standards for non-potable reuse.

26.3.2.1 Effluent Quality

The WWTP was found to produce an effluent having low concentration of BOD (8 mg/L), TSS (10 mg/L) and turbidity (1.2 NTU). The significant removal were observed in TKN (97%; effluent TKN 1.9 mg/L), ammoniacal nitrogen (as NH_4-N) (99% removal; effluent NH_4-N: 0.6 mg/L), and phosphorus (PO_4-P) concentration (96% removal; effluent PO_4-P: 0.1 mg/L), which is very impressive in biological nutrient removal systems. The observed effluent fecal coliform concentration of 230 MPN/100 mL was significantly lower than the WHO and NRCD fecal coliform standard of 1000 MPN/100 mL for the reuse of the treated effluent for unrestricted irrigation and to direct discharge the treated effluent in surface waters, respectively.

26.3.2.2 Nitrogen and Phosphorus Removal

To verify the conditions for denitrification, the dissolved oxygen concentration was measured in the reactor during the start-up of settling sequence. It was observed that only after 15 min, the DO concentration reached at 0.1 mg/L into the SBR basin; thereafter, favorable condition takes place for continued denitrification in sludge blanket zone. Furthermore, favorable conditions (anoxic/anaerobic conditions) were observed in bioselector basin cells, which favor the phosphorus release in this bioselector cells. This finally leads to the luxury uptake of phosphorus in SBR basin and low phosphorus concentration in effluent.

26.3.2.3 Sludge Settling

In order to study the reactive settling, the zone settling study was also carried out in SBR basin. Only after 15 min, the sludge settled up to 75% (2.8 m) of original water depth (3.8 m) (sludge settling velocity = 11 m/h). No sludge bulking or pin point floc was observed in the SBR basins, which resulted in excellent sludge settling and good effluent quality.

26.3.2.4 Biomass in SBR Basins

The observed SOUR value of 47 mgO_2/g VSS/h is very good, which represents to the existence of very healthy biomass into the SBR basin and indicates the rapid oxidation of substrate.

A low F/M ratio (0.14 per day) means that the microorganisms in the SBR reactor are starved, generally leading to a more efficient wastewater treatment. The benefit of operating at the low F/M resulting in a reduced and mineralized (stabilized) waste sludge generation. At this sludge loading, process resistance to hydraulic and organic shock loading is very high. The hazard of low F/M filamentous bulking is eliminated by the purpose designed multicell selector.

The observed protozoan fauna of *Arcella*, *Vorticella*, *Aspidisca*, and *Rotaria* confirms that the WWTP is performing satisfactorily, i.e., the good effluent quality, good nitrification, and existence of process favorable conditions, i.e., good floc formation, sludge settling, and DO in the SBR basins.

26.3.2.5 Remark

On the basis of the overall assessment, it can be said that the SBR-based WWTP is performing very well under full flow capacity conditions. The finally treated effluent can be discharged safely in surface waters. Furthermore, merely increasing the chlorine dose to 1–2 mg/L can satisfy the US EPA requirement for non-potable reuse such as urban reuse (landscape irrigation, vehicle washing, toilet flushing, use in fire protection, and commercial air conditioners), food crop irrigation, and recreational impoundments.

26.3.3 45 MLD Mundhwa WWTP, Pune

The plant was receiving the average flow of 45,000 m^3/day (as per the data collected by WWTP's staff); however, on the day of monitoring and sampling, the plant received a flow of 2068 m^3/h or 49,635 m^3/day (50 MLD appx.). Despite receiving a flow more than the designed capacity (45,000 m^3/day), the WWTP was producing the satisfactory effluent quality in terms of BOD, suspended solids, nitrogen, phosphorus, and turbidity that not only fulfills the Indian effluent discharge standards but also almost fulfills US EPA standards for non-potable reuse.

26.3.3.1 Effluent Quality

The WWTP was found to produce an effluent having low concentration of BOD (5 mg/L), TSS (7 mg/L) and turbidity (2 NTU). The significant removal were observed in TKN (95%; effluent TKN 2.3 mg/L), ammoniacal nitrogen (as NH_4-N) (96% removal; effluent NH_4-N: 1.3 mg/L), and phosphorus (PO_4-P) concentration (70% removal; effluent PO_4-P: 0.7 mg/L), which is very impressive in biological nutrient removal systems. The effluent fecal coliform concentration of 7.5×10^3 MPN/100 mL was found significantly high than the WHO and NRCD fecal coliform standard of 1000 MPN/100 mL for the reuse of the treated effluent for unrestricted irrigation and to direct discharge the treated effluent in surface waters, respectively. Thus, the chlorine disinfection with a dose of 2.5 mg/L is necessary to fulfill the standard criteria.

26.3.3.2 Nitrogen and Phosphorus Removal

To verify the conditions for denitrification, the dissolved oxygen concentration was measured in the reactor during the start-up of settling sequence. It was observed that only after 5 min, the DO concentration reached at 0.1 mg/L into the SBR basin; thereafter favorable condition takes place for continued denitrification in sludge blanket zone. Furthermore, favorable conditions (anoxic/anaerobic conditions) were observed in bioselector basin cells, which favor the phosphorus release in these bioselector cells. This finally leads to the luxury uptake of phosphorus in SBR basin and low phosphorus concentration in effluent.

26.3.3.3 Sludge Settling and Characteristics

In order to study the reactive settling, the zone settling study was also carried out in SBR basin. After a settling period of 20 min, the sludge depth was reduced from 5 m (at zero time) to appx. 90% of original water depth, i.e., 4.5 m (sludge settling velocity = 13 m/h). No sludge bulking or pin point floc was observed in the SBR basins, which resulted in excellent sludge settling and good effluent quality.

Sludge characterization revealed that due to high NPK [N = 34.00 g/kg (3.4%), P = 11 g/kg (1.1%), and K = 2 g/kg (0.2%), the dewatered sludge can be used as a fertilizer after drying and or/composting. Most of the heavy metals are under the limit of ceiling concentration as suggested by US EPA.

26.3.3.4 Biomass in SBR Basins

The observed SOUR value of 84 mgO_2/g VSS/h is very good, which represents to the existence of a very healthy biomass into the SBR basin and indicates the rapid oxidation of substrate.

A low F/M ratio (0.14 per day) means that the microorganisms in the SBR reactor are starved, generally leading to a more efficient wastewater treatment. The benefit of operating at the low F/M results in a reduced and mineralized (stabilized) waste sludge generation. At this sludge loading, process resistance to hydraulic and organic shock loading is very high. The hazard of low F/M filamentous bulking is eliminated by the purpose designed multi cell selector.

The observed protozoan fauna consisting of *Arcella*, *Vorticella*, *Aspidisca*, *Rotaria*, *Suctorian* confirms that the WWTP is performing satisfactorily, i.e., the good effluent quality, good nitrification, and existence of process favorable conditions, i.e., good floc formation, sludge settling, and DO in the SBR basins.

26.3.3.5 Remark

On the basis of the overall assessment, it can be said that the SBR-based WWTP is performing very well under full flow capacity conditions. The finally treated effluent can be discharged safely in surface waters after disinfect with a chlorine dose of 2.5 mg/L. Furthermore, merely increasing the chlorine dose 5 mg/L can satisfy the US EPA requirement for non-potable reuse such as urban reuse (landscape irrigation, vehicle washing, toilet flushing, use in fire protection, and commercial air conditioners), food crop irrigation, and recreational impoundments.

26.4 Performance Overview of 84 SBR-Based WWTPs

26.4.1 Treatment Capacity

Eighty-four SBR-based WWTPs under operation in different states (Gujarat, Rajasthan, Punjab, Mumbai, Haryana, Madhya Pradesh, Uttarakhand, Uttar Pradesh, Goa, and Tamil Nadu) of India were monitored. The treatment capacity of the plants ranged from 1 MLD to 245 MLD. Out of 84 WWTP, the 45 plants were operating at 1–25 MLD capacity, 18 plants at 25–50 MLD, 7 plants at 50–75 MLD, 6 plants at

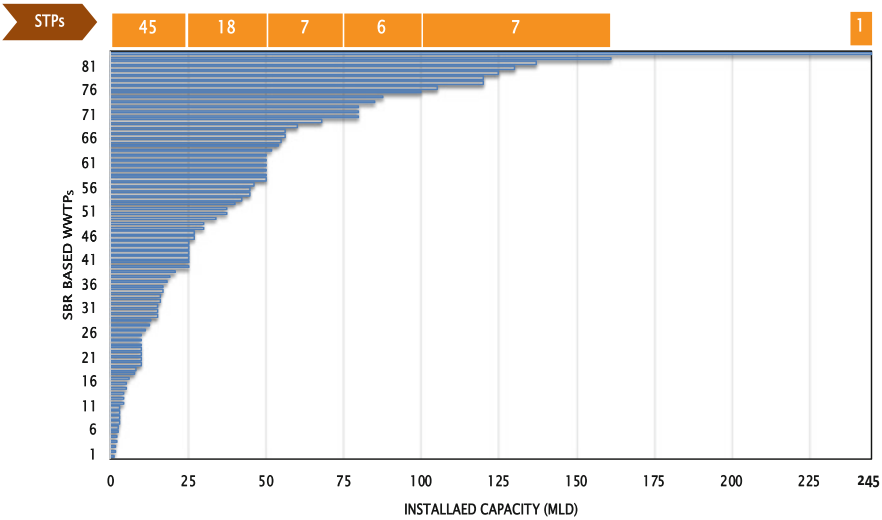


Fig. 26.2 SBR-based wastewater treatment plants

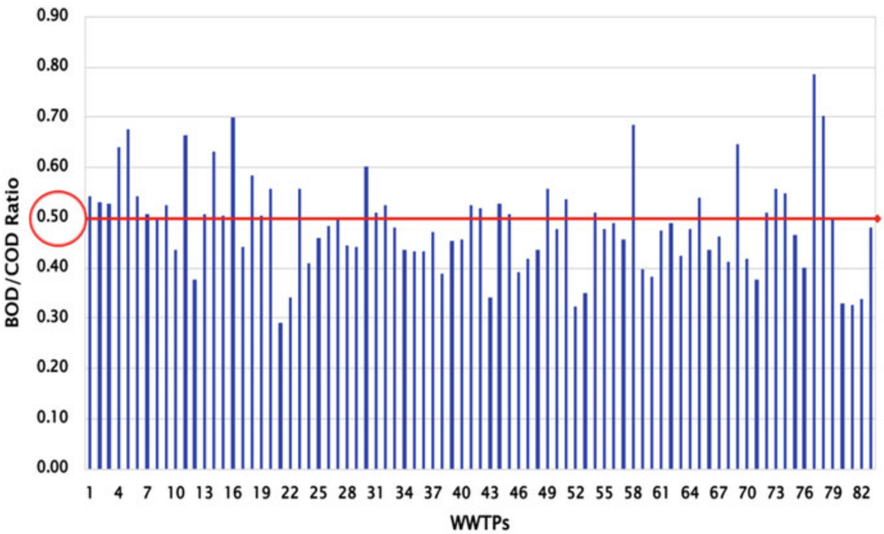
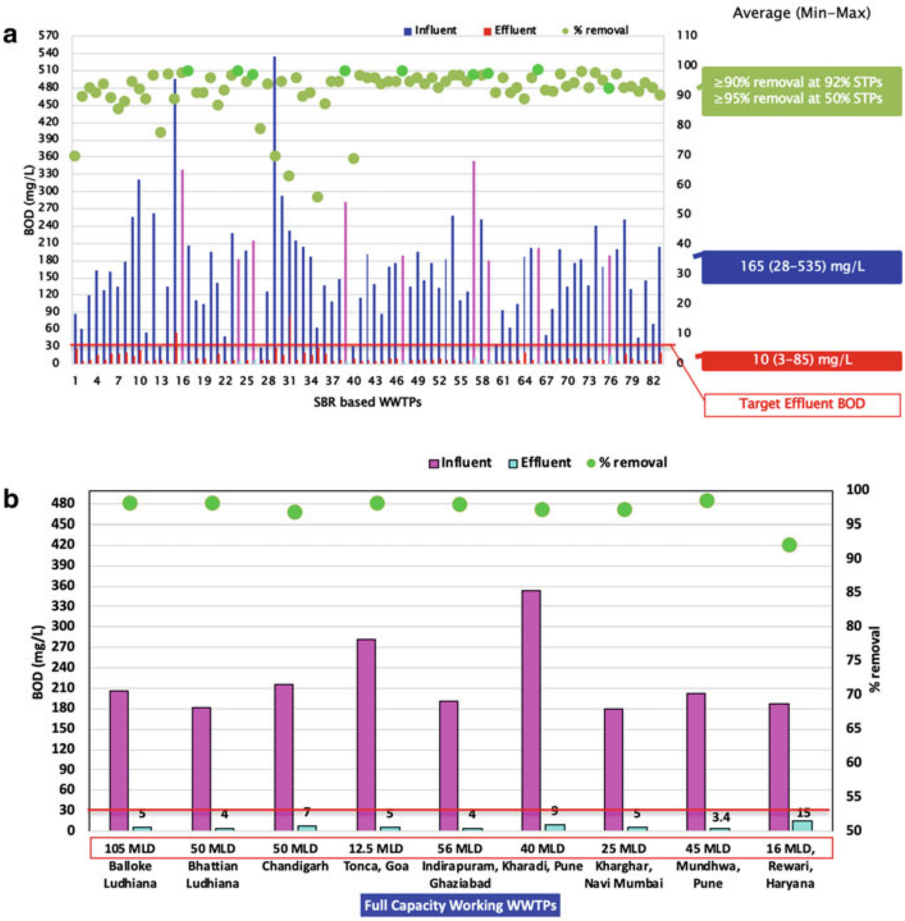


Fig. 26.3 BOD to COD ratio in raw wastewater

75–100 MLD, 7 plants at 100–161 MLD, and 1 plant at 245 MLD capacity (Fig. 26.2). The BOD to COD ratio in raw wastewater ranges from 0.3 to 0.8 with an average BOD/COD ratio of 0.5, which shows the biodegradable nature of wastewater (Fig. 26.3).

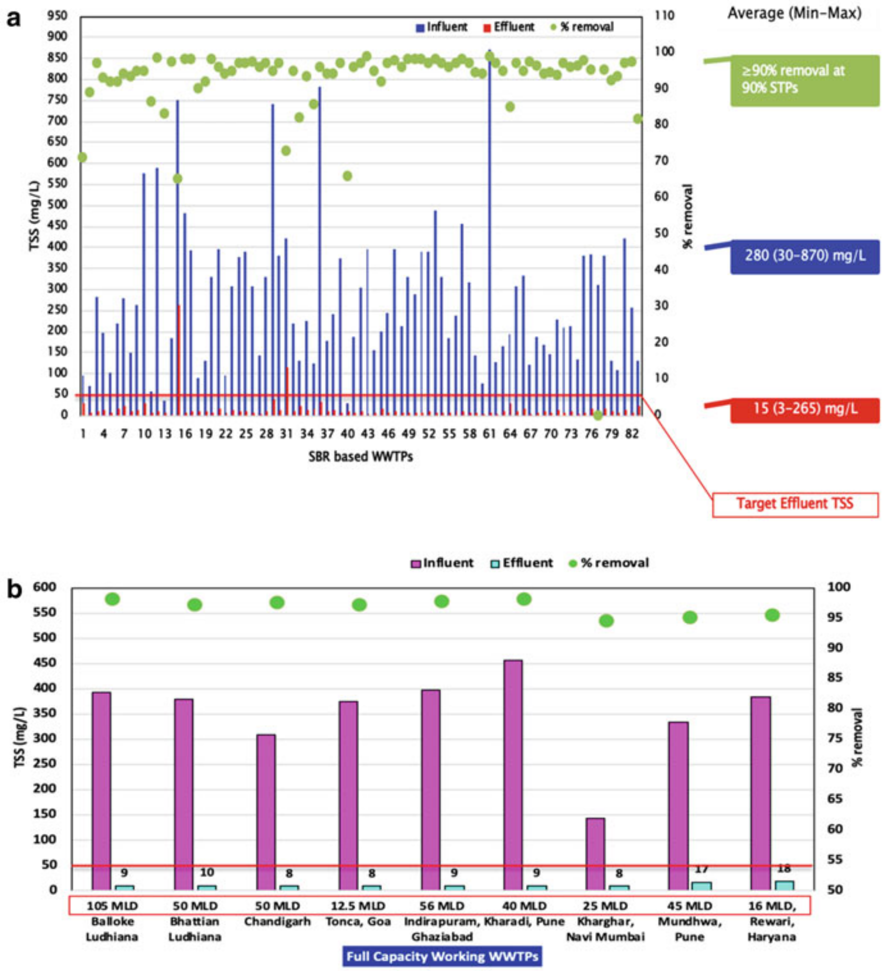
26.4.2 Organics Removal

Figure 26.4a shows that the raw wastewater BOD ranges from 28 to 535 mg/L (avg. 165 mg/L). The effluent BOD ranges from 3 to 85 mg/L (avg. 10 mg/L). More than 92% WWTPs achieve the BOD removal of ≥90%; however, 50% WWTPs achieved ≥95% BOD removal. The finding reflects the excellent BOD removal performance of SBR-based WWTP. Total nine WWTPs working at full capacity were observed to achieve an average BOD removal of 97% and producing the effluent of excellent



BOD (mg/L)	Inlet	Outlet	% removal
Avg	222	6	97
Min	180	3.4	92
Max	354	15	98

Fig. 26.4 (**a**) BOD profile in influent and effluent of WWTPs. (**b**) BOD removal at WWTPs working with full capacity



TSS (mg/L)	Inlet	Outlet	% removal
Avg	352	10	97
Min	144	8	94
Max.	457	18	98

Fig. 26.5 (**a**) Influent and effluent TSS profile and percentage TSS removal in WWTPs. (**b**) TSS removal at WWTPs working with full capacity

quality having avg. BOD value of 6 mg/L (Fig. 26.4b), which is significantly lower than the Indian effluent discharge standard of 30 mg/L.

Figure 26.5a reflects that TSS values in influent ranges from 30 to 870 mg/L (avg. 280 mg/L); however, the effluent TSS is significantly lower (avg. 15 mg/L) and fulfill the Indian effluent discharge standard of 50 mg/L. More than 90% WWTPs achieve the TSS removal of ≥90%. Total of nine WWTPs working at full capacity were observed to achieve an average TSS removal of 97% and producing the effluent of excellent quality having avg. TSS value of around 10 mg/L (Fig. 26.5b), which is significantly lower than the Indian effluent discharge standard of 50 mg/L.

26.4.3 Nitrogen and Phosphorus Removal

Figure 26.6a, b shows that the raw wastewater NH_4-N and PO_4-P ranges from 0.5 to 109 mg/L (avg. 35 mg/L) and 0.42 to 11.5 mg/L (avg. 3.7 mg/L), respectively. The effluent NH_4-N and PO_4-P ranges from 0 to 55 mg/L (avg. <5 mg/L) and 0.1 to 4.5 mg/L (avg. 1.6 mg/L), respectively. More than 80% WWTPs achieve the NH_4-N removal of ≥85%. The PO_4-P removal ranges from 12% to 95% with an average removal of 55%. The SBR-based WWTPs show the excellent performance regarding the NH_4-N and PO_4-P removal.

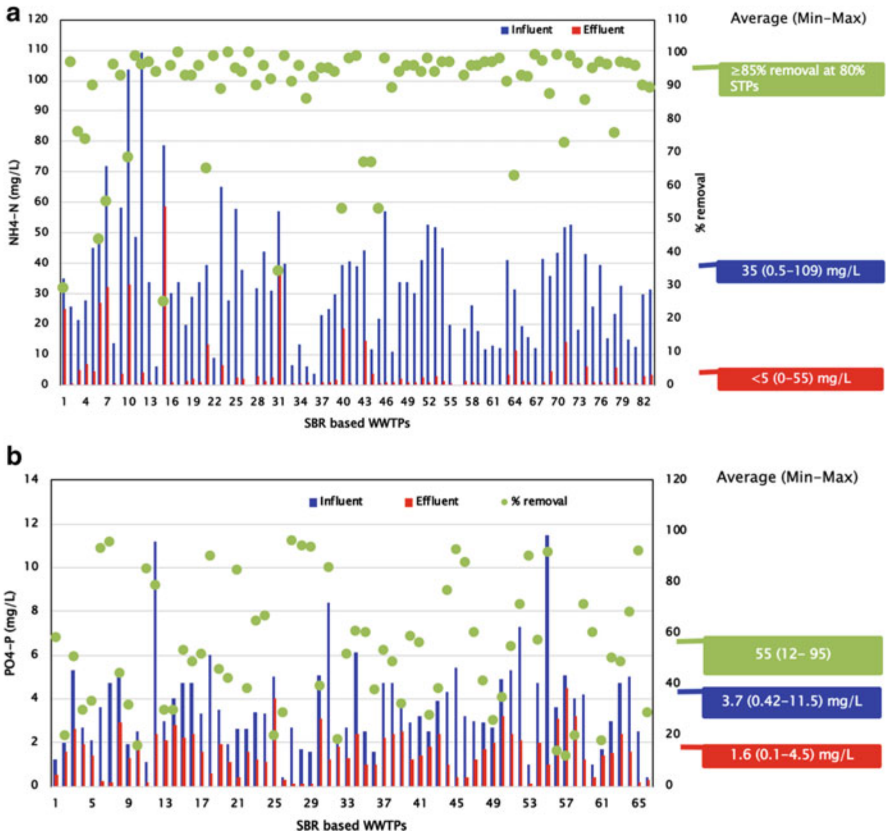


Fig. 26.6 (**a**) Influent and effluent NH_4-N profile and percentage NH_4-N removal in WWTPs. (**b**) Influent and effluent PO_4-P profile and percentage PO_4-P removal in WWTPs

26.5 Energy Analysis

Table 26.2 indicates the share of the energy required for different processes in SBR and ASP. It can be clearly observed that due to energy efficient machines such as pumps, motors, and blowers, SBR requires much less energy (kWh/m^3) than conventional activated sludge (CAS) process.

Figure 26.7a, b shows the energy distribution for the various units (excluding the energy requirement for pumping sewage into the plants) in ASP and SBR plant. This energy distribution pattern provides valuable information for identifying the areas with higher energy consumption that require maximum focus to reduce the energy consumption by a significant margin. Aeration was found to be the most energy-intensive step in both ASP and SBR consuming 61% and 53% of the total electricity requirement, respectively. All the operations in SBR are controlled by programmable logical controllers (PLCs), variable frequency drives (VFD) for pumps and motors, and automated DO control. Thus, comparison of both the technologies showed that large improvements in energy consumption can be made by modernization of the plants and processes including automated DO controls, fine bubble diffusers, efficient blowers, and adjustable speed drives.

26.6 Conclusion

The WWTPs working with cyclic technology were found to be efficient in producing the excellent effluent quality in terms of significantly low concentration of organic pollutants (BOD, COD, and TSS), nutrients (TKN, ammoniacal nitrogen, and phosphorus), and indicator bacteria (fecal coliforms). The MLSS in SBR basins showed good settling characteristics and beneficial protozoan population. The effluent not only completely fulfills the Indian effluent discharge standards but also almost fulfills US EPA requirement for non-potable reuse standards. However, the chlorine dose of 1–2 mg/L in chlorination tank can satisfy the US EPA requirement for non-potable reuse of treated effluent, such as recreational impoundments, cooling water, horticulture, and toilet flushing. The final disposed sludge was observed to be rich in nutrients (NPK) and thus can be utilized as an organic fertilizer.

Table 26.2 Energy required in different treatment stages

	ASP		SBR	
Treatment stage	kWh/day	kWh/m^3	kWh/day	kWh/m^3
Pretreatment	154.41	0.009	197.60	0.007
Primary treatment	105.84	0.006	100.80	0.004
Secondary treatment	2576.88	0.143	2603.22	0.096
Chlorination	564.00	0.031	697.00	0.026
Sludge handling	257.85	0.014	359.32	0.013
Building and lighting	317.00	0.018	117.00	0.004

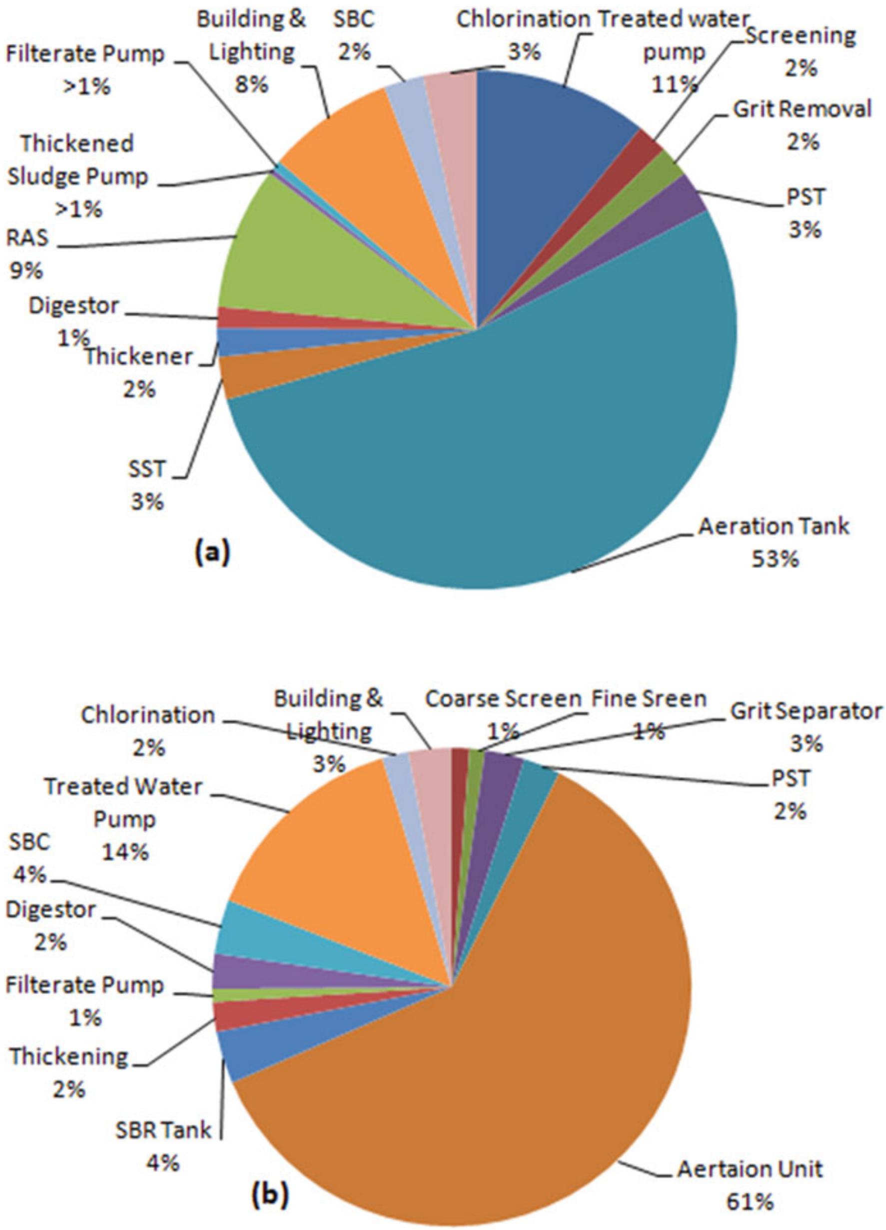


Fig. 26.7 Energy split of all the units in (**a**) SBR and (**b**) ASP

Energy audit of two most commonly used wastewater treatment technologies, i.e., ASP and SBR, revealed that SBR process is 42% and 24% more efficient in cost and energy as compared with ASP due to its state-of-the-art energy saving modern devices such as automation through PLC control and VFDs. It can be said that non-clogged fine bubble type diffuser systems in SBR with OUR-based controlled blower operation made aeration in SBR more energy efficient.

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Biodegradation of Soap Stock: As an Alternative Renewable Energy Resource and Reduce Environmental Pollution

27

Kamlesh Kumar R. Shah and Gayatriben B. Patel

Abstract

Cotton (*Gossypium hirsutum L.*) is an essential natural fiber crop cultivated for the production of cotton, cooking oil, and supplementary products. In India, it plays a key role in the Indian farming and textile industries market. Modest information was available on the utilization of cotton seed by-products and the dumping of waste. Cottonseed meal may be toxic to cattle because of gossypol present in it. Soap stock, a rich source of fatty acids, is an undesirable by-product formed during the processing of crude oil to edible oil and is separated by centrifugation. It has 5%–10% of the crude oil mass augment higher values to by-products, but it creates handling, storage, and disposal problem. A gelatinous dark brown cottonseed oil soap stock was oily sludge generated from oil refineries, which is composed of hazardous waste alkanes and aromatic hydrocarbons. It was playing a significant role in environmental pollution due to low oil–water solubility. Disposals of waste soap stock were done by microbes to reduce environmental problems. Microbe and its metabolic product (enzymes) can degrade soap stock and generate value-added renewable by-products like biodiesel, biogas, and bio-oil. Fatty acid methyl esters were produced using biological catalysts or whole-cell catalysts for transesterification of soap stock. It was used as an alternative best carbon source for the production of biosurfactants and microbial lipases. Bio-oil is a renewable energy resource, production from soap stock via pyrolysis, or continuous fast microwave catalytic

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co-pyrolysis process. That's all biomass conversion to value-added products is a potential purpose in soap stock treatment to reduce oil pollution.

Keywords

Biodiesel · Bio-oil · Biosurfactants · Cottonseed oil soap stock · Gossypol · Oily sludge

27.1 Introduction

In cotton (*Gossypium hirsutum* L.), a member of the family *Malvaceae* and 50 species were identified, which can be divided into two types: cultivated and wild cotton. Only four are cultivated, with the remaining 46 growing wild types. *Gossypium hirsutum*, *Gossypium herbaceum*, *Gossypium barbadense*, and *Gossypium arboreum* are four common cultivated cotton species. The fiber quality of these four *Gossypium* species was different. The cotton bud is the key part used in the cotton plant, which is the starting unprocessed raw material for a broad assortment of product formation in textiles, edible oil, paper, livestock feed, and medicinal products. The massive cotton production resulted in the accumulation of huge waste after the harvesting and ginning process (Fig. 27.1). This is a great challenge to manage the disposal of waste effectively. Cotton by-products have significant trade values. Cotton plant waste products (non-cotton fiber) were produced during the ginning process, which contains cotton gin trash (CGT), post-harvest field trash (PHT), crushed seeds (oil-extracted cake), and soap stock with additional values. First, the waste management of PHT is used in usual farming, where the cotton plant is slashed and left in farm benefited to land. In the ginning operation system, CGT was the most cost-effective burden of waste and 10% of the total weight. Waste CGT was mainly low valued used as biomass feedstock for commercial bioenergy/biofuel, fertilizer, incinerated material (ash) improving soil carbon, and compost to protect soil moisture with improving yield. Cotton fibers have represented 35%–40% total weight of the ginned cotton, which is used in fabric preparation. Besides, the high-cost effective matter was a cottonseed, which was used as human food (refined oil) and protein meals in the feed industry. The total weight of ginned cotton constitutes about 55% of cottonseeds (Egbuta et al. 2017).

Cotton is the most significant commercial crops of India, the foremost natural source of fiber cultivated for its lint and seed oil. Cotton is a source of fiber, oil, and protein. Currently, cotton is a fiber cum oil yielding crop. Cottonseed oil is broadly used for human consumption. Cottonseed oil is rich in both saturated (C14:0, 16:0, etc.) and unsaturated (C 18:1, 18:2, 18:3) fatty acids. The cottonseed oil has a ratio of 2:1 of polyunsaturated to saturated fatty acids and generally consists of 65%–70% unsaturated fatty acids including 18%–24% monounsaturated (oleic), 42%–52% polyunsaturated (linoleic), and 26%–35% saturated (palmitic and stearic). Cottonseed oil processing involves the removal of linters, decortications, and separation of the hull, expelling, solvent extraction, and finally refining of the oil. The crude oil is

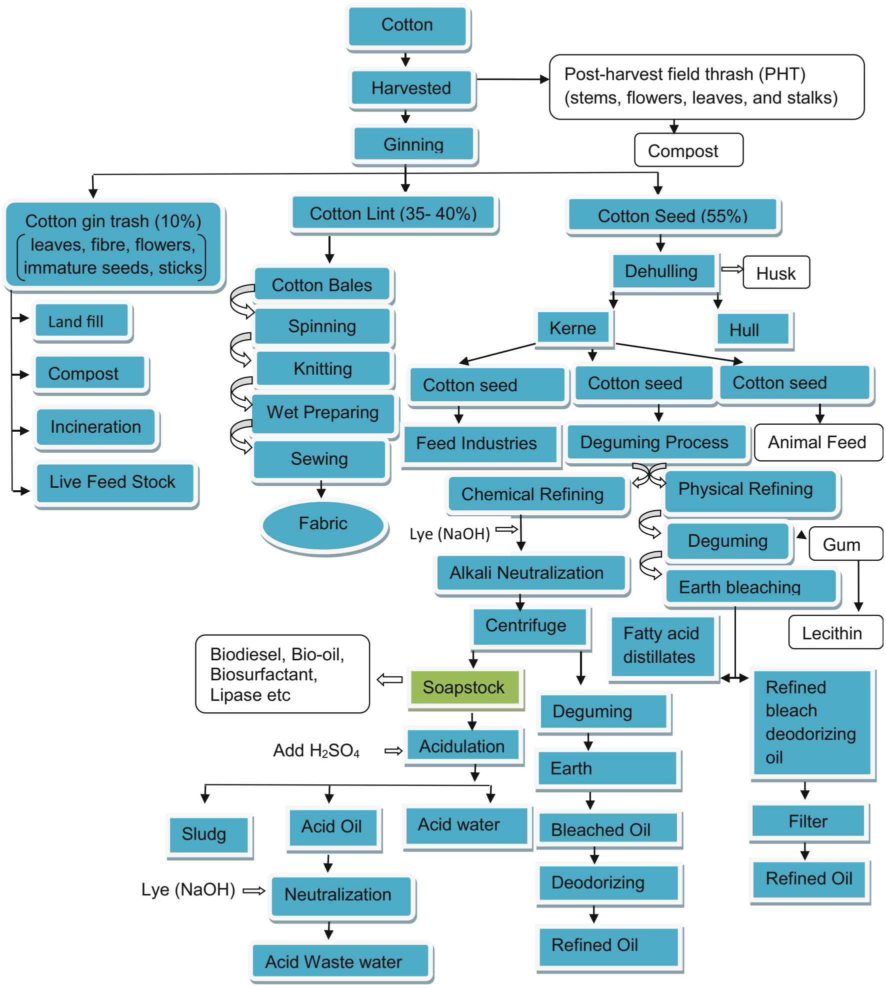

Fig. 27.1 Cotton ginning process and its by-product managements

extracted by solvent from cottonseed (Agarwal et al. 2003). In vegetable oil refining, chemical and physical processes such as degumming and neutralization are involved. Bleaching and deodorization will remove undesirable flavor and color causing substances from oil. By separating these unwanted materials (soap stock, lecithin gum, and other impurities), the quality edible oil can be maintained and obtain oxidative stable oil (Pantoja et al. 2018).

27.1.1 Cotton Seed Oil Soap Stock

Soap stock is a gelatinous dark reddish-brown waste, the lipid-rich high-value waste by-product produced from the oil refining process. Soap stock was engendered, from dark reddish-brown crude cottonseed oil, a strong typical odor. The oil refined with alkaline caustic soda solution reduces free fatty acid content and color from the oil. In this process, neutralization of free fatty acids with NaOH produces soap with other impurities and separated by centrifugation is the soap stock. The cottonseed oil soap stock is dark reddish-brown, but in the refining, process color varies from light dirty yellow to dark green due to the amount of alkaline solution. It also affects soap stock consistencies due to which it may vary from soft pasty to hard putty mass. In India, the alkali refining process generates a high amount of soap stock about 16%–20%, as compared with the United States around 6%–7% soap stock found in the cottonseed oil. As an increased amount of soap stock, material has caused the problem of storage, higher fatty acid content, and coloring matter. The soap stock value is determined by total fatty acid content that varies according to the refining process used because caustic lye dissolved the majority of impurities present in soap stock.

Compared with refined oils, soap stock cost is being low cost at around one-tenth of the charge of refined oil with a cheap source of fatty acids (Pantoja et al. 2018) and consists of low-quality lipid (Davis et al. 2002), complex mixture of nonfatty substances (mucilage, traces of metals, and alkali) and fatty, lipophilic substances (soap, neutral fat, phospholipids, pigments, and other unsaponifiable substances) (Satya Sree et al. 2014). Many industrial companies purchased soap stock for a variety of end consumers. Based on its fatty acid content, the applications of soap stock include its use as a feed, feedstock for chemical reactions, the nutrient source for microorganisms, use as an enriched fat supplement in concentrated diets feed, and fertilizer ingredient.

Oily sludge was generated in crude oil refinery industries consisting of waste hazardous alkanes, aromatic hydrocarbons; this waste disposal generates a serious problem when hydrocarbons persist in the ecosystems. Hydrocarbon has considered being recalcitrant because of its hydrophobic nature (low water solubility) and low volatility. The generated sludge is not only one problem but during normal operation, investigation, leakage, and spillage of crude oil resulting in pollution of locations such as oil wells, sumps, and pits, tank batteries, gathering lines, and pump stations. Unfortunately, there is insufficient information available regarding cotton by-product utilization (Roy et al. 2014).

27.2 Soap Stock Utilization as an Alternative Renewable Energy Source

The sustainability of petroleum-based fuel supply has put on broad attention from the global center of the population. Biodiesel, a renewable fuel, has a huge scene in satisfying an increasing transport fuel demand. The global energy disasters and inadequate fossil fuels, along with remarkable environmental problems, have posed several challenges to the environment-friendly substitute of fossil fuels(Sang 2003).Soap stock has low commercial value; however, it is used in biodiesel production. It's a good alternative for the cost reduction of biofuels, to solve environmental problems (Piloto-Rodríguez et al. 2014). Soap stock can be effectively renewed into an incredibly useful product such as biodiesel; simultaneously, waste minimization can be carried out practically (Satya Sree et al. 2014).

27.2.1 Utilization of Renewable Substrate for Bioenergy/Biofuel Production

Biofuel, from vegetable oil, has huge potential as an alternative energy source to put back fossil fuels. The biofuels are environmentally friendly and free from nitrogen and sulfur. Biofuel is obtained from biomass and farming or cultivated area foodstuffs such as cotton, palm, vegetable, soybean, and rubber seed oil. In biofuel production, various methods such as pyrolysis, gasification, and transesterification, catalytic cracking reaction, and hydrocracking reaction can be used (Sang 2003).

27.2.1.1 Soap Stock Waste Substrate to Biodiesel Production

Biodiesel, an alternative fuel for the diesel engine, is biodegradable and less toxic. These renewable fuel sources are currently accessible; it is used directly without diesel engine modification, not as much of emission into the environment. Biodiesel has an enormous potential in fulfilling an ever-increasing transport fuel demand. Biodiesel is formed by the use of the transesterification of triglycerides with an alcohol in the presence of a proper catalyst. However, researchers declared some significant challenges associated with productions, which are the feedstock cost and the variety of suitable methods for efficient production of fuel from different feedstock with given attention to parameters such as the molar ratio of alcohol to oil, type and amount of catalyst, reaction temperature, reaction time, reaction medium, and type and the relative amount of solvents (Gebremariam and Marchetti 2017).

On other hand, the majority of researchers have to pay attention to low-price oils as feedstock for biodiesel production such as waste oils and nonedible oils and usage of waste oils can decrease biodiesel production costs by 60%–90%(Pantoja et al. 2018). To biodiesel, the cost reduction of biodiesel was produced from high free fatty acid that contain soap stock which is used as feedstock (Su and Wei 2014). Several types of research have been reported on the conversion of soap stock into biodiesel. Hence, renewable and environment-friendly alternatives to fossil fuels should be explored. A key biomass source is one of the renewable and environment-friendly energies and presents several advantages over traditional fossil energy. The advantages of biodiesels are clean, portability, ready availability, renewability, higher combustion efficiency, lower sulfur, aromatic content, higher cetane number, higher biodegradability, and nontoxic diesel substitute, whereas the disadvantages of biodiesel included moisture absorption, low calorific value, corrosiveness, and high viscosity mainly that arises due to the presence of oxygen. So we need to seek a potential way to manufacture high-value products from soap stock, which is a precious subject. Soap stock is a dark gelatinous material of oil refining. Researchers have a focus on soap stock as a source of biomass energy conversion to biodiesel (Dai et al. 2017).

Piloto-Rodríguez et al. (2014) reviewed biodiesel production from by-products, and waste material vegetable oil refining industries such as soap stock, acid oil, and fatty acid distillate in the cost-effective way of diminishing usual oil consumption and environmental harms. In assessment, biodiesel was obtained by covering by-products, not only widely used acid/base catalysis but also solid and enzymatic catalysis. Zahan and Kano (2018) reviewed focus on the use of palm oil, its by-products, and mill effluent for biodiesel production, as an excellent raw material, because biodiesel has similar properties to the regular petrodiesel.

Biodiesel Production: Two-Stage Process

Haas et al. (2000) reported methods with the aim that the fatty acids present in soap stock were esterified to fatty acid esters using low-priced reagents and to present an inventive path for soap stock utilization in biodiesel production. This process involved two steps, in alkaline hydrolysis the first step is the saponification of lipid-linked glycerides and phosphoglycerides into fatty acid esters, whereas in the second step acid (sulfuric acid) catalyzed esterification reaction results in the free fatty acid sodium salts followed by water washing treatment. Further, the fatty acid sodium salts were mixed with sulfuric acid and excess methanol resulting in the quick synthesis of fatty acid methyl esters (FAME) with $>99\%$ fatty acid esters. Su and Wei (2014) revealed that free fatty acids (FFAs) are present in a higher amount in soap stock. It cannot effectively transfer into biodiesel directly in two-stage process esterification followed by transesterification, whereas one stage process lipase-catalyzed methanolysis efficiently enhance biodiesel production.

Biodiesel Production: One-Stage Process

In the two-stage process, high FFA soap stock oil was reduced by pretreatment with acid catalysts such as sulfuric acid via acid-catalyzed esterification of FFAs. However, acid uses several forms of problem such as complex neutralization, corrosion, and environmental issues, whereas alkaline catalysis required feedstocks with lower free fatty acids in biodiesel production. In comparison, lipases were catalyzing both esterification and transesterification reactions in biodiesel production from soap stock oil in one stage. The advanced process has improved the production efficiency (changing two-stage to one-stage) in mild reaction conditions. Lipase catalyst was nontoxic to afford the green and sustainable process (Nurfitri et al. 2013).Conversely, lipases have come forward as a great tool for converting a wide range of feedstock oils to biodiesel. Lipase enzymatic catalysis (in both free and immobilized form) is feasible to produce biodiesel under milder conditions, while it creates fewer wastes than the conventional chemical process. The production of biodiesel using immobilized lipases improves reusability and operational stability, resulting in higher conversion rates and shorter reaction times, respectively. For biodiesel, different immobilization methods for lipases could be useful: adsorption, cross-linkage, entrapment, encapsulation, and covalent bonding (Luna et al. 2016).

The most widely used immobilized lipases involved in biodiesel production are Novozym 435 isolated from *Candida antarctica* and immobilized on a macroporous acrylic resin. Another Lipozyme TL IM isolated from *Thermomyces lanuginosus* is immobilized on a gel of granulated silica (Luna et al. 2016). Also, Lipozyme RM IM is isolated from *Rhizomucor miehei* and immobilized on an anionic resin (Soumanou and Bornscheuer 2003). Finally, the uses of enzymes will be altered, with special importance in the modification of fats and oils to the production of biodiesel. Response surface methodology (RSM) was also used in biodiesel production. In analysis, immobilized *Candida rugosa* lipase on chitosan catalyzed the preparation of biodiesel from rapeseed soap stock oil with methanol carried out and optimized (Shao et al. 2008).

Su and Wei (2014) developed the one-pot process. Lipase catalyzed soap stock oil to enhanced biodiesel production via one-pot esterification and transesterification reaction. Lipase catalyzed soap stock methanolysis reaction carried out into a solvent-free system in mild reaction condition in one stage. However, from biodiesel, tertiary alcohol was separated and reused. In advance improved process, different water absorbent was used to increase yield with solve the water activity problem. Furthermore, this simple process yield could be increased and reused to reduce the cost of biodiesel. Pantoja et al. (2018) contributed their work on biodiesel production from buriti oil soap stock. The process of methyl esters production was the acidulation of soap stock with sulfuric acid (H_2SO_4) and esterification of the fatty acids using methanol and H_2SO_4 as a catalyst. Due to the quality of the fuel produced, the transesterification method is the most preferred way to produce biodiesel from diverse feedstock types.

Gardy et al. (2019) reviewed nano-catalyst-based biodiesel production from cheap nonfood feedstocks like waste cooking oil, where nanomaterial-based catalysts such as solid acid and base catalysts were future perspectives of production of biodiesel. Further nanocatalysts were no toxicity, high surface area, reusability, higher stability, and the simplicity of purification. These solid catalysts have added high amounts of FFAs, being able to simultaneously esterify FFAs and transesterify triglycerides in feedstocks like waste cooking oil to biodiesel.

The drawback of lipase as a catalyst: In lipase free fatty acid catalysis process, water by-product was produced, which resulted in significantly decrease in biodiesel yield with an effect on the activity and stability of lipase. That was the reason for too low conversions of feedstock to the products. To overcome this problem, develop a modern system that has a water adsorbent system, to improve lipase-catalyzed reaction system, and conditions. This updated method is helpful for further progress in industrial biodiesel production at a low cost from the soap stock oil in a green and sustainable way (Pantoja et al. 2018).

27.2.1.2 Soap Stock Waste Substrate to Bio-oil and Biochar Production

Twenty-first century researchers are paying attention to waste material conversion into a valuable product. They have been concerned about the interest in the development of renewable energy because of the over-consumption of fossil fuels with increasing world demand. Oil refineries generated soap stock waste by-products, from which researchers cost-effectively harvested renewable energy biodiesel. But, biodiesel was obtained from soap stock having some drawbacks including high viscosity and low caloric value. Therefore, it's mandatory to focus on high-valued product formation from low-valued soap stock (Dai et al. 2017).

Bio-oil, a renewable energy resource, is one of the most promising alternatives to fossil fuels produced from biomass. Biochar, as carbonized biomass, is obtained from sustainable sources during pyrolysis. Biochar persists to be utilized as a soil conditioner in soils to sustainably enhance their agricultural and environmental value under present and future management. Pyrolysis is one of the environment-friendly, thermochemical conversion techniques, which alter biomass into the bio-oil (liquid), biochar (solid), and gas with promising flexibility in production and marketing (Uzoejinwa et al. 2018). It is based on the conduction, convection, and radiation heat transfer principle, where energy is delivered from the surface to the inside (Wang et al. 2019). For some improvement of the quality lower yield of the oxygen-containing compound and higher yield of hydrocarbon in bio-oil, it was done by using HZSM-5 typical zeolite catalyst that did well in deoxygenation (Jae et al. 2011).

Microwave-assisted cocatalytic fast pyrolysis has been accepted as one of the most potential technologies for biomass utilization. It is an eco-friendly and cost-effective technology to convert biomass into liquid fuels through one reactor with only a single step (Duan et al. 2019). Microwave-assisted co-pyrolysis of low hydrogen-to-carbon and high hydrogen-to-carbon effective ratio materials with the aid of HZSM-5 and MCM-41 is a promising technique to improve the bio-oil quality. The co-pyrolysis, high through technology is coupled downdraft reactor, and the microwave-absorbent bed enhances the efficiency of process operation and improves the qualities of bio-oil. With the application of the downdraft reactor, the chance of contact between the pyrolysis vapors increases; this helps enhance the synergistic effect. Compared with electric heating, microwave fast heating rate has the advantage in no temperature gradient, no hysteretic effect, and volumetric heating (Wu et al. 2020). The microwave heating technology to biomass pyrolysis is a method for bio-oil upgrading, the aromatic compound increased in bio-oil with the application of downdraft reactor and silica (SiC) (microwave absorber) (Wang et al. 2019). Soap stock is one of the best by-products of oil industries and feedstock for co-pyrolysis for bio-oil production (Wang et al. 2019).

Santos et al. (2010) researched that fatty wastes material from the agribusiness can be altered into biofuels by the pyrolysis process. Three industrial fatty wastes (soybean soap stock, beef tallow, and poultry waste) were utilized in the pyrolysis process in the absence of the catalyst. These vegetable and animal fatty waste by-products were thermally cracked, distilled, and form diesel-like fractions of linear and cyclic paraffin and olefins with oxygenated compounds, carboxylic acids and esters; it has been contributed to decreasing the waste and biofuel use concerned to reduce the pollution caused by the use of fossil fuels.

According to Dai et al. (2017), bio-oil and biochar were produced from soap stock through a microwave-assisted co-catalytic fast pyrolysis process when combining the effect of in situ and ex situ catalysis. In the in situ process, silica wool (Sic) and HZSM-5 catalyst bed are prepared at the desired temperature in quartz; the reactor was to add soap stock, bentonite, and HZSM-5 mixture for pyrolysis. In ex situ catalysis, only soap stock dry powder added to the catalyst bed reactor was placed in the pyrolysis vapor outlet. Herein the integrated co-catalysis process, pyrolysis temperature (500 °C) was raised by the dual thermal condition of Sic particle heat and microwave heat. In the microwave-assisted co-catalytic fast pyrolysis (MACFP) process, the bentonite is used to increase the yield of bio-oil and porous structure quality of biochar in the optimal experimental conditions of the co-catalytic process.

Wang et al. (2019) demonstrated that fast microwave-assisted ex situ catalytic co-pyrolysis of effective material *Chromolaena odorata* and soybean soap stock with aid of ex-situ catalyst HZSM-5 was a promising technique to improve the quality of bio-oil by decreasing the oxygen content and high aliphatic content. *C. odorata* and soybean soap stock were given a synergistic effect on the property of bio-oil and bio-char.

Duan et al. (2019) investigated renewable jet-fuel range hydrocarbons production from lignin and soap stock co-pyrolysis with agricultural waste-derived activated carbon catalyst. Ex situ catalytic fast co-pyrolysis was done using the corn stover-derived activated carbon catalyst in a facile fixed-bed reactor. The soap stock works as the hydrogen donor, which exhibited a synergistic positive effect with lignin on enhancing the production of valuable aromatics in the obtained bio-oil. Additionally, biomass-derived activated carbon catalyst has the vigorous catalytic ability to transfer pyrolysis vapors into high-density jet fuel-ranged aromatic hydrocarbons rather than phenols with the assistance of soap stock solid waste. The reported study may provide a novel route of converting solid wastes into value-added jet fuels and hydrogen-enriched fuel gases, which will move on the utilization of renewable biomass.

Wang et al. (2019) reported a new modified continuous fast microwave pyrolysis system, where the quartz reactor has a spiral stirring blade in microwave pyrolysis modified furnace. In microwave absorbent bed (SiC) and HZSM-5 catalyst heated at the desired pyrolysis temperature (550 °C) than *Alternanthera philoxeroides* and peanut soap stock feds to the reactor. In U-type condensers, the bio-oil fraction was collected. In a new continuous fast microwave catalytic co-pyrolysis system, *Alternanthera philoxeroides* and peanut soap stock were given considerable synergistic effect on the production of an increased amount of aromatic bio-oil but reduce the yield of bio-oil.

Jiang et al. (2020) have done an investigation on the microwave-assisted catalytic fast co-pyrolysis (MACFP) process; soap stock and biomass (soybean straw) were pyrolyzed in the presence of composite catalysts ZSM-5 and SiC in the downdraft reactor. Novel combine composite catalyst with the synergistic effect of soap stock and soybean straw helps in augmenting the quality of bio-oil such as decreasing the proportion of oxygen-containing compounds and increasing the proportion of aromatics in bio-oil. Jiang et al. (2020) reported the latest methods; SiC powder and ZSM-5 composite catalysts were able to synthesize hydrocarbon-rich bio-oil. These composite catalysts are involved in generating high heat, which forms excellent pyrolysis vapors from soybean soap stock in a tandem microwave reaction system (two microwave devices: microwave pyrolysis and microwave ex situ catalytic reforming). In the first microwave pyrolysis, gas is produced and moved through the catalyst bed in the second microwave system and then condensed at the U-type condenser pipes. The experiment was repeated for the accuracy of the results.

27.2.1.3 Biofuel Purification Techniques

After by-product (glycerol) separation, the crude biodiesel needs to be purified to meet the standard specifications before marketing. Otherwise, impurities are present in the biodiesel which not only affect its diesel engine performance but also complicate its handling and storage. So before marketing, biodiesel purification is a crucial step. Biodiesel purification methods can be classified based on the nature of the process. Equilibrium-based, affinity-based, solid–liquid separation processes, membrane base, and reaction-based separation techniques are various biodiesel purification methods (Fig. 27.2), into which an appropriate combination of methods is regularly required to achieve healthy biodiesel in purification technology.

Numerous upgrading methods can be planned to upgrade bio-based oils by the deoxygenating process via hydrodeoxygenation and decarboxylation/decarbonylation pathways to produce renewable biodiesel with excellent fuel properties (Bateni et al. 2017).

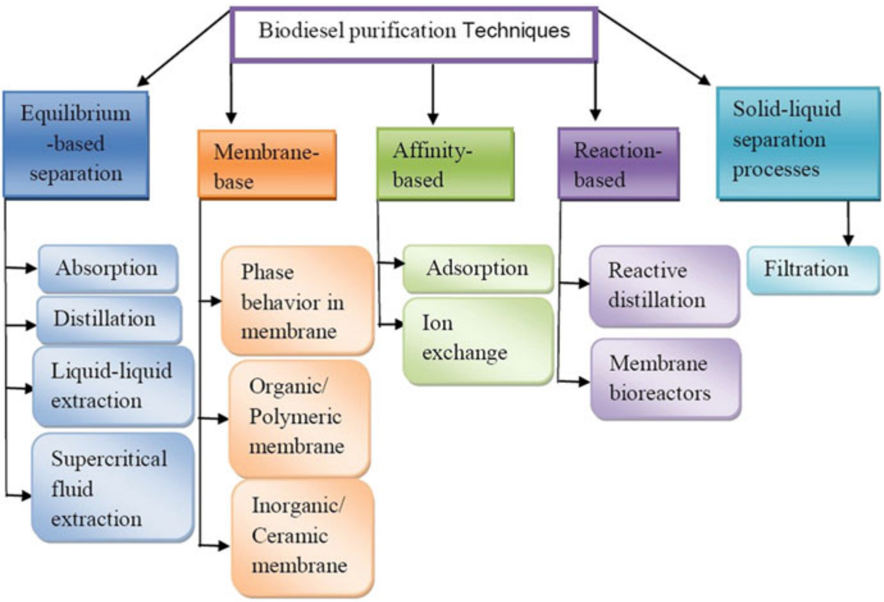

Fig. 27.2 Purification techniques of biodiesel classified based on the nature of process

27.2.1.4 Biofuel Production: Catalytic Cracking and Hydrocracking Process

Hydrocracking and catalytic cracking are also technologies that are extensively used to produce biofuels from vegetable oils. In thermal cracking reactions, pyrolysis is not desirable for high-molecular-weight hydrocarbons, converted into hydrocarbon compounds with a lighter molecular weight at high temperatures. In thermolysis, coke formation occurs with a large amount of gas and naphtha of low quality as a result of the over-cracking process. In contrast, in the catalytic cracking process, the catalysts can decrease the decomposition temperature, by reducing the activation energy of the cracking; consequently, it will make the whole process more efficient as it reduces pollution through lower energy consumption and minimizes unnecessary by-products, like heteroatom substances. In the hydrocracking process, high-molecular-weight hydrocarbons are transformed into hydrocarbon compounds with a lighter molecular weight with immediate or sequential hydrogenation and carbon bond breaking. The catalytic cracking of oil shows a potential alternative route for environmental-friendly liquid fuels. Heterogeneous catalysts such as HZSM-5, MCM-41, CaO, and multiporous composite MC-ZSM-5/MCM-41 have been used as catalysts for these processes (Sang 2003).

Sihombing et al. (2020) investigated the synthesis of bio-gasoline (bio-petrol) from the hydrocracking of MEFA rubber seed oil. Nonedible oils, for instance Rubber seed oils, are achieved from rubber seeds; which have the core linoleic acid 39 of 67% and oleic acid of 23.52%. Co and Co-Mo metal are loaded onto Sarulla Natural Zeolite, which has been activated and calcined (SNZ-Cal), catalyst oxidized at 500 °C for 2 h within oxygen gas flow on hydrocracking of MEFA rubber seed oil. The Co/SNZ-Cal and Co-Mo/SNZ-Cal catalyst was added in zeolite carriers during a wet impregnation method using Co $(NO_3)_2{\cdot}6H_2O$ and $(NH_4){\cdot}6Mo_7O_{24}{\cdot}4H_2O$ precursor salts, which provide larger surface area and pore diameter and smaller pore volume that show the highest catalytic activity and selectivity in the MEFA hydrocracking process of rubber seed oil to produce biogasoline fractions and biodiesel fractions.

Sang (2003) reported a single step direct process; catalytic cracking of palm oil was demonstrated as a potential alternative route for the production of environmental-friendly liquid fuels. Palm oil has been cracked at atmospheric pressure and reaction temperature of 450 °C to produce biofuel in a fixed-bed microreactor in the presence of catalysts like microporous HZSM-5 zeolite, mesoporous MCM-41, and composite micromesoporous zeolite. These catalysts were useful for catalytic cracking of the used vegetable/waste palm oil, which was easily obtained from fast-food restaurants. These catalysts passed reactions that influence catalyst pore size and acidity of the composite material over biofuel production due to their shorter chain length compared with crude palm oil. Products such as gas, organic liquid, water, and coke were obtained during catalytic cracking. The organic liquid product was composed of hydrocarbons resultant in olefins and paraffin (gasoline, kerosene, and diesel). Michał et al. (2013) reported the fermentation process; *Clostridium acetobutylicum* was producing biobutanol from biomass and biodegradable waste (sugar beets, sugarcane, wheat, corn, straw, or wood waste) by chemical reaction. Biobutanol is a mixture of isomeric alcohols like n-butanol (1-butanol), sec-butanol (2-butanol), isobutanol (2-methyl-1-propanol), and tertiary-butanol (2-methyl-2-propanol) and has similar fuel properties to gasoline.

27.2.1.5 Novel Biofuel (Green Diesel) Production

Researchers face the problem of managing the glycerol produced during biodiesel synthesis, which causes technical and commercial problems in biodiesel production. These biodiesel drawbacks are associated with glycerol by-product obtained during the transesterification process, which goes beyond at least 10% by weight of oil used as raw material. Therefore, in the future, widespread use of biofuel will develop new technology products with high quality of biofuel from naturally derived feedstocks. The alternative methods should be developed for the production of high-quality diesel fuel (novel biofuel), which fulfills the needs to be compatible with petroleum-derived diesel fuel and with offered transportation infrastructures. Further, the renewable liquid biofuels could be produced by research on glycerol free process, production practices, and engineering developed to produce alternative high-quality diesel fuel from vegetable oils, by hydrotreating triglycerides in conformist oil refineries (green diesel) in addition to novel biofuels that integrate glycerol into their composition (Gliperols, DMC-Biods, and Ecodiesels) and nearby relevant technologies for their productions (Calero et al. 2015). Glycerol triacetate (triacetin), a biodiesel-like biofuel, patented as Gliperol® by the Research Institute of Industrial Chemistry Varsow (Poland). Gliperol improves the yield, the effectiveness, and the economic feasibility of the conventional biodiesel process. After Gliperol was patented, further research on basic catalysts, such as potassium hydroxide, potassium methoxide, and polyethylene glycolate, has been carried out (Estevez et al. 2019).

In the integration of glycerol as a biofuel, the FAMEs are achieved; at the same time, several glycerol derivatives generate instead of glycerol. That glycerol derivatives appear to be the best choice to pass up the enormous amount of glycerol produced in the usual transesterification process. Furthermore, deal with the problem associated with the separation and cleaning of residual glycerol dissolved from the FAMEs mixture—raw biodiesel. These derivatives of glycerol revealed similar properties as biodiesel, glycerol triacetate (Gliperol), glycerol carbonates (DMC-Biod), or monoglyceride (like EcoDiesel), which are obtained during processes (Estevez et al. 2019). Numerous oxygenated biofuels that integrate glycerol as soluble derivatives, very similar to biodiesel, have been described, such as Gliperol, DMC-Biod, or Ecodiesel (Luque et al. 2008).

Likewise, alternative high-quality diesel fuels, generally known as “green diesel,” have also been obtained by several processes, i.e., cracking or pyrolysis, hydrodeoxygenation, and hydrotreating of vegetable oils. All these biofuels can be considered as “renewable diesel,” although their composition is similar to that of fossil fuel (Abdulkareem-Alsultan et al. 2019).

Xu et al. (2005) demonstrated that a new route of enzymatic interesterification (lipase-catalyzed interesterification of soybean oil) of triglycerides for biodiesel production with methyl acetate instead of methanol as the acyl acceptor enhances the stability of the immobilized lipase greatly. Santos et al. (2010) carried out fatty wastes such as soybean soap stock, beef tallow, and poultry industry waste, which were used in pyrolysis in the absence of catalysts. In pyrolysis, fatty waste organic mixtures of hydrocarbons and oxygenated compounds were obtained and isolate diesel-like fractions such as olefins, paraffin, and some oxygenated compounds such as carboxylic acids and esters. Casas et al. (2011) worked on sunflower oil interesterification reaction with methyl acetate, which was done for biodiesel and triacetin production.

27.2.1.6 Soap Stock Waste Substrate to Biogas Production

Biogas is methane-rich flammable gaseous fuel produced by the breakdown of organic matter. As an international situation increasing the costs of fossil fuels with a fall in the emissions of greenhouse gases, companies would like to decrease the energy utilization of fossil fuels and focus on biogas recovery, which can respond to this craving. In the management of industries wastes such as by-products and effluents to reassess the current improvement for some types of by-products (biogas), anaerobic digestion is one potential solution. The anaerobic digestion of by-products generated during the production and refining of vegetable oils conversion to biogas through methanization as an alternative to conventional solutions to reducing the energy consumption of fossil origin on refinery sites (Torrijos et al. 2008).

Thanikal et al. (2015) developed the process of refined vegetable oil and the anaerobic digestion of the soap stock sludge form during the refining of sunflower oil, to reduce its pollution load, to characterize and apply anaerobic digestion, and to treat the refined oil sludge and produce biogas. The biodegradability of soap stock sludge is possible, but it would be slow, as anaerobic digestion of soap stock sludge could be an environmentally friendly treatment of this waste. Torrijos et al. (2008) stated that refining crude oil; generate by-products, and residues are very interesting substrates due to their high-fat content on methane production from reactors co-digesting a mixture of grass, cow dung, and fruit and vegetable waste. Three by-products, one soap stock, one used winterization earth, and one skimming of aero-flotation of the effluents, were used for co-digestion to very high methane yields.

Lipolytic novel *Staphylococcus haemolyticus* strain enzymatically hydrolyzed the oil and grease present in a by-product from the vegetable oil refining industries (soap stock) before the anaerobic biological treatment stage, involved in biogas production. The enzymatic pretreatment was playing as a very promising alternative for treating soap stock, having high-fat contents to biogas (Cherif et al. 2014).

27.3 Soap Stock Utilization of the Renewable Substrate in the Production of High-Value Compounds

Soap stock is a by-product of the oil refining plant, which presents a great opportunity for harvesting biomass energy in a cost-effective and promising way. Soap stock is treated to produce an enviable and saleable product with no environmental pollutants (Dai et al. 2017).The low commercial value by-products are truly appropriate to the extraction or isolation of several industrially useful high-value compounds in a wide range of applications (Piloto-Rodríguez et al. 2014).

27.3.1 Soap Stock Waste Substrate to Lipase Enzyme Production

Recently, research has been focused on the selection of suitable economic substrates for the fermentation process. Agro-industrial waste is an alternative substrate; if used, it may help in solving the problems of disposal and pollution. The use of cottonseed and soybean cakes as substrates to produce microbial lipase by *Yarrowia lipolytica* is an essential nutrient composition for microorganisms' growth. The cottonseed and soybean cakes are concerned in increasing interest as abundant and cheap renewable feedstocks, and additionally, it could condense the human impact on the environment (Farias et al. 2014).

Damaso et al. (2008) worked on the production of lipases by solid-state fermentation (SSF) using the *Aspergillus niger* mutant 11T53A14 from agro-industrial residue supplemented with by-products from corn oil and olive oil refining process (soap stock, stearin, and fatty acids) as substrate. Among them, soap stock was the best inducers for lipase activity. Ribeiro dos Santos et al. (2014) researched about lipase production solid-state fermentation (SSF) using a mutant strain of *Aspergillus niger,* where canola, sunflower, and corn soap stocks were used as lipid substrate. Alkaline soap stocks were demonstrating the importance of lipid substrate to improve the lipase activity. Rade et al. (2020) revealed novel lipase production from thermophilic fungus *Rasamsonia emersonii* (*Re*Lip). New fungal biocatalyst tolerates extreme conditions such as the presence of methanol, high temperatures, and acidic medium. This biocatalyst was hydrolyzing macaw oil (a highly oil-producing plant, presents high contents of free fatty acids), an attractive feedstock for the production of "drop-in" biofuels.

27.3.2 Soap Stock Waste Substrate to Biosurfactant Production

Biosurfactants are defined as a class of surface-active molecules synthesized by microorganisms. Renewable sources utilized for biosurfactant production pay attention as an alternative to petroleum-based surfactants. Some advantages of biosurfactants over synthetic surfactants are high surface activity, low toxicity, biodegradability, and effectiveness. The applications of rhamnolipid biosurfactant showed bioremediation and heavy metal removal, biocontrol agent, and microbial enhanced oil recovery (Pathania and Jana 2020).

Partovi et al. (2013) demonstrated methods of biosurfactant production using cheaper oil-rich waste soap stock using microorganisms. *Pseudomonas aeruginosa* produced pure and less expensive biosurfactant and environmental-friendly (green) compound with commercial value and reduced oily waste entering into the earth's surface. The biosurfactant products, rhamnolipids, were detected by the easy infrared spectrophotometry method. Nitschke et al. (2010) demonstrated that low-cost oil waste material soybean, cottonseed, palm, babassu, and corn were utilized by *Pseudomonas aeruginosa*, to generate rhamnolipids. *Pseudomonas aeruginosa* LBI strain was utilized by soybean oil soap stock as the sole carbon source for the production of rhamnolipids. *Candida antarctica* and *Candida apicola* were produced glycolipids when supplemented with soap stock (Bednarski et al. 2004).

Partovi et al. (2013) worked on the fermentation process, where extracellular biosurfactants were produced from *Pseudomonas aeruginosa* MR01 linking to the biodegradation of soybean oil refining wastes. Three soybean oil refinement wastes, acid oil, deodorizer distillate, and soap stock, were utilized by microbes in biosurfactant production. Nitschke et al. (2010) worked on rhamnolipid surfactant production from soybean oil soap stock by *Pseudomonas aeruginosa*. This strain utilized soap stock as an alternative carbon source and resulted in rhamnolipid, useful in environmental and food industry applications. The rhamnolipids from *Pseudomonas aeruginosa* are the most effective mixture of the homologues species Rha2C10C10 and RhaC10C10.

Pathania and Jana (2020) worked on *Pseudomonas aeruginosa*, which is involved in the synthesis of rhamnolipid from a mixed substrate (fried oil and glucose). Microbes utilized that sugar and lipid moieties occurred through different pathways based on carbon sources directly. Obtain the sugar and lipid moiety without undergoing the gluconeogenesis and FAS II pathway, respectively, and enhance biosurfactant production. Furthermore, the microbe was isolated from the exhaust chimney site (fried oil condensate) to use fried oil as a substrate and further screened for its ability to utilization mixed carbon sources in biosurfactant production. These biosurfactants demonstrate excellent surface-active and thermochemical stability properties. Inexpensive biosurfactant products with high yield and productivity could be potential by isolation of mixed carbon source utilizing strain and optimization of waste substrates from oil/soap stock and sugar/corn syrup industries in media. The foremost problem associated with biosurfactant is the lack of structural variation and economical process using low-cost materials and low productivity. New strain isolation and fermentation process might be creating issues partially.

27.4 Biological Degradation of Soap Stock to Reduce Environmental Pollution

Now the day foremost, environment problem is oil pollution. Environments were damaged by the extensive release of various oily products that contain aromatic hydrocarbons during spillages and leakage from underground tanks, steamer's origins of widespread contamination of surface soils, groundwater, seas, and oceans. Most of them are toxic, harmful to aquatic and terrestrial life (Musale and Thakar 2015). When soil is contaminated with oil, it loses its fertility for more than 20 years. The texture of soil and physicochemical characteristics are affected. Additionally, the mites and other insects can't survive in the soil so oil contaminates land leading to the main imbalance in the food chain. Oil contamination has also an unfavorable effect on seed germination, and the farmers are not competent to cultivate crops for years (Mandal et al. 2011). The characteristic of soap stock from alkali refining of vegetable oils has turned into trouble with oil refineries as increased restrictions on environmental pollution (Dai et al. 2017). Cottonseed soap stock was a 7% oil-rich compound (Patel and Shah 2018). Bioremediation/biodegradation is emerging as one of the most promising technologies for the removal of petroleum hydrocarbons from the environment (Musale and Thakar 2015).

The researchers are interested in encouraging the people to use green technology; a biological methods in the clean-up technologies, for hydrocarbon- polluted sites without addition of chemical to the environment. The bioremediation/biodegradation suggests a viable alternative for this purpose to the petroleum industry. Petroleum hydrocarbons are naturally biodegraded pollutants, removed by applying hydrocarbon utilizing oleophilic bacteria, which are ubiquitously present in the environment. In microbial degradation, Microbes can utilize pollutants through enzymatic activities and metabolic processes to enhance the rate of pollutant degradation. Pollutant utilizing microbial population is an increase at contaminated site of environment, the first line of defense against oil pollutions (Varjani et al. 2013).

27.4.1 Microbial Degradation of Soap Stock to Control Oil Pollution

Bioremediation is one of the techniques to remove hydrocarbon from contaminated sites and include importance for industrial and environmental applications(Musale and Thakar 2015). Bioremediation is the technique that augments the natural rate of biodegradation of pollutants through selected microorganisms. Biostimulation is a process that enhances the growth and activity of microbes at the site of pollution through nutrition addition, whereas the bioaugmentation process adds more selected microorganisms at the pollution site to control pollution. By these techniques, we can reduce environmental pollution by the use of microbes or microbial consortia. An indigenous microbial consortium was developed; *Pseudomonas* strains PS-I, PS-II, and PS-III were used for the cleanup of oil-contaminated soil by bioaugmentation of polycyclic aromatic hydrocarbon (PAH)–polluted soil with PAH-degrading strains or consortia. In the investigation, PS degrading strains or consortia were able to reduce garden soil polluted with crude oil (Mittal and Singh 2008).

Chakrabarty researched genetic cross-linking that fixed plasmid genes in place and created a new, stable, bacteria species called *Pseudomonas putida*. *Pseudomonas putid*a was capable of consuming oil, carried on plasmid much faster than the previously oil-eating strains of microbes. Multi-plasmid hydrocarbon-degrading *Pseudomonas* is the first patented living microorganisms; new microbes could digest hydrocarbons in an oil spill. The developed genetically engineered *Pseudomonas*, "an oil-eating bacteria," was known as "superbug" (Pandey and Arora 2020). Varjani et al. (2013) demonstrated that bioremediation is cost-effective and considered an environmentally safe method for treating oil-polluted sites. From crude oil-contaminated samples, *Arthrobacter*, *Halomonas*, *Pseudomonas*, *Bacillus*, *Klebsiella*, *Proteus*, *Aspergillus*, *Neurospora*, *Rhizopus*, *Mucor*, *Trichoderma*, etc. were reported as hydrocarbon utilizing bacteria (HUB). HUB was capable of utilizing crude oil as a carbon source.

Actinomycetes are an important oil degrader; *Rhodococcus* sp. were isolated from the oil-polluted coastal region, which degrades aliphatic and aromatic fraction of crude oil. From studies, suggestion for cleanup of chronic oil-contaminated coaster region is possible by spraying cheaper nitrogen and phosphate to a substantial natural flora capable to increase the degradation of oil-polluted coastal region hydrocarbons at tropical ambient temperature. *Rhodococcus* sp. were isolated hydrocarbon degraders from a chronically oil-polluted marine site. It was able to degrade the aliphatic fraction of Assam crude oil in seawater supplemented with cheaper nitrogen source and phosphorus as revolutions (Sharma and Pant 2001).

In India, Oil and Natural Gas Corporation Limited (ONGC) is a public sector petroleum company. It is a company contributing 77% of India's crude oil and 81% natural gas production. Bioremediation technology has facilitated Oil and Natural Gas Corporation Limited (ONGC), India to petroleum hydrocarbon contaminated hazardous oily waste management in an environment-friendly approach. In situ and ex situ bioremediations were carried out for oil-contaminated effluent pits, sludge pits, oil spilled land and tank bottom, and effluent treatment plant (ETP) oily sludge, which contaminated heavy paraffinic, asphaltic, and light crude oil and emulsified oily sludge/contaminated soil. The microbial consortium was developed; different fractions of total petroleum hydrocarbon (TPH)/oily waste were biodegraded to environment-friendly end products (Mandal et al. 2011).

27.4.2 Biocatalyst Application in Controlling Environmental Pollution

Lipase (triacylglycerol ester hydrolases, E.C. 3.1.1.3) enzymes catalyze the hydrolysis of long-chain triacylglycerol to glycerol and free fatty acids (FFA). Lipases, interfacial activation, could occur at the lipid–water interface, unique structural characteristics of these hydrolytic enzymes. Lipolytic enzymes are illustrated to be an extremely potent alternative for degrading lipid-rich waste generated by industries. Further application of lipid waste pretreatment reduces the concentration of an organic substance, color, and suspended solids (Cherif et al. 2014).

Microbial lipases are already well-known for their infinite potential on the subject of usage in different industries. In the last few decades, interest in lipase production has increased with its vast potential in industrial applications as food additives (flavor modification), fine chemicals (synthesis of esters), wastewater treatment (decomposition and removal of oil substances), cosmetics (removal of lipids), pharmaceuticals (digestion of oil and fats in foods), leather (removal of lipids from animal skins), and medical (blood triglyceride assay) detergent formulation (removal of fatty residues). The lipase utilized in each application is specific, based on its substrate specificities such as fatty acid, alcohol, position (regio-), and stereospecificity, temperature, and pH stability. The lipase characteristic exhibits a high activity toward lipids with fatty acid residues of C_8 to C_{18} chain length (Prazeres et al. 2006).

In this regard, the biotechnological process can add value to waste (soap stock) by biodiesel production via lipase enzyme esterification (Botton et al. 2018; Wang and Cao 2011).

Soap stock with a high percentage of moisture ferments in hot weather and white fungus grows and utilizes it as the sole source of carbon. Patel and Shah (2020) reported that cotton oil soap stock neutral fats were enzymatically hydrolyzed on the approach of biological alteration of cotton oil soap stock. *Oospora lactis* utilized cotton oil soap stock as a carbon source during cultivation. Lipase produced by *Oospora lactis* precipitated with isopropanol, and its culture filtrate was used in hydrolysis of fatty material. Patel and Shah (2018) isolated *Bacillus* sp. from enriched cottonseed soap stocks samples. Isolated *Bacillus licheniformis* and *Bacillus pumilus* were good in lipase production, which will be used on enzymatic degradation of soap stock. *Fusarium oxysporum* produced alkaline lipase, which is potentially suitable for lipid stain removal applications in industrial laundry and household detergents (Prazeres et al. 2006). Novel alkaline lipase was produced by *Acinetobacter calcoaceticus* using olive oil as the sole carbon source. These innovative hydrolytic enzymes are stable over a pH range of 6.0–10.0 applicable as an additive in detergent formulations (Wang et al. 2012).

Haas et al. (2000) have developed combined two-step methods successively that enhance the conversion efficiency of soap stock into simple alkyl esters of soap stock fatty acid. In the first alkali-catalyzed by nonenzymatic steps, alcohol and KOH were transesterified glycerides and phosphoglyceride linked fatty acids of soap stock. On the secondary enzymatic steps, lipase esterified the remaining (not esterified in alkali transesterification step) free fatty acids of the substrate to alkyl fatty acid esters. In the combined process, the degree of conversion of soap stock fatty acid to alkyl ester was 81%. Wang and Cao (2011) reported a novel process of fatty acid ethyl ester (FAEE) production from soap stock and acyl acceptor diethyl carbonate through transesterification and esterification reaction. Lipase acts as a catalyst, which reacts with environment-friendly short-chain diethyl carbonate with soap stock and converted into FAEE. The FAEE produced has low viscosity, less toxic, and highly lubricant. A most significant aspect of this research was to reduce the production cost when costly alcohol use is replaced by cheaper diethyl carbonate in addition to frequent reuse of enzymes.

27.4.3 Biosurfactant Application in Controlling Environmental Pollution

Biosurfactant, nontoxic, biodegradable, and low-molecular-weight surface-active compounds are produced from microbes by utilizing cheaper substrates. Microorganisms were produced, and biosurfactant boasts potential effect on the utilization and degradation of hydrocarbon. This microbial surfactant solubilizes hydrocarbon and additionally enhances the bioavailability of hydrocarbon to microbes. Biosurfactants have potentially reduced the surface and interfacial tension of liquid, solid, and gases to its solubilization in a liquid solution at extreme temperature, pH, and salt concentrations. The microbial surfactant has distinguishing characteristics and better performance achieved attention and significance in various fields such as enhanced oil recovery, environmental bioremediation, food processing, and pharmaceuticals. Along with all biosurfactants, rhamnolipid has revealed improved performance in the bioremediation of hydrocarbons (Musale and Thakar 2015).

Microbial treatment of soap stock by *Staphylococcus* sp. strain produced biosurfactants and extracellular lipase when soap stock was used as an alternative carbon source. These potent metabolic product biosurfactant and lipolytic enzymes were potentially applicable in soap stock treatment (Patel and Shah 2020). Maneerat (2005) isolated two different *Acinetobacter* strains, which produce two extracellular capsular heteropolysaccharides from commercially obtainable and cheap fatty acid mixture (soap stock oil) served as the carbon source in a controlled fed-batch fermentation process. *A. calcoaceticus* RAG-1 produces emulsions. On the other hand, *A. calcoaceticus* A2 generates bidisperse (PS-A2), which is another extracellular zwitterionic heteropolysaccharides. This microbial polysaccharide disperses the big solid limestone granules forming micron-size water suspension with observation to their industrial application as specific dispersing agents.

27.4.4 Soap Stocks Use as Animal Feedstock Preparation

Waste disposal is gradually more difficult and costly. As a result, proper handling methods of soap stock are proposed. National Research Council stipulation involves that before soap stock is added to a commercial feed, it has been neutralized. On the other hand, the difficulty of handling wet soap stock, avoiding microbial spoilage, and shipping costs frequently preclude such disposition neutral dried soap stock (NDSS) and source of industrial fatty acids by splitting the glycerides by continuous high-temperature hydrolysis, as a source of energy and xanthophyll in poultry rations(Beal et al. 1972).

Peña et al. (2014) reported that three soybean by-products might build a high fiber and energy feed for ruminants. The blend of soybean hull, soy lecithin, and soap stock were containing excellent fiber and fat that feeding values to raise the growth of beef calf and lactation in dairy cattle. Cottonseed oil soap stock was used in manufactured feed grade oil, giving antidiarrheal effect when incorporated into a calf milk replacer diet. This feed-grade oil was prepared by methylation of cottonseed oil soap stock, which consists of fatty acid of methyl esters with free fatty acids and plant pigments. In cottonseed oil soap stock, the free form of gossypol was found, having toxic properties. A higher amount of soap stock when used as feed supplements to animal adversely affect the health of the animal due to gossypol, practically under certain conditions to use soap stock(Nagalakshmi et al. 2007).

27.4.5 Soap Stock with Other Waste Treatment Reduce Pollution

Microwave pyrolysis is more efficient, faster, and easier to control and industrialize, and it is a highly sustainable method for disposing of biomass(Wang et al. 2019). Duan et al. (2019) researched the co-pyrolysis of lignin and soap stock for upgraded liquid oils. In lignin waste treatment, lignin was directly incinerated that because of environmental pollution and energy waste, it's difficult to exploit and degrade the lignin structure. Currently, the pyrolysis of lignin and soap stock biomass into liquid oils, called bio-oils or pyrolysis oils, has received considerable attention. However, the key for high-efficiency utilization of the bio-oil is to improve the alternative petrochemical yield and overcome its low quality, such as large amount of oxygen, high acidity and viscosity, and poor heating value.

A novel route of converting soap stock and corn stover waste resources into valuable high-density jet fuel-ranged hydrocarbons was developed, which will contribute to advancing the utilization of renewable biomass and in the meantime turning waste soap stock into value-added fuels. A novel route was converting solid wastes into value-added jet fuels and hydrogen-enriched fuel gases, which will advance the utilization of renewable biomass. (Duan et al. 2019).

In nutshell, cottonseed soap stock will utilize by microbes to lipase production most effective way to in degradation of oil pollutants in the environment. Besides, microbial lipases and biosurfactants play a vital role in lipid waste degradation. However, these potential lipases will be used in oily sludge conversion to renewable biofuel generation and choice of the root in biological degradation as an alternative renewable energy resource with a reduction in environmental pollution. Biodiesel and green diesel (biomass-based biofuel) nowadays are continuously used in the market, which reduces the utilization of fossil fuel to decrease the prices of petroleum-derived fuel.

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Influence of Nanomaterials in Combined Microbial Fuel Cell-Electro-Fenton Systems as a Sustainable Alternative for Electricity Generation and Wastewater Treatment

28

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Abstract

The supply of energy from sources other than conventional ones and the pollution of aquatic bodies are global problems. Combined microbial fuel cell (MFC) and electro-Fenton (EF) systems are an emerging alternative that includes bioenergy production, which can use to drive the EF process toward the elimination of pollutants present in wastewater, avoiding the energy expenditure that would be incurred if only the EF process were carried out. This chapter shows MFC, EF, and MFC-EF's fundamentals, emphasizing the nanomaterials developed so far for their use as anodes and cathodes in MFC-EF systems, mainly carbon-based. In the specific case, the anodic electrodes, the porosity significantly influences on the biofilm's adhesion to its surface and on electron's transfer, guaranteeing that the EF process is carried out. The most used nanomaterials are carbon nanotubes and graphene in cathode due to their large specific surface area and electrical conductivity. Likewise, the solid iron promoters of nanometric size are recently reported and incorporated into the medium or as cathode modifiers. Finally, it included a cost/benefit analysis according to the maintenance of microorganisms, preparation of electrodes, and their useful life, cell design, and the mineralization of pollutants. The limitations, advantages, and areas of an opportunity of the MFC-EF systems that can become in the future a versatile and dominant

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technology for the generation of bioenergy and the elimination of pollutants in wastewater pointed out.

Keywords

Cost-benefit · Electro-Fenton · Microbial cell · Nanomaterials · Wastewater

28.1 Introduction

Industrial development has made man's lifestyle transcend over time; however, it caused environmental alterations, for example, in water bodies, which we are facing today. Likewise, man's life would not be the same without the presence of electricity generated by fossil fuels, which have supplied energy demand for several decades worldwide. Fossil fuels are about to run out, and that new power generation alternatives must be developed to meet the needs of the population. Hence, the contamination and reduction of the quantity of drinking water is a latent problem, which is why the need to look for alternatives that can improve the quality of the wastewater arises to give it a second use in activities in which it is not essential to use water drinkable, and that in turn also generate electricity. In this sense, one of the research topics that have attracted the most attention for several decades is developing bioelectrochemical systems (BES). BES are a promising technology for the generation of renewable energy, obtaining bioproducts, degradation of pollutants in the water, and diminished air pollutants; if they are also combined with pollutant degradation processes such as electro-Fenton (EF), there are self-sustaining systems that allow both the generation of electricity and the efficient treatment of water. In these systems, nanotechnology has played a significant role since it is the science that atomically modifies the properties of materials for different applications with greater use. Nanotechnology to treat wastewater and power generation in recent years has excellent height because of nanomaterial's contributions (membranes, electrodes, and iron nanoparticles). In this sense, this chapter addresses the following topics:

1. BES for power generation and water treatment, where classification is shown and importance in generating bioenergy and wastewater treatment, as well as fundamentals of the EF process, reviewing its efficiency in treating different organic pollutants.
2. Combined processes between microbial fuel cells (MFC) and EF, analyzing nanomaterial's importance in the manufacture of electrodes, separators, and iron catalysts. Cell design parameters, operational parameters, and biological parameters are also analyzed. The advantages and disadvantages of improving efficiency in water treatment and bioenergy generation are indicated.
3. Finally, a cost/benefit analysis is presented in terms of the maintenance of microorganisms, preparation of the electrodes and their lifetime, cell design,

and mineralization of pollutants from which one can have a view to implementation on a large scale.

28.2 Combined MFC-EF Processes for Power Generation and Pollutant Removal

28.2.1 Bioelectrochemical Systems for Power Generation and Water Treatment

Depending on the application, BES are classified:

MFC: microbial fuel cells, which generate electricity.
CDM: microbial desalination cells used to desalinate seawater.
CEM: microbial electrolysis cells, which produce inorganic chemical compounds such as hydrogen.
CESM: microbial electrosynthesis cells, of which organic chemicals are obtained.
CCS: sediment fuel cells, a new technology used to convert organic chemical energy stored in a sediment into electrical energy.

Of the cells mentioned above, MFCs are interesting because they convert the chemical energy of the contaminating organic matter into bioelectricity, using biocatalysts that allow the redox reactions to be carried out within the cell. However, the development of these systems is complicated because the water to be treated is generally problematic due to all the polluting organic compounds present. The operation times are considerably longer by a step of conditioning the cell before the operation. Subsequently, they must consider the cell's operating time for power generation and wastewater treatment, so it is essential to evaluate the operating conditions, with primary attention to the electrode material, which will optimize its operation and scaling to treat large volumes of water. The way to take advantage of the electric current generated or the storage of the hydrogen produced should be examined.

28.2.2 Electro-Fenton Process a Useful Alternative for Wastewater Treatment

In this way, while MFCs are an alternative for electricity generation, in terms of water treatment, electrochemical advanced oxidation processes (EAOP) are used for the degradation of organic pollutants, for example, electro-Fenton (EF), which is based on the Fenton reaction, that is, the mixture of hydrogen peroxide (H_2O_2) and an iron salt ($FeSO_4$) to generate hydroxyl radicals ($^{\bullet}OH$), according to Eq. (28.1):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH \quad (28.1)$$

$^{\bullet}$OH radical (E° $^{\bullet}$OH/H_2O = 2.8 V/SHE) is a strong oxidant that reacts nonselectively with organic pollutants until mineralization (Brillas 2014), i.e., CO_2, water, and inorganic ions. According to what is described, H_2O_2 must be supplied continuously, which eventually becoming impractical inconvenient. In EF, H_2O_2 is electrogenerated in situ at the cathode by reducing O_2 via two electrons (Eq. 28.2), while Fe^{2+} is added to the medium, which allows the formation of $^{\bullet}$OH and Fe^{3+} (Eq. 28.1). Fe^{2+} allows the formation of $^{\bullet}$OH and Fe^{3+}; in the same pH, the predominant species of iron (III) is Fe $(OH)^{2+}$ (Eq. 28.3), which can be reduced to Fe^{2+} according to Eq. (28.4). This cathodic regeneration is a relevant advantage of EF:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (28.2)$$

$$Fe^{3+} + H_2O \rightarrow Fe(OH)^{2+} + H^+ \quad (28.3)$$

$$Fe(OH)^{2+} + e^- \rightarrow Fe^{2+} + {}^{-}OH \quad (28.4)$$

In this sense, the material most used as an anode is a boron-doped diamond, while carbonaceous materials are used as a cathode to favor the production of H_2O_2. When Fe^{2+} is added in the form of salt, the EF process is known as homogeneous, but it can also be immobilized on the cathode as detailed in Sect. 28.7, known as heterogeneous EF. The group of Professor E. Brillas is one of the most active in this area since the 1990s (Brillas et al. 1998), testing the efficient degradation of pollutants such as colorants, pesticides, and drugs in different reactor arrangements. EF has also studied wastewater treatment, reaching high percentages of total organic carbon (TOC) removal (Huang et al. 2020). It is evident that EF is well proven; however, a significant drawback is the energy expenditure required for its operation.

28.2.3 MFC-EF Combination System Overview

The MFCs can supply the energy required for the EF process to be carried out; in other words, a synergistic connection can be established between MFC and EAOPs, such as EF. This type of combination has attracted the interest of research groups, which is reflected in the considerable increase in MFC-EF publications, especially in the last 5 years, according to Fig. 28.1.

From the work of Feng et al. (2010), it was determined that the combined MFC-EF systems are characterized by being energetically sustainable because the cathodic reaction of EF is carried out with the supply of electrons from the anode of the MFC.

In fact, in MFC-EF, the electrons released in the anode chamber are conducted by a resistance to the cathode and are consumed by reducing electron acceptors, such as oxygen, carrying out the cathodic reaction in the MFC. From the reduction of

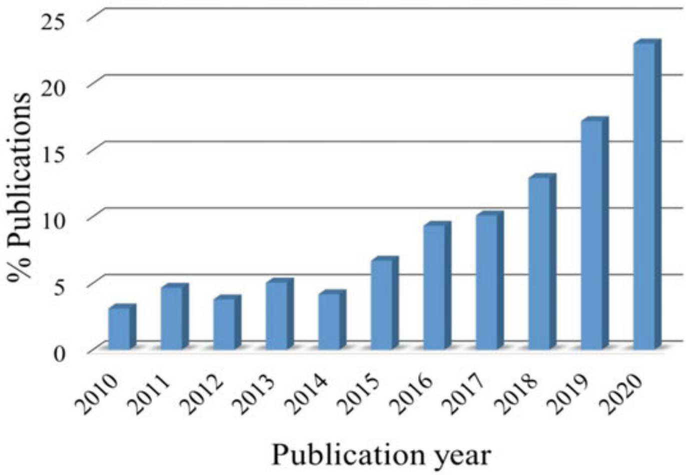

Fig. 28.1 The trend of published studies on MFC-EF combined systems in the last 10 years. (Search source https://www.sciencedirect.com/ (accessed July 2020))

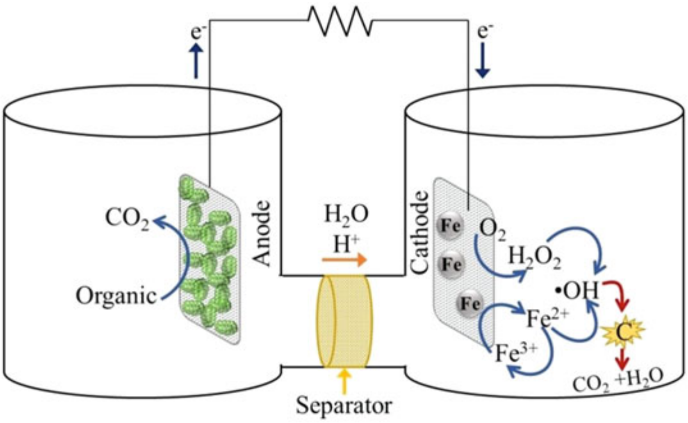

Fig. 28.2 Representation of the MFC-EF components

oxygen, H_2O_2 is produced in the cathode chamber, and it is possible to carry out the EF process by adding iron; see Fig. 28.2.

The MFC-EF process in the future will be one of the main processes used for the treatment of wastewater containing emerging organic compounds. The EF process has indeed been widely studied, and despite this, on some occasions, it does not mineralize the organic load of pollutants; it only removes the color of the wastewater, which makes it necessary to give a pre- or post-treatment to the contaminated water, making the treatment process more expensive (Li et al. 2018). Therefore, in recent years, the degradation by MFC-EF of different pollutants such as dyes, pharmaceuticals, and wastewater has been studied. Treatment times are characterized by being prolonged (72 h or more), which highlights the need to optimize the reactor's configuration to increase the electrical output, which can favor both removal efficiency and treatment times. Published studies reveal the feasibility of using combined systems for the degradation of organic pollutants with promising applications to wastewater treatment so that there is a significant decrease in energy consumption (as can be seen from Sects. 28.3 to 28.7), high efficiency, low toxicity, and clean treatment, as the mineralization of the pollutant is achieved without production of sludge.

In Fig. 28.2, the typical MFC-EF reactor is composed of two chambers; in one of them, the anode is placed, and in the other, the cathode, using a membrane or separator between both chambers. The anode chamber contains nutrients to keep the microorganisms that form the biofilm on the anode, while the cathode chamber contains the organic pollutant in the supporting electrolyte to promote ionic conductivity; likewise, in order to favor the production of H_2O_2 in the cathodic compartment, it is required feed air. Thus, continuous efforts are made to improve the operation of these systems in development, such as nanotechnology, which provides new possibilities for the manufacture of anodes, cathodes, membranes, and the supply of iron for the Fenton reaction (see Fig. 28.2). In this sense, each of these components is described below, emphasizing nanomaterial's use for their manufacture.

28.3 Anodic Nanomaterials Suitable for Use in Microbial Fuel Cells

The MFC-EF's good performance depends on the oxidation/reduction reactions in each of the chambers. The electrochemical materials are essential for the reactions to be carried out efficiently and lower resistivity to transfer the electrons. Precisely, in the anodic chamber, microorganisms form a biofilm that grows on the electrode's surface, transferring the electrons to it. This process links the relationship with electrochemistry, which is a crucial factor determining the conversion of organic matter present in the wastewater into electrical energy. Once the importance of the formation of the biofilm on the anodic electrode is known, it is necessary to choose an anodic material that presents high biocompatibility with the microorganisms, and electro-active surface area, which could be affected by all the parameters mentioned above. Three essential processes occur at the anode, as can be seen in Fig. 28.3:

(a) Formation of the biofilm.
(b) Oxidation of the matter.
(c) Extracellular transfer of the electrons from the microorganisms to the electrode.

Other important features for choosing an anodic electrode are:

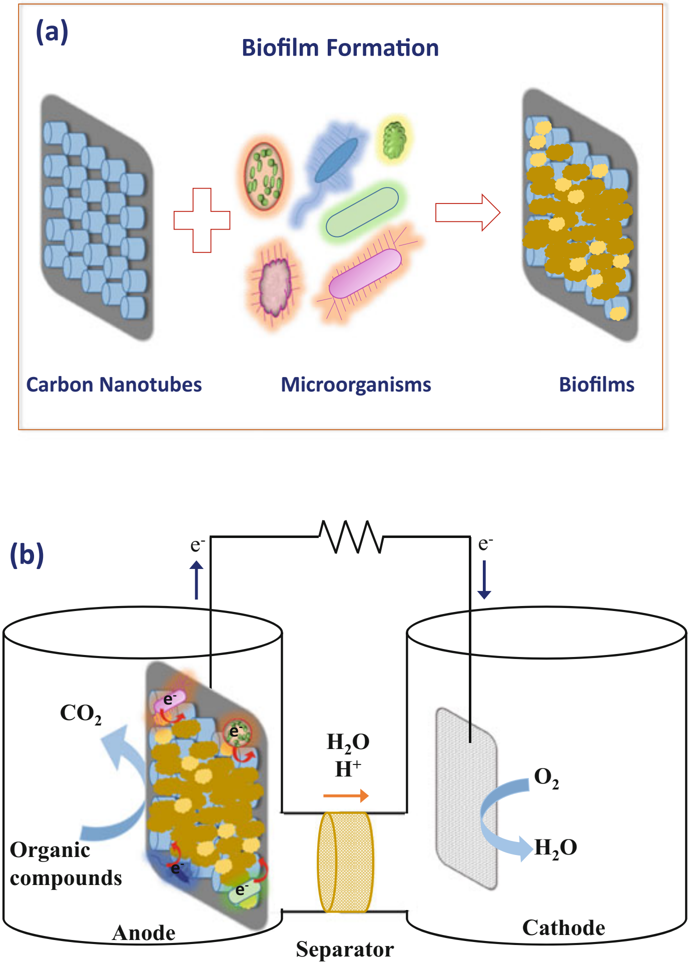

Fig. 28.3 Representation of (**a**) a biofilm construction from carbon nanotubes and microorganisms; (**b**) the oxidation of the matter with the extracellular transfer of the electrons from the microorganisms to the anode electrode surface

1. To present a sizeable electroactive surface area, where there are more active sites so that microorganisms can adhere or be close to them and carry out the catalytic activity by performing the electron transfer reaction.
2. Good electrical conductivity to accelerate the electron transfer process.
3. Excellent biocompatibility, i.e., the material has a structure that allows the biofilm to adhere uniformly to its surface.
4. The electrode should have low toxicity to microorganisms. For example, iron-based materials are not suitable for use in MFCs because they corrode quickly (because in some cases the wastewater is at a very acidic or basic pH), releasing

iron ions into the environment, which will damage the immune system of the microorganisms, causing them to be damaged and in some cases killed.
5. The material must be chemically stable during the long period of the cell's operation to avoid replacement.
6. Flexibility for easy handling.
7. Durability performance for long-term operations.
8. That the raw material of the electrode is abundant in nature and is economical.
9. Preferably, after the experiment is finished, the electrode's maintenance should be simple and low cost.
10. The thickness of the electrode should be moderate to prevent the biofilm that is formed from being too thick to block the internal structure of the electrode pores, which would diminish the effectiveness of the reaction.
11. The material is recommended to be economical and suitable for commercial application since removing pollutants and the systems's output power will depend on these characteristics.

All these characteristics depend on the material studied and the working conditions of each cell. Besides, in most articles, we speak in a qualitative and not quantitative way when referring to these data.

Various materials are developed for use in these systems; however, no material is 100% efficient for general application in the degradation of organic matter present in wastewater and power generation. Most of the electrodes used as anodes are carbon-based because they have a large surface area, chemical stability, high conductivity, and biocompatibility with microorganisms.

Among the commercial carbon electrodes that can be found are cloth, paper, felt, brush, plate, rod, activated carbon, granular carbon, and reticulated vitreous carbon, which have specific characteristics that favor the recognition of microorganisms to form biofilms, such as the following:

(a) Graphite electrodes have high mechanical resistance, but because they have a flat surface, the biofilm does not adhere well to its surface, affecting the water's mineralization and power output of the system.
(b) The carbon felt has the characteristic of being flexible and high electrical conductivity, so microorganisms adhere easily. However, due to its thickness, the formation of the biofilm makes it difficult to diffuse the substrates, which is unfavorable for the microbial colonization formed inside of the electrode.
(c) Carbon paper and cross-linked vitreous carbon are of a rough surface with poor mechanical stability.
(d) Carbon brushes have better performance but require a high-cost metal (usually titanium, which is non-toxic to microorganisms) to support the carbon fibers in brush form, which makes them more expensive to use, making them unsuitable for commercial scale.

As for materials that are different from carbon, stainless steel mesh, platinum alloys, platinum, titanium, and copper, among others, are used. Whether they are carbon anodes or not, the geometry of these electrodes can be flat, three-dimensional, packed, and in the shape of a brush, which gives them a high contact area, an increase in active sites, and therefore current density, as well as a decrease in power density (PD).

On the other hand, although stainless steel electrodes comply with most of the characteristics mentioned above of carbon-based anodic electrodes, the biofilm formed on their surface is easily removed by gravity and by the long periods of operation of the cell. Therefore, it is challenging to have favorable results from organic matter degradation and bioelectricity generated.

In this aspect, nanotechnology, specifically in the synthesis, design, and development of nanomaterials, has contributed to diverse materials with different applications in affordable technologies to wastewater treatment. Nanomaterials have unique characteristics such as high surface area, mechanical properties, higher chemical reactivity, lower cost (depending on the material), and their possible efficient regeneration for reuse. These characteristics together allow nanomaterials to be perfect for use in wastewater remediation. However, conventional routes of nanomaterial synthesis use hazardous and, in some cases, volatile chemical materials. Therefore, extreme care must be taken with the reagents used to avoid that the contamination generated by obtaining nanomaterials is less than the one that is desired to be removed from the water to be treated. Otherwise, the development of the material will not be profitable or environmentally friendly. Of course, different ways of synthesizing efficient and environment-friendly nanomaterials for water remediation have been sought.

Table 28.1 shows several studies on nanomaterial's performance for use as anodes in bioelectrochemical systems for wastsewater treatment and power generation. In some cases, these nanomaterials compared with non-nanostructured materials show a significant difference in performance; for example, the materials described by Liang et al. (2020) compared with a carbon-felt electrode without nanoparticle impregnation gives a chemical oxygen demand (COD) removal of 45.6% for phenanthrene and 42.3% for pyrene, showing an electrical power of 0.49 + 0.07 kJ, operating at the same conditions as the other electrodes. The increase in the degradation of organic matter and electric energy generation happens because nanomaterials have more available catalytic areas and more biomass is generated, which is better distributed over its surface. Thus, nanomaterials can be an excellent option to be used as anodes in a bioelectrochemical system for wastewater treatment and power generation, with their respective considerations.

28.4 Formation of the Biofilm on the Anode

A fundamental and essential part of the development and implementation of MFC-EF systems is forming the biofilm generated by the microorganisms used in the anodic zone, either from a pure microorganism or from a mixed culture.

Bacteria's transport to the anode begins with a micro-colonization fixed on the electrode; over time, micro-colonies are formed on the surface to form the biofilm. A

Table 28.1 Nanomaterials reported in microbial fuel cells for wastewater treatment and power generation with the corresponding chemical oxygen demand (COD), power, and operation time

Nanomaterial	COD (%)	Power (kJ)	Power density (W m^{-2})	Power per unit volume (W m^{-3})	Operation time (h)	Reference
Graphite felt impregnated with graphite oxide	71.20 Phenanthrene 69.60 Pyrene	0.87 ± 0.04	–	–	110	Liang et al. (2020)
Graphite felt impregnated with graphene	73.00 Phenanthrene 68.20 Pyrene	0.98 ± 0.14	–	–		
Graphite felt impregnated with carbon nanotubes	78.10 Phenanthrene 66.70 Pyrene	0.57 ± 0.06	–	–		
Granular activated carbon on titanium mesh	96.50	–	0.73	–	730	Liu et al. (2019)
Carbon cloth impregnated tungsten carbide	95.50	–	3.26	–	480	Liu et al. (2020)
SnSb@rGO	48.95	–	6.25	–	330	Divya Priya et al. (2020)
Sb-SnSb@rGO	49.23	–	7.6 0	–		
Sb-SnO_2@rGO	48.04	–	1.32	–		
PANI/MWNT	95.30	–	0.11	–	–	Zhang et al. (2020)

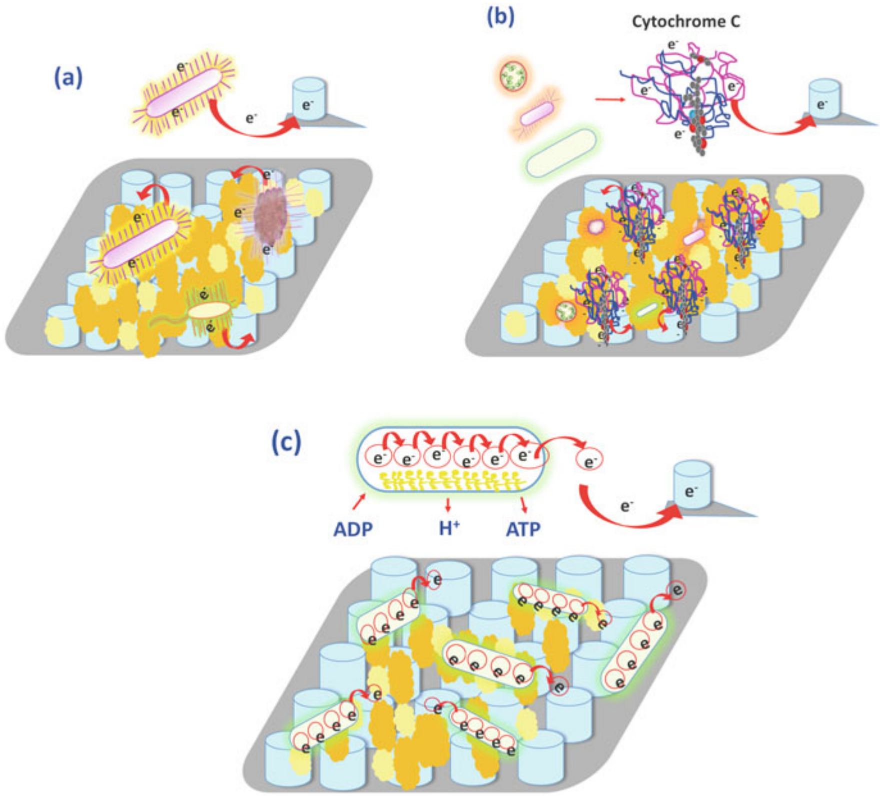

Fig. 28.4 Representation of direct electron transfer by pilis (**a**), cytochrome type C (**b**), and indirect transfer employing electron shuttles (**c**)

biofilm is then a concentration of microorganisms with cells mutually attached through a matrix or polymer network that they produce to embed themselves or adhere to a surface. The polymeric matrix comprises extracellular polysaccharides, proteins, and genes having a continuous and sequential formation process. The process of biofilm formation is complex and is directly affected by the operating conditions of the system. The biofilm acts as a biocatalyst to hydrolyze the substrate and produce both electrons and protons in the MFC. The transfer of electrons can occur by mechanisms associated with (Fig. 28.4):

1. Direct electron transfers by pilis growth, which are small, highly conductive villi, called "microbial nanowires."
2. Direct transfer by cytochrome type C from the proteins bound to the separator.
3. Indirect transfer employing electron shuttles, such as oxidized and reduced shuttle molecules.

On the other hand, bio-electroactive film's efficiency in microbial fuel cells depends mainly on three parameters: cell design, operational parameters, and biological parameters.

28.4.1 Cell Design Parameters

When designing the system, specific requirements must be considered, such as:

1. The material with which the cell will be built: usually, the most used at the laboratory level have been (a) plexiglass, (b) polyvinyl chloride (better known as PVC), and (c) glass. However, if the design of the cell is to be scaled for real applications, the materials to be used must be designed to withstand the pressure that the electrolyte will exert on its walls and to know the properties (pH, composition, temperature, etc.) of the electrolyte present to avoid problems of leakage or corrosion.
2. The electrodes (this subject is dealt with in-depth in Sects. 28.3 and 28.6 of this chapter).
3. The configuration of the cell and each of the chambers is important since the electrode's geometry, the internal resistance, and the electrical conductivity will depend on it.
4. The distance between the electrodes should be as close as possible to avoid potential drops within the system.
5. The separator's use will influence ion transport, forming the biofilm, and avoiding the contamination caused between each cell chamber.
6. The type of substrate, its concentration, temperature, and pH to avoid damaging the cell materials and ensure that the substrate is to the microorganism's liking.
7. Finally, yet notably, the microorganism's characteristics must be known because some of them require a specific temperature to guarantee their growth and multiplication.

28.4.2 Operating Parameters

Among the operating parameters are the variation in temperature and pH. The pH in the anodic chamber plays a vital role in the growth of microorganisms and the formation of biofilm and maintains the balance of redox reactions. Neutral pH is optimal for the survival of microorganisms; any increase or decrease in pH (extreme acids or bases) results in a substantial decrease in biofilm performance due to changes in cytosolic pH, ion concentrations, separator potential, and proton transport (Zakaria et al. 2019). The temperature influences the system's electrocatalytic performance because very high or shallow temperatures affect the survival of microorganisms; in this case, the control temperature will favor the growth, development, and survival of the system's microorganism.

28.4.3 Biological Parameters

The biological variables that influence the formation of biofilms are the type of microorganisms (gram-positive or gram-negative), their structure, the composition of the strain that can be affected by the inoculum preparation, pure or mixed microbial culture, the metabolic pathways, and the electron transfer route. The type of substrate influences the biofilm's properties and thickness because it is its source of carbon and energy (Saba et al. 2017). It is also essential to consider the microbial resource, its availability, diversity, abundance, microbial interactions, including competition, growth rate, specific electron transfer rate, ability to synthesize redox mediators, ability to produce nanowires and perform direct electron transfer, and bioremediation rate in terms of substrate utilization and substrate specificity. It has also been found that bacterial cells can communicate with cells of other species, causing them to organize themselves in a structured and functional way, allowing them to regulate their life cycle according to the environment surrounding them in the biofilm through the detection of quorum sensing. Quorum sensing can be considered a natural mechanism to improve the current generation, biofilm dynamics, and the electron transfer process. Immobilization of quorum sensing depends on cell's entrapment and connections in the electrode morphology (Christwardana et al. 2019).

On the other hand, when there are mixed microorganisms in the cell, it can affect the current production giving some points higher than others do, making the system unusable for real applications (Saba et al. 2017). At the same time, some microorganisms die, and others are born again, so the layers of dead cells delay the transfer of electrons and increase internal resistance; this makes the system reach the equilibrium conditions of operation in a long time and decreases the generation of electrical energy and the degradation of organic matter (Christwardana et al. 2019). Hence, if it is required that the microorganisms have significant efficiency, chemical or genetic modifications can be made (Palanisamy et al. 2019).

Once all the above is understood, an important aspect that must be considered in the formation of the biofilm is how the electrode will be prepared, which can be by anode inoculation or by inoculation of the microorganism(s) and then placed in the cell, depending on the choice, and the pros and cons of each methodology must be

Table 28.2 Microorganisms used in MFC for power generation

Microorganism	Power density (W m^{-2})	Reference
Shewanella oneidensis MR-1	0.23 ± 11.6	Bian et al. (2018)
Geobacter, *Geothrix*, and *Pseudomonas*	3.26	Liu et al. (2020)
Shewanella putrefaciens	1.46	Varanasi et al. (2016)
Bacillus subtilis with sub species *spizizenii*	0.01	Mathuriya and Pant (2019)
Consortium	0.49 ± 8	Cheng et al. (2017)

evaluated (Palanisamy et al. 2019). Table 28.2 shows the microorganisms most used in MFCs for the transfer of electrons to the EF system.

28.5 Nanomembrane Separators of the Chambers in the MFC-EF Systems

One of the components that play a significant role in developing and installing an MFC-EF system is the separator, which will isolate or separate the anodic zone (where the fuel is located) from the cathode reduction reaction (in this case, the EF process). The effluent used in each chamber has different pH, concentration, and atmospheric condition; it is also sought that there is no passage of oxygen from the cathode to the anode, that there is no diffusion of the substrate, that the microorganisms are only in the anodic zone, and that there is no crossing of CO_2 from one zone to another, to avoid a short circuit between the electrodes.

Nowadays, this type of system works at a laboratory level that does not use a separator; they are of a single camera. These systems have shown electrochemical activity to give treatment to the residual water. However, with very low coulombic efficiency, they could not be used in an MFC-EF system because considering only the MFC without membrane, they have disadvantages like the loss of substrate, the possible death of the anaerobic microorganisms by the crossing oxygen, a competition of reactions in the cathode by the presence of the substrate that feeds the microorganisms, and the growth of biofilm in the cathode electrode. Another problem that occurs in cells without a membrane is the loss of power when several cells are connected in series, i.e., the sum of the voltage when divided into the total of the cells is not the same as it is a single cell, which is less (Duteanu et al. 2010).

The separators can be classified into permeable and semipermeable. Permeable allows the flow of liquid through their structure; that characteristic makes them nonselective concerning ions or neutral molecule's transport. Semipermeable allow the selective passage of certain species under molecular size or charge. Depending on the use required, the choice of the separator will be made. On the other hand, since MFC-EF systems began to be investigated for their various applications, the most widely used separators have been ion exchange membranes (cations, anions, and bipolar) due to their high ion conductivity efficiency shown in the cell. Although they present high-cost problems, they do not tolerate high operating temperatures and, in most cases, are not compatible with the effluents to be degraded (Palanisamy et al. 2019).

The main disadvantage of the anion exchange membrane (AEM) is that it allows the substrate's passage from the anodic to the cathode zone due to the concentration gradient. Therefore, most of the MFC-EF systems use the cation exchange membrane (CEM), but they present difficulty maintaining a low pH in the cathode, causing inhibition in the EF process because at pH greater than three, the iron precipitates, altering the operation of the membrane and the cathode. Part of the current research is focused on developing separators or membranes that do not modify the reaction medium's pH. In this sense, a bipolar membrane could be used to maintain a stable pH in the supporting electrolyte without adding acid to decrease the pH (Li et al. 2017).

The separators are mostly made of polymers, but due to their characteristics, it is necessary to develop new materials to meet the requirements of a specific MFC. The cell separators can be made of polymers (Mathuriya and Pant 2019), ceramic (You et al. 2019), and zeolites embedded in polyvinyl chloride (Nagar et al. 2019). Even the exchange membranes are embedded with inorganic additives (SiO_2, TiO_2, ZrO_2, etc.) to modify their properties (Nagar et al. 2019; Palanisamy et al. 2019). The ceramics support high temperatures, which is an advantage, however, show problems when designing their porosity; the color (which depends on its composition and combination of clays) influences the size of its porosity and therefore the efficiency of the system. The advantage is that, if the manufacturing process of ceramic-based separators is optimized (thickness, density, porosity, and pore size), the cost of the MFCs will be significantly reduced, and their implementation to real systems would be more accessible to the population. In the case of polymers, the conditions of the wastewater to be treated are very aggressive and quickly degrade the polymer separator. In general, most of the separator materials need to be in the cells for several days to activate and do their work, which involves time and money.

In every material development, particular characteristics are sought to solve a problem. Important properties and characteristics that should be examined in the development and implementation of a separator in an FMC are as follows:

(a) The yield, which may be focused on ionic conductivity, pore size, uniform porosity, and overall electrochemical activity, is present.
(b) Pore size, depending on the diameter of the pore, it will or will not allow the passage of ions, substrate, microorganisms, and oxygen from one chamber to the other.
(c) All MFC separators should be of uniform porosity to avoid leakage and contamination from one chamber to another.
(d) That under the system's operating conditions, the separator presents high ion exchange capacity.
(e) The design of the chamber separator is flexible and fits the design of the reactor.
(f) The separator material is intended to be stable under different operating conditions, such as temperature, pH, and pressure.
(g) Investigate the effects of the pollutant to be treated on the surface of the separator.
(h) Cleaning and regeneration techniques of the separator should be easy to improve its lifetime due to the biomass's biofouling.
(i) The internal resistance present should be low to prevent the loss of potential in the cell from being less.
(j) The thickness of the separator is essential in the ion transport, if it is too thick, it will make the passage of the ions difficult, and if it is too thin, it will not withstand the temperature and pressure of the system.

(k) Carry out studies on the separator to verify that the absorption of water or electrolyte does not affect its mechanical resistance and ionic conductivity, preventing the crossing of ions (Shabani et al. 2020).

Another critical aspect to be considered in a separator is to avoid bacterial growth on its surface, which prevents adequate ion transfer. However, this problem is not so easy to eradicate due to the nature of the system itself and its time to find the right conditions for its operation. The separator's maintainance would make the process more expensive and would break the balance of adaptation of the experimental conditions reached up to that moment, considering that the good functioning of an MFC depends on the adaptation time of the microorganisms in the environment. Finally, despite the rapid development of separators in recent years, their practical use in MFCs and MFC-EF is affected by the aforementioned limitations. Although significant progress has been made in understanding these material's structure and properties, there are still challenges for their practical application, especially in achieving low manufacturing costs.

Nanotechnology has also been implemented to develop nanometric materials to be used in the membranes, as nanomembranes and electro-spinning fiber nano-membranes are based on polymers, biopolymers, carbon, and other materials (Al_2O_3, TiO_2, Ag, and carbon nanotubes with different compounds), which are embedded to improve their mechanical, thermal, and anti-pathogenic properties (Jie et al. 2015). Most of these nanomembranes are designed to be used when the wastewater has been previously treated or used in tap water to eliminate microorganisms pathogenic for human beings. They are not widely used in MFC-EF systems because the pore size can be affected by substrate particles or biofilm growth on the membrane surface, preventing the passage of protons from the anode to the cathode.

It is necessary to clean the membrane during its use, which generates high costs for the system:

1. The experiment has to be suspended to see the conditions in which the separator is located.
2. If the adsorbed species can be easily removed, it is only necessary to use ultrasound or the autoclave to re-sterilize the membrane (as long as the membrane material can withstand the sterilization temperature).
3. If it is not impossible to remove the pollutants or microorganisms, the nanomembrane will become unusable.

28.6 Carbonaceous Nanomaterials as Cathode for the Production of H_2O_2

For EF processes, the reduction of O_2 is required via two electrons (Eq. 28.2), a favored reaction in acidic media. Also, the cathode material has a fundamental role, and a cathode free of noble metals and low activity to the oxidation of H_2O_2 is necessary, as the carbon. Carbonaceous materials are generally used, characterized as being abundant, nontoxic, and inexpensive.

Oxygen reduction reaction (ORR) and subsequent production of H_2O_2 in systems MFC-EF are of great importance because the peroxide is essential to effect the Fenton reaction (Eq. 28.1), and thus degradation of organic pollutants is efficient. The generation of peroxide depends on the carbonaceous material properties such as structure, morphology, and composition. It is intended that the materials exhibit high surface area, high porosity, the presence of oxygenated functional groups, and contact angles less than 90 ° (Zhao et al. 2018).

There is growing interest in using carbon-based nanomaterials, such as nanotubes and graphene, due to its reactivity toward the ORR. Carbon nanotubes (CNTs) are composed of sp^2-hybridized carbon atoms with structures of cylindrical shape, hollow, which are usually closed at one end and with a diameter in the order of nanometers. They are classified as SWCNT (single-walled) and MWCNT (multi-walled). All these features confer excellent chemical, physical, mechanical (with too high elastic modulus), optical, and electronic properties (Yazdi et al. 2016).

It has been observed that CNTs used as electrodes have high electron transfer kinetics, which favors ORR and, therefore, hydrogen peroxide production. The electrocatalytic activity of ORR in CNT is considered to be due to oxygen-containing functional groups such as quinone groups (Zhang et al. 2014). Besides, the large surface area and pore size are related, allowing easy diffusion of oxygen to the cathode. According to various studies, the use of CNT-cathode increases H_2O_2 production, accelerating organic pollutant's degradation in EF process (Table 28.3).

Methodologies to prepare the CNT-based cathode are followed where the nanomaterial is dispersed in a mixture consisting of a solvent (ethanol) and nafion. The paste/suspension formed is pressed on a stainless steel mesh or also on nickel foam. H_2O_2 production using CNT varies in ranges from 20 mg L^{-1} h^{-1} to 1291.3 mg L^{-1} h^{-1} (Chen et al. 2016b). Some other proposals evaluated polypyrrole composites/MWCNT electrochemically synthesized (Babaei-Sati and Basiri Parsa 2017). With this cathode, 33.73 mg L^{-1} h^{-1} of H_2O_2 were produced, which may be associated with the following: (1) carbon atoms can be active sites both in the pyrrole ring and in MWCNT where oxygen is adsorbed to be subsequently reduced and (2) electrical conductivity and electroactivity of the polymer in combination with the electrical conductivity of MWCTN favor electron transfer toward ORR. The feasibility of using CNT is evidenced if the H_2O_2 production achieved with these nanomaterials is compared with activated carbon, with a generation of 22.62 mg L^{-1} h^{-1}, whereas with a graphite plate, only 8.6 mg L^{-1} of H_2O_2 is produced.

Some other composites are Fe-CNT, where the iron is integrated into the cathode (Sect. 28.7); in these cases, the cathode preparation is similar to that mentioned above with the difference that iron is added to the suspension (CNT, iron, polytetrafluoroethylene (PTFE), and ethanol). The paste is assembled, for example, in Ti meshes and stainless steel. With these electrodes, PD values of between 80 and 283.32 mW m^{-2} are achieved, which may be the energy required to supply the EF process (see Table 28.3). This type of nanomaterials can be used during different

Table 28.3 Degradation of pollutants by EF and MFC-EF with the corresponding anode and cathode, total organic carbon (TOC) removal, and energy consumption or power density

Pollutant	Anode/cathode	Degradation (%)	TOC removal (%)	Energy consumption/power density	Reference
EF					
Phenol	Pt/graphene	78 (after 3 h)	50 (after 8 h)	0.04 kWh g^{-1} TOC^{-1}	Mousset et al. (2016)
Amoxicillin	Pt sheet/modified graphite felt	60 (after 90 min)	–	1.14 kWh m^{-3}	Kalantary et al. (2018)
Tetracycline	CNT/FeOCl-CNT	99 (after 1.5 h)	59 (after 1.5 h)	0.34 kWh m^{-3}	Li et al. (2020b)
Pig farm wastewater	Graphite rod/CeO_2-doped CNT	–	72 COD (after 80 min)	–	Kuang et al. (2020)
MFC-EF					
Sulfamethoxazole	Carbon felt/γ-FeOOH graphene	97 (after 40 h)	–	472.21 mW m^{-2}	Y. Wang et al. (2019)
Methylene blue	Carbon fiber brush/graphite plate	99 (after 28 h)	74 (after 28 h)	7469.90 kWh (kg TOC)$^{-1}$	Zou et al. (2020)
Erythromycin	Carbon cloth/CNT-γ-FeOOH/stainless steel	89 (after 48 h)	87 (after 48 h)	193.00 W m^{-2}	Li et al. (2020a)

cycles of use without affecting H_2O_2 production efficiency since, according to some studies, the surface area decreases only 15% after five cycles.

For its part, graphene is a two-dimensional (2-D) material composed of hexagonal sp^2 hybrid carbons, with a layer of monatomic thickness. Its properties are large surface area (2675 $m^2\ g^{-1}$), high mechanical resistance (~1 TPa), high thermal and electrical conductivity, flexibility, and chemical stability. These properties allow them to have diverse applications such as photonics, electronics, and the generation and storage of energy (Ambrosi et al. 2014). Graphene has higher electrical conductivity compared with CNT because graphene has every atom available, which gives it high electrochemical activity, making it an excellent candidate for use as an electrode in MFC and EF. In this sense, as an anode in MFC, it achieves high PD compared with a carbon cloth electrode (Liu et al. 2012), while as a cathode in EF, it has shown high production of H_2O_2 and consequently improves the efficiency of removal of pollutants (Chen et al. 2016a).

The methodology for manufacturing cathode-based graphene is similar to CNT. To date, ORR performance has been compared with graphene, oxidized graphene, and reduced graphene and 2-D material stacking to result in 3-D materials with improved properties and characteristics. Pristine graphene considering graphene monolayer, graphene multilayer, and graphene foam as cathode has also been evaluated; two parameters that play an essential role in the activity of the ORR are the increase in the specific surface area that facilitates the diffusion of O_2 and the increase in the electrical conductivity of graphene materials, graphene foam being the material with the highest production of H_2O_2 (6.39 mg $L^{-1}\ h^{-1}$). With this type of material, it is noteworthy that the energy consumed in the degradation of pollutants is almost two times less than carbon felt (CF) and eight times less than with graphite (Mousset et al. 2016).

In general, the ORR mechanism in graphene can be related mainly to epoxy groups (C-O-C). However, the carbon atom next to the oxygen atom in the epoxy group has Lewis basicity, and Lewis base sites can adsorb oxygen molecules. These active sites together favor the catalytic performance of graphene toward ORR as schematizes in Fig. 28.5 where it is considered that, in graphene, adsorption is favored by one end of the O_2 molecule, while CNT adsorption is through two oxygen atoms simultaneously a carbon atom, that is, a triangular adsorption (Dong et al. 2018).

Quinone group is thought to facilitate the ORR; therefore, the interest arises to functionalize graphene with quinone giving satisfactory 75 mg $L^{-1}\ h^{-1}$ of H_2O_2. In this case, the proposed mechanism exemplified in Fig. 28.3 explains a first step with the generation of superoxide ($Q^{\bullet-}$) through the electron transfer reaction that subsequently forms the intermediate superoxide with the adsorbed molecular oxygen ($Q\text{-}O_2^{\bullet}$) to later lead to the ORR. The intermediate superoxide absorption is more accessible at the edge plane of the oxidized carbon surface where the electron transfer rate is higher than the basal plane (Divyapriya et al. 2017).

The combination of CNT-graphene has been evaluated, and on the one hand, graphene can improve the surface area, promoting adequate adsorption capacity. In contrast, the presence of CNT can improve the general resistance of the material

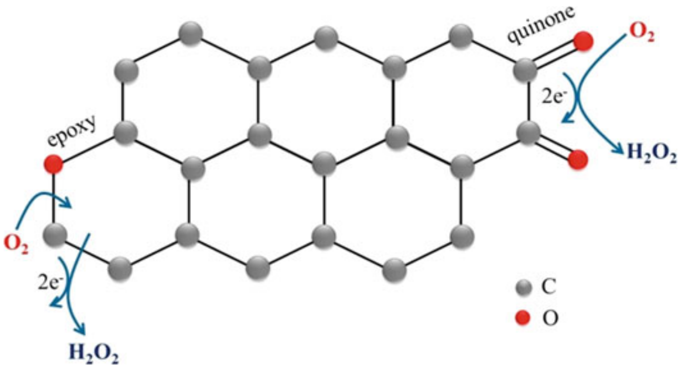

Fig. 28.5 Schematic representation of the ORR's possible mechanism in graphene for the electrochemical generation of H_2O_2

obtained, which favors the production of H_2O_2 and the degradation of pollutants in EF systems (Chen et al. 2016b).

It the combined MFC-EF systems, the peroxide production is not high. Despite it, the feasibility of wastewater treatment without adding additional energy is evidenced by the studies grouped in Table 28.3, although it must be considered that the treatment time is longer than EF.

Thermodynamically reducing O_2 to H_2O_2 is not entirely favorable, as is the disintegration of H_2O_2 to H_2O. For this reason, there are still challenges to be achieved from which high levels of H_2O_2 production are achieved. So far, efforts to increase H_2O_2 production are focused on modifying carbonaceous nanomaterials with N and P; for example, to electrocatalyst the ORR using substrates that do not involve the addition of other chemicals and to use green synthesis methodologies, which in that sense electrochemical methods can be an excellent option.

28.7 Iron-Based Nanomaterials as Catalysts

Fenton process carries out in the cathode chamber, in addition to the electrogenerated H_2O_2, a source of iron is required, which not only may be added either in salt form (Fe^{2+}) but also can be supplied in solid form such as pellets, nanoparticles or even as part of the cathode.

When an iron salt is used, Fe^{2+} is always regenerated at the cathode by reducing Fe^{3+}. The drawback is that, first, generated waste sludge to the end of the EF process must be removed and second, the homogeneous Fenton reaction is carried out at pH 3 to prevent the iron salt precipitates, leading to a step of acidifying the solution to be treated and a step of neutralization at the end of treatment. Therefore, today there is interest in EF processes where solid iron catalysts are used, and it is even proposed that iron forms part of the cathode. The use of iron in situ has the advantage of its easy recovery after treatment and the self-supply of a constant amount of iron

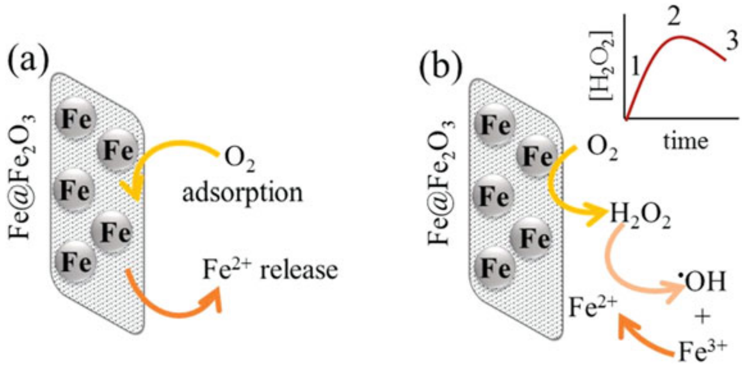

Fig. 28.6 Proposed mechanism for the Fenton reaction in situ using Fe@Fe_2O_3/CF adsorption O_2 stage (**a**) and H_2O_2 production (**b**)

ions during the EF process. The challenge now is to develop catalysts with minimal leaching, high catalytic performance, and high stability. In this sense, the use of nanomaterials offers, due to their nanometric size, a higher proportion of atoms and active size, so they could interact and react with other atoms and molecules.

Of the catalysts used, the literature reports the use of iron oxide–based nanowires, Fe@Fe_2O_3, synthesized under ambient conditions with sodium borohydride in an aqueous solution. This catalyst can be supported in CF, although materials at a nanoscale such as CNT are also used. Thus, using a cathode, Fe@Fe_2O_3/CNT is degraded, for example, 90% of rhodamine B dye in 120 min, while when using only CNT, there is no degradation (Ai et al. 2007). The concentration of Fe ions leached from the electrode to the medium has been evaluated, and it has been found that the concentration does not increase linearly and tends to remain constant after some time, indicating that the catalyst can be reused.

The Fenton reaction (Eq. 28.1) can occur on the same cathode surface between adhered Fe and the H_2O_2 generated in this same area, although leaching of iron ions can occur and contribute to the homogeneous Fenton reaction. The mechanism suggested that using this type of composites proposes a first step where the O_2 is adsorbed on the cathode and corrosion of the Fe@Fe_2O_3 resulting in the formation of Fe^{2+}, Fig. 28.6a. The ORR occurs via two electrons generating H_2O_2, Fig. 28.6b, a period that can be characterized by three stages (Fig. 28.6b): the first one increases in the production of H_2O_2 (1), then an equilibrium stage with the rate of generation and decomposition of H_2O_2 are equal (2), and finally the concentration of H_2O_2 decreases markedly due to its gradual consumption in the Fenton reaction (3); stages take a few minutes to even hours. The $^{\bullet}OH$ generated from Fenton reaction reacts with the pollutant to give H_2O and CO_2, and Fe^{3+} is reduced to Fe^{2+} on the cathode surface. There is evidence that the electrogenerated H_2O_2 reacts rapidly with iron and that even this reaction is faster than the production rate of peroxide (Zhuang et al. 2010); therefore, the rapid generation of H_2O_2 is essential to continue the Fenton reaction. The best performance, with materials of the type Fe@Fe_2O_3, can be

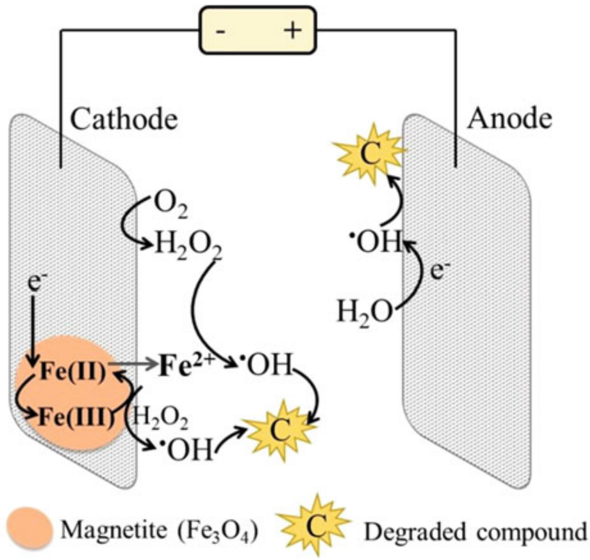

Fig. 28.7 Proposed mechanism for the degradation of pollutants by EF using Fe_3O_4/cathode

related to the reaction between iron leached, and H_2O_2 is kinetically more favorable than the conventional reaction between Fe^{2+} and H_2O_2; in addition to the zero-valent iron of Fe@Fe_2O_3/CF, it can react with dissolved O_2 to form reactive intermediates, which promotes the cathodic reaction.

On the other hand, synergies have currently been generated between catalysts that are incorporated into the cathode as graphene/Fe_3O_4 (Divyapriya et al. 2017) and iron oxychloride-CNT (Li et al. 2020b). In these cases, the interaction between nanocatalysts and carbonaceous nanomaterials such as graphene and CNT potentiates both properties, making them the combinations of interest to be explored in EF processes.

The oxidation mechanisms will depend on the physicochemical characteristics of iron minerals such as magnetite (Fe_3O_4), goethite (α-FeOOH), maghemite (γ-Fe_2O_3), and hematite (α-Fe_2O_3). For example, minerals with the presence of iron in trivalent and divalent forms such as magnetite are more active than those that contain only iron in its divalent or trivalent form, such as hematite or pyrite (Ganiyu et al. 2018). The mechanism proposed in materials such as magnetite embedded in the cathode shown in Fig. 28.7 where •OH generation may occur from the reaction of H_2O_2 with Fe in solid form or with dissolved Fe^{2+}. The generation of oxidizing species by these pathways allows significant degradation of pollutants such as phenol resulting in 90% COD removal after 240 min (Pujol et al. 2020).

On the other hand, magnetite's catalytic activity is increased by its nanosize and used without support, which comes into direct contact with the solution to be treated (heterogeneous EF). It has the advantage that it can be easily separated by applying an external magnetic field, making it an excellent candidate for large-scale wastewater treatment applications. Using F_3O_4, for example, 55% removal of amoxicillin at pH 7 is achieved after 90 min (Kalantary et al. 2018). The nanomaterial activity in the EF process may be due to the solubility and availability of Fe^{2+} in the octahedral structure of the nanoparticles that improve the EF process's efficiency.

The challenge is to obtain iron nanoparticles under synthetic conditions that control the chemical composition, dimension, and morphology, which has been achieved through electrochemical synthesis.

28.8 Cost-Benefit of MFC-EF Cells

A cost-benefit analysis is a tool that allows an evaluation of the relationship between investment costs (start-up, maintenance, and depreciation of materials due to wear or reuse of the MFC-EF) and the benefits associated with their performance. This study will indicate the profitability of using the MFC-EF for power generation, and wastewater treatment via EF providing information on their strengths and weaknesses, focused on savings. It should be noted that conventionally treating wastewater is very expensive, consuming about 3% of the electrical energy produced worldwide (Munoz-Cupa et al. 2020) addition to investment costs, maintenance, and analysis of the quality of treated water. For its part, Munoz-Cupa's research indicates that an MFC's investment cost is approximately 30 times higher than that of a conventional wastewater treatment plant (Munoz-Cupa et al. 2020).

Abourached's work indicates that a stock of 10 cells connected in series has a capital cost of 4.2 million dollars and its operational cost is 2.2 million dollars to maintain 113.14 m^3 of wastewater (Abourached et al. 2016). Therefore, the investment cost of designing an MFC-EF is grouped into the costs of the electrode materials, catalysts, and the separation membrane of the anode and cathode chambers. The electrodes and catalysts that have given better results according to their electrical conductivity contain precious metals, and the most used separators are those of polymers.

According to research by Sun et al. (2015), it considers that the lifetime of Cu, Pt, stainless steel, and Ti-based electrodes are 10 years and that their depreciation will be 90% of the original price. In the case of the proton exchange membrane, Nafion will have a lifetime of 2 years and depreciate to 50%, but this study was focused on the operation of the proton exchange membrane in fuel cells, where the fuel and oxidant are not dependent on a pH and are not corrosive compared with the electrolytes used in the MFC-EF. As a result, electrodes have to determine the performance and cost of an MFC, making their design and synthesis one of the biggest challenges to be solved. Carbon-based materials are an excellent alternative to use in the MFC-EF systems because they are low cost (carbon felt sheets, 20×20 cm, costs $181.00 in Fuel Cell Store).

Besides, the work published by Mustakeem in 2015 mentions that CNTs are an excellent alternative to be used as anodic electrodes, but they are costly compared with stainless steel electrodes and graphite rods (Mustakeem 2015). However, quantifying these material's lifetimes in FMCs is not as simple since it will depend on the cell's operating conditions (Sects. 28.4.2 and 28.4.3). Something important that can be mentioned is that the MFC without membrane decreases its cost and maintenance but presents the problems mentioned in Sect. 28.5 and is not suitable for use in MFC-EF systems.

Typically, MFC-EF systems use the proton exchange membrane as a separator, which provides a stable environment for the growth of microorganisms, preventing the transfer of the OH radical and pollutants from the cathode to the anode. Currently, the cost of a Nafion membrane sheet (0.41 $\times$ 1.23 m) is $870.00 at Ion Power Inc., limiting its use in commercial systems because of the size and cost. Studies by Zou et al. show that 38% of the total cost of an MFC is for the membranes and 99.92% of the energy consumption is due to aeration of the cathode by using the pump and extended treatment times (Zou et al. 2020).

Xu et al. (2018) conducted a comprehensive cost-benefit analysis of microbial fuel cell implementation that considers the following costs: $4.7 labor; $158 assembly and failure testing; $15.8 per operating cycle for routine maintenance, including labor and maintenance of devices and instruments (including installation and maintenance of pumps for continuous feed of wastewater at the anode and cathode); $24.5 for monitoring of water quality; $15.8 for repair or purchase of an accessory (once every 3 months system repair), and estimated $0.13/kWh of electricity used in the cell.

Also, a cost per unit of power and a profit model considering the income are described: (a) for electricity production, (b) for wastewater treatment, and (c) for the unit of power produced; the cost-benefit ratio and sensitivity analysis is characterized by assessing the uncertainty of an investment project (Xu et al. 2018).

However, most of the works published so far only report the power generated, the power density, and the percentage of chemical demand removed from water treatment in the MFC-EF (Table 28.3). Wang et al. (Wang et al. 2020) indicated that until today, there is no detailed study of coulombic efficiency analysis because it requires a lot of system operation time and the expense for excessive consumption to measure the samples and it is necessary to pay the labor to who will make such measurements.

Once the cost-benefit analysis is understood, it is essential to emphasize that in order to have a higher performance in the wastewater treatment in MFC-EF systems, it is necessary: to modify and implement anodic and cathode nanomaterials that improve current and power density, increase of H_2O_2 production rate, organic load mineralization efficiency, continuous operation, the microorganisms used to adapt to the experimental conditions and transfer the electrons efficiently, and the construction of the system design that is low cost.

28.9 Conclusions

The combined MFC-EF systems for the generation of electricity and the treatment of polluted water are a type of self-sustainable treatment in energy terms that have shown exemplary performance in the degradation and mineralization of organic pollutants why their study is increasing. Today, this technology still has some limitations and challenges to become a cost-effective option for treating wastewater.

Therefore, there is a strong tendency to explore mainly in nanomaterial's synthesis and properties for use as a membrane, anode, cathode (CNT and graphene), iron catalyst, and interaction between the microorganisms and the anode to form the biofilm finally. It still improves the synthesis conditions that allow controlling the chemical composition, size, and morphology of these nanomaterials.

The production of H_2O_2 in combined MFC-EF systems has allowed the Fenton reaction to take place, and consequently, organic pollutants are degraded. However, it is necessary to continue exploring this topic since the decomposition of H_2O_2 at the cathode is a problem.

The current knowledge on MFC-EF is based on cells or reactors at the laboratory level, so there is still much work to optimize the process to finally conclude the design of reactors that allow the treatment of large volumes of pollutants, as well as wastewater, which is still very limited. The generation of technical knowledge, coupled with a complete cost-benefit analysis, will visualize the application and real feasibility of this type of system that currently has promising results.

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Role of Bio-Selectors in Performing Simultaneous Nitrification and Denitrification in Sequencing Batch Reactor-Based STPs of India

29

Ghazal Srivastava, Ankur Rajpal, and Absar Ahmad Kazmi

Abstract

This study aims to enhance nutrient removal efficiency and functional microorganisms' role in governing simultaneous nitrification and denitrification (SND) using the well-designed compartmented selectors with the SBR aeration tanks. Simultaneous nitrification and denitrification were observed in ten wastewater treatment plants of India; they are based on C-Tech–based SBR technology associated with highly effective bioselectors favoring lower SVI and large floc formation. There are numerous factors responsible for the SND process: "carbon source" (soluble COD (sCOD), readily biodegradable COD (rbCOD), soluble BOD_5), "dissolved oxygen concentration (DO)," and "floc size." SND is an increasing function of rbCOD as high rbCOD values flourish high denitrification rates. For SBRs treating domestic wastewaters, rbCOD fractions observed are $13.12 \pm 3.36\%$. Increased DO in the reactor's bulk liquid negatively affects SND, but at lower DO (0–2.5 mg/L), anoxic mass fractions exist in the center of larger flocs that causes SND. The relationship between SND and rbCOD/sCOD (%) for all the SBR plants showed an increasing linear trend at $R^2 = 0.7$. Detailed analysis of 3 MLD SBR, Roorkee, showed excellent results at $R^2 = 0.81$ and achieved the optimum total nitrogen removal of 83% at COD/TN of 9. Relationships of rbCOD/TN and BOD_5/TKN with effluent NO_3-N revealed satisfactory denitrification rates in SBR plants. Storage products, acting as an active substrate for SND, were observed in the aeration tank's sludge as blue-black granules of polyhydroxybutyrates by optical microscopy. This study revives the process performance improvement strategies in selector-based SBRs.

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Keywords

Dissolved oxygen · Flocs · Polyhydroxybutyrates · Selectors · SND

29.1 Introduction

The study revolves around the two essential parts for the analysis of bio-selector-based SBRs: first, the role of compartmented anoxic bio-selectors in the sludge settling characteristics, nutrient removal, and performing simultaneous denitrification and denitrification in the cyclic advanced technology-based SBRs, and second, specific requirements of the occurrence of simultaneous nitrification and denitrification in these kinds of systems. It is demonstrated that conventional SBRs are devoid of this selector zone, which helps numerous areas in their efficient advancement (Metcalf and Eddy 2003). It was suggested that optimal selector designs would incorporate multiple basins to account for varying flow and loadings over a facility's life to provide the optimal conditions for kinetic selection of floc forming microorganisms. It has been observed that a sequencing batch reactor alternated under oxic and anoxic conditions, and the selector effect can be achieved due to the high initial substrate concentrations in the SBR under anoxic conditions. Anoxic selectors exhibit an excellent high uptake rate of readily biodegradable COD (selector effect) (Mangrum 1998).

The term selector was introduced by Chudoba et al. (1973). It consists of several mixing tanks, where the recirculation of activated sludge and influent mix before reaching the aeration basin of the SBRs (Chudoba et al. 1973). The recycling stream, rich in biomass, is mixed together with a soluble, biodegradable substrate in the selector, and the readily metabolizable organic substrates are removed (Metcalf and Eddy 2003). There are three selectors, depending on the electron acceptor in the biodegradation reaction: aerobic, anoxic, and anaerobic. Usually, in these advanced SBR treatment systems for nutrient removal, anoxic (/anaerobic) selectors are incorporated; nitrates are formed in the biological reactor and transferred through an internal recycle stream to the selector. In anoxic selectors, dissolved oxygen should be kept at less than 1 mg/L in the selector, and the nitrate supply should be within the range of 6–8 kg NO_3-N/kg COD. The HRT is recommended to be around 60–90 min (Jenkins et al. 2004; Metcalf and Eddy 2003). In anoxic/anaerobic selectors, polyhydroxyalkanoates (PHA) storage, hydrolysis of stored inorganic phosphates, and stored glycogen fermentation are the primary metabolic activities removing soluble substrate (Jenkins et al. 2004). As presented by Jenkins et al. (1993), small amounts of the substrate are oxidized in the selector during the formation of internal storage products. Typical storage products are polyhydroxyalkanoates (specifically PHBs) and carbohydrates (Gaudy Jr and Gaudy 1988). The potential for PHB (poly-β-hydroxybutyrate) to serve as the electron donor for effective simultaneous nitrification and denitrification (SND) is investigated in a sequencing batch reactor (SBR) using a mixed culture and acetate as the organic substrate (Third et al. 2003).

SBR system incorporated by alternating aerobic and anoxic/anaerobic stages in the reaction period allows for effective removal of suspended solids and organic matter removal. It achieves a satisfactory reduction of nitrogen by nitrification–denitrification processes and phosphorus by release and uptake (Lim et al. 2000). The selector and RAS pumps provide the following (Minnesota Pollution Control Agency 2006): firstly, it creates an anoxic/anaerobic atmosphere to affect denitrification of nitrates present in the RAS, and secondly, it suppresses filaments from proliferating by creating an anoxic fill condition by which natural selection of floc-forming bacteria takes place, resulting in excellent settling characteristics of the sludge (SVI $<$ 120 mL/g) (SFC Environmental Technologies Pvt Ltd 2020). Thirdly, it causes more extensive floc formation and simultaneous nitrification and denitrification. SND has been referred to as a biological process for nitrogen removal, where nitrification and denitrification occur concurrently in the same aerobic reactor (Jimenez et al. 2010). There are various factors responsible for this process to happen (Pochana et al. 1999, Dutta and Sarkar 2015), where the primarily important factor is carbon supply or source (sCOD, rbCOD, and soluble BOD) and specifically C/N ratio, where SND is an increasing function of rbCOD (Khursheed and Kazmi 2011). The second factor is “dissolved oxygen concentration” control. Increased DO in the reactor’s bulk liquid negatively affects SND; however, the relationship is not directly obvious with denitrification and nitrification rates increasing at DO levels up to 0.8 mg/L (Pochana et al. 1999; Yan et al. 2019). SND is a decreasing function of DO concentration at DO$>$0.8 mg/L (Pochana et al. 1999). Furthermore, the third important factor influencing SND is “floc size.” Larger sludge flocs reflect an increase in SND activities (Pochana et al. 1999). Implications of simultaneous nitrification and denitrification include consideration of the factors that affect floc size and distribution. Larger-sized floc formation occurs where the floc reaction profile allows for the nitrification in the peripheral sections, and denitrification prevails in the floc‘s inner parts (Mustafa et al. 2009). The direct role of nitrification in the competition between floc, formers and filaments, can be neglected because of low-energy yield compared with carbonaceous compounds’ metabolism. Besides, nitrifying bacteria are not involved in floc formation. On the contrary, they need well-established flocs to which they can attach. The soluble organics in the raw sewage are sequestered as intracellular compounds in the biomass. This stored substrate is then used for simultaneous nitrification and denitrification (SND) and enhanced biological phosphorous removal (EBPR) under managed cyclic aeration sequences in a sequencing batch reactor in the aeration zone. Still, the concentration of volatile fatty acids in the influent to the secondary process must be sufficient to support the nitrate’s denitrification in the return sludge and support the energy cycling of the BioP organisms responsible for selecting against filamentous organisms (Parker et al. 2003).

29.2 Materials and Methods

29.2.1 Study Area and Sample Preparation

The SBR full-scale plants are from the Indian states like Uttarakhand (Roorkee and Haridwar), Uttar Pradesh (Varanasi), Punjab (Abohar), Haryana (Karnal), and Gujarat (Rajkot, Ahmedabad, and Dwarka). The SBR monitored under this study are summarized in Table 29.1. A thorough analysis of anoxic selectors is carried out in 3 MLD SBR plant.

Some of the SBR plants have pure domestic sewage as their influent wastewater, and some are dealing with the inclusion of industrial sewage or drain water with municipal wastewater. A detailed analysis of 3 MLD SBR, Roorkee was performed in the study. An essential feature of this institutional STP is the deodorization system's additional odor control for sump well and pretreatment units and advanced tertiary treatment facility (fiber disc filtration and UV disinfection) due to its location closed to the residential area. The design quality of raw sewage for the 3 MLD SBR, Roorkee is BOD_5 ~ 200 mg/L, COD ~450 mg/L, TSS ~ 407 mg/L, TKN (as N) ~ 34 mg/L, and TP (as P) ~7 mg/L. After the experimental analysis, it differed with time; BOD_5 is observed as 163.1 ± 57 mg/L, COD as 400.9 ± 129 mg/L, TSS as 236.8 ± 79 mg/L, TN as 33.6 ± 9 mg/L, TKN as ~32.8 mg/L, and TP as

Table 29.1 SBR-based sewage treatment plants used in the study

S. no.	Location of sewage treatment plant	Capacity (MLD)	Technology	Raw/industrial sewage
1	IIT Roorkee	3	Selector-based SBR	Municipal wastewater
2	Jagjeetpur, Haridwar	27	Primary clarifier + selector-based SBR	Municipal wastewater
3	Abohar, Punjab	25	Selector-based SBR	Dairy wastewater + Domestic sewage
4	Rajkot, Gujarat	70	Selector-based SBR	Municipal wastewater
5	Rajkot, Gujarat	56	Selector-based SBR	Municipal wastewater
6	Ahmedabad, Gujarat	60	Selector-based SBR	Municipal wastewater
7	Gharaunda, Karnal, Haryana	7	Selector-based SBR	Municipal wastewater
8	Karnal, Haryana	10	Selector-based SBR	Municipal wastewater
9	Dwarka, Gujarat	7.4	Selector-based SBR	Municipal wastewater but with high TDS (>1800 mg/L)
10	Goithaha, Varanasi	120	Selector-based SBR	Municipal wastewater

6.1 ± 2.4 mg/L. The ratio between VSS: TSS is found as 0.54. The wastewater pH was 7.18 ± 0.35, and the final treated effluent after disinfection was 7.37 ± 0.23. The designed and actual wastewater characteristics of the SBR plants are shown in Tables 29.2 and 29.3.

29.2.2 Physicochemical Parameters' Analysis

Onsite monitoring of DO, ORP, and SV30 in the bio-selectors and aeration tanks of 3 MLD SBR plants is appropriately executed. A portable DO meter (Hach 110Q multimeter, Hach, USA) was used to measure DO and temperature in the aeration tanks and selectors. The pH was measured by a portable pH meter (HQ11d pH Meter, Hach) and ORP by the portable ORP meter (Srivastava and Kazmi 2020). Complete performance evaluation of the plants in terms of COD (all possible fractions), BOD (soluble and suspended), TSS, VSS, NH_4-N, NO_3-N, TN, PO_4-P, TP, SND%, and sludge operational parameters have been performed according to *Standard Methods* (APHA, AWWA, WPCF 2005). SV30 is measured by optical measurement with a measuring cylinder and timer. Grab samples of 0.5 L will be used for analyzing the parameters according to *Standard Methods* (APHA, AWWA, WPCF 2005). The fraction of rbCOD is calculated using the modified floc filtration method (Wentzel et al. 2000).

29.2.3 Microscopic Analysis for Protozoa and PHB Identification

Microscopic examination for protozoa, metazoan, filamentous, and foaming organisms, bacteria, sludge flocs have been observed at 10X, 20X, 40X, and 100X magnification. The qualitative microscopic studies were performed in mixed liquor sludge samples of the SBR aeration tank. Nearly, twenty-five microliter subsamples of sludge were studied under phase contrast, i.e., Radison RXLr5 illumination at 40× and 100× magnifications. PHB identification is being carried out using Sudan Black B dye staining at 100× magnification (with immersion oil) by using an optical microscope (Optika microscope) (USEPA 1987; Sharma and Dhingra 2015). PHB visualization was carried out in the SBR aeration tank's sludge samples of Roorkee and Varanasi plants. Qualitative microscopic observations were carried out in a mixed liquor sample of all the SBR plants' aeration tanks. Twenty-five microliter subsamples of sludge were examined under phase contrast (Optica, Italy) illumination at 20× and 40×. Four replicates of the final volume 1 mL were tested. Enumeration of microbiota was done according to scale where (+) = 5–10 protozoans/mL, (++) = 10–100 protozoans/mL, (+++) = 100–1000 protozoans/mL, and (++++) = >1000 protozoans/mL.

Table 29.2 Designed and actual wastewater characteristics of the SBR plants

Plants	25 MLD		70 MLD		56 MLD		60 MLD		7 MLD		10 MLD		7.4 MLD		120 MLD		27 MLD	
Parameters	DES[a]	ACT[a]	DES	ACT	DES	ACT	DES	ACT	DES	ACT	DES	ACT	DES	ACT	DES	ACT	DES	ACT
Flow (MLD)	25.0	20.0	70.0	51.3	56.0	19.7	60.0	43.3	7.0	2.94	10.0	2.52	7.4	0.83	120.0	22.3	27.0	27.7
pH	6.0–8.5	7.15	5.5–9.0	7.11	5.5–9.0	7.09	6.5–8.5	7.04	5.5–9.0	7.3	5.5–9.0	7.09	5.5–9.0	7.87	5.5–9.0	7.01	5.5–9.0	7.2
COD (mg/L)	475	2286	650	98	500	265	450	301	500	398	400	401	425	216	500	326	450	324
BOD_5 (mg/L)	203	856	325	61	250	88	251	86.4	250	161	250	169	254.5	112	200	230	250	161
TSS (mg/L)	450	1029	550	42	400	97	304	87	450	254	350	246	387.5	132	400	312	400	246
TKN (as N) (mg/L)	45	57	45	23	40	34.2	40	46.6	50	56.3	35	69.4	50	41.2	40	57.2	60	27
TP (Total as P) (mg/L)	5	6.4	6	4.2	6	3.7	8	6.1	7.1	5.8	5	8.2	7.1	2.6	8	4.46	15	3.9

[a]DES corresponds to Inlet parameters as per design basis (average), and ACT corresponds to actual values observed in the inlet (average/after sampling and analysis)

Table 29.3 General designed and actual wastewater characteristics in three MLD full-scale SBR

Parameters	Designed wastewater quality	Actual wastewater quality
pH	5.5–9.0	7.2 ± 0.35
COD	450	401 ± 129
BOD_5	200	163 ± 57
TSS	407	237 ± 79
TKN (as N)	34	33 ± 5
TP (as P)	7	6.1 ± 2.4

29.3 Results

29.3.1 Performance Evaluation

Wastewater treatment plants located in different parts of India have pH in their acceptable limits, and COD, BOD, TSS, TN, and TP removals are shown in Fig. 29.2. Some regular and thorough study was undertaken for 3 MLD SBR, as it is constructed near the campus of IITR. Composite sampling was also conducted for two of these plants: 7 MLD STP at Gharaunda in Karnal district of Haryana state and 25 MLD STP at Abohar in Fazilka district Punjab.

In Fig. 29.1, it can be observed that the plants 25 MLD Abohar and 7.4 MLD Dwarka have exceeded the current effluent standards in terms of organic matter, nitrogen, and suspended solids and resulted in turbid effluent. In the Abohar plant, the sewage effluent quality was influenced by the mixing of dairy wastewater, which exceeded the plant's design's inlet capacity. Hence, the plant became unable to treat immensely contaminated wastewater. At Dwarka, 7.4 MLD SBR plant was being observed containing higher total dissolved solids (>1800 mg/L) of wastewater and could not satisfy the effluent standards except phosphorus. Plants like 70 MLD SBR at Rajkot, 60 MLD SBR at Ahmedabad, and 10 MLD SBR at Karnal, Haryana, successfully removed all contaminants except total nitrogen and total phosphorus the current NGT standards. The rest of the plants, including 3 MLD SBR at Roorkee, 27 MLD SBR, Haridwar, Uttarakhand, 7 MLD Karnal, Haryana, 56 MLD SBR, Rajkot, Gujarat, and 120 MLD SBR, Varanasi, Uttar Pradesh, is excellently fulfilling the criteria as per the latest NGT norms (NGT 2019) for organic matter, suspended solids, and total nitrogen due to having favorable conditions prevailing in anoxic bio-selectors and aeration tanks. Complete removal of all parameters (along with TP $\leq$ 1 mg/L) was only fulfilled by 120 MLD SBR, sewage treatment plant at Varanasi, Uttar Pradesh. It operates at a minimum recirculation ratio (18%), and a sufficient anaerobic mixing time of 45 min is being provided for 3 h cycle (Table 29.4). Nitrogen removal and SND in all the SBR plants are represented in Fig. 29.2.

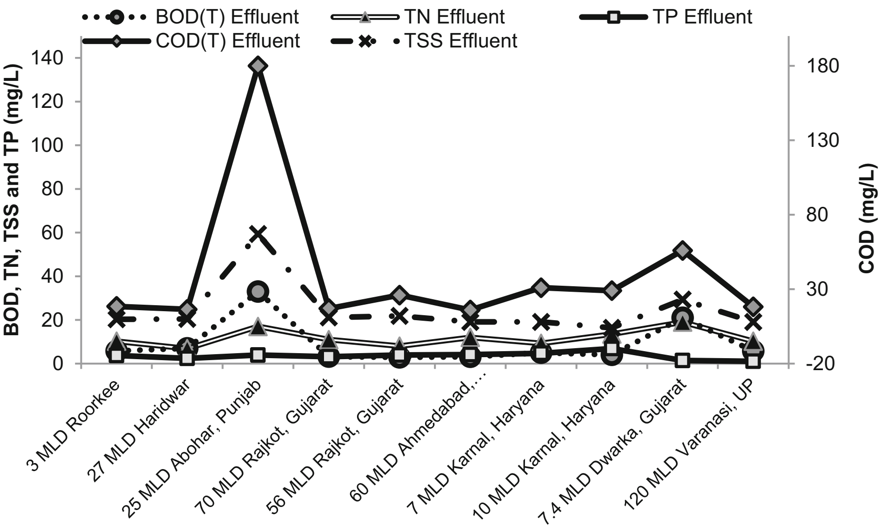

Fig. 29.1 BOD_5, COD (T), TN, TP, and TSS Effluents in the SBR plants

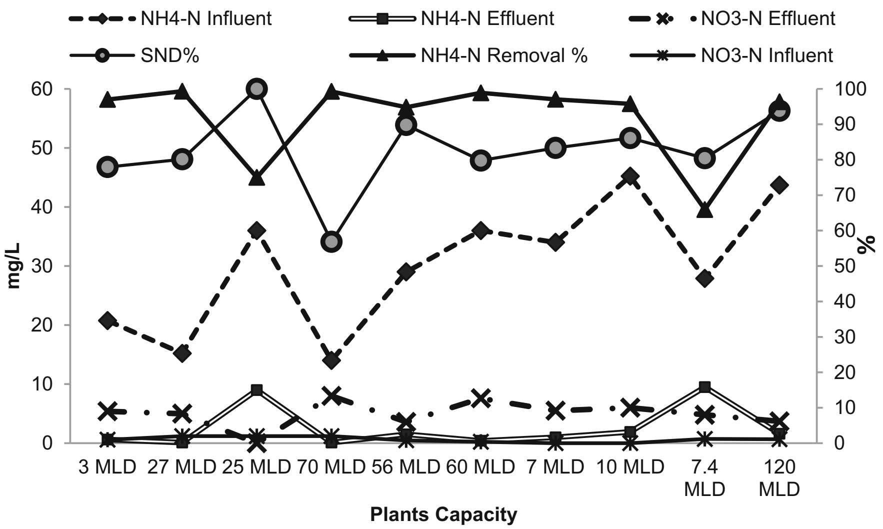

Fig. 29.2 Nitrogen removal and simultaneous nitrification and denitrification in the SBR plants

29.3.2 Role of Anoxic Selectors in Excellent Sludge Settling Characteristics

Obtaining reliably high compaction characteristics and low SVI values have enormous economic consequences on selector-based systems' design and operation.

Table 29.4 Process design parameters in SBR plants with bio-selectors

Location	Capacity (MLD)	No of basins	Range of ORP (mV) (extreme compartment of multicell selector from RAS and in aeration tank)	DO Profile (mg/L) (aeration, settling/ decanting (S/D))	Total HRT (hours)	Retention time in selector (minutes)	Selector compartments/ basin	SRT (days)	Cycle time (minutes)	Recirculation ratio (%)
Roorkee	3	2	−80 to −110, +40 to +150	0–2.48, 0.02–0.22 (S/D)	18.11	60	3	15	244	25%
Haridwar	27	4	−85 to −120, +50 to 160	0–2.55, 0.02–0.25 (S/D)	11.1	35	1 selector / basin	–	180	22%
Goithaha, Varanasi	120	6	−183.6 to −207.6, +80 to +160	0–2.4 (max 2.8) 0.02–0.22 (S/D)	14	45	9	10.4	180	18%
Abohar, Punjab	25	4	−60 to −100, 70 to160	0.19–2.3, 0.34–0.12 (S/D)	14.62	60	6	10.3	162	25%
Gharaunda, Karnal Haryana	7	2	−50 to −120, 40 to 150	0.06–4.58. (max 6.08), 0.0–0.3 (S/D)	17.88	65	6	13.54	180	25%
Karnal, Haryana	10	2	−50 to −140, 70 to 150	0.19–2.1, 0.19–0.34 (S/D)	14.37	55	6	13.22	180	21%
Ahmedabad, Gujarat	60	4	−50 to −140, 70 to 150		15.07	50	6	15.29	180	25%

(continued)

Table 29.4 (continued)

Location	Capacity (MLD)	No of basins	Range of ORP (mV) (extreme compartment of multicell selector from RAS and in aeration tank)	DO Profile (mg/L) (aeration, settling/ decanting (S/D))	Total HRT (hours)	Retention time in selector (minutes)	Selector compartments/ basin	SRT (days)	Cycle time (minutes)	Recirculation ratio (%)
				0.00–2.4, 0.19–0.22 (S/D)						
Rajkot, Gujarat	70	6	−30 to −110, 70 to 150	0.00–2.00, 0.19–0.22 (S/D)	18.07	65	6	11.81	180	20%
Rajkot, Gujarat	56	4	−40 to −100, 70 to 150	0.00–2.02 0.00–0.42 (S/D)	15.05	60	6	12.4	180	18%
Dwarka, Gujarat	7.4	2	−50 to −100, 60 to 140	0.00–2.00, 0.87–0.76 (S/D)	14.26	50	6	12.21	180	25%

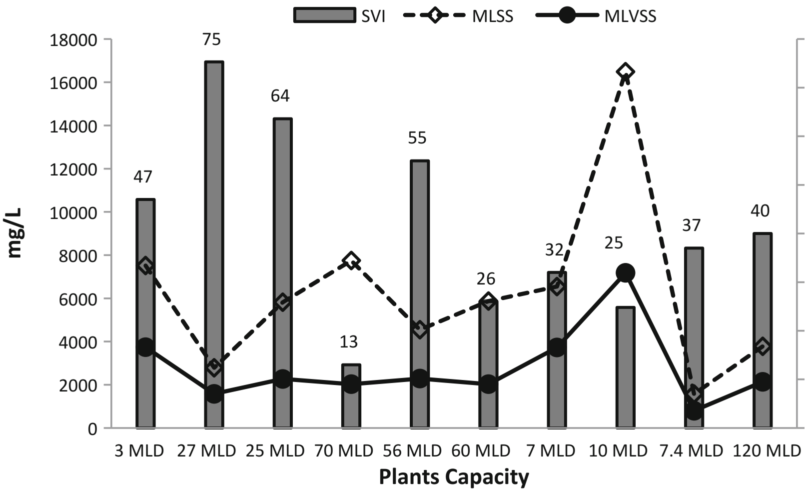

Fig. 29.3 MLSS, MLVSS, and SVI in the SBR plants

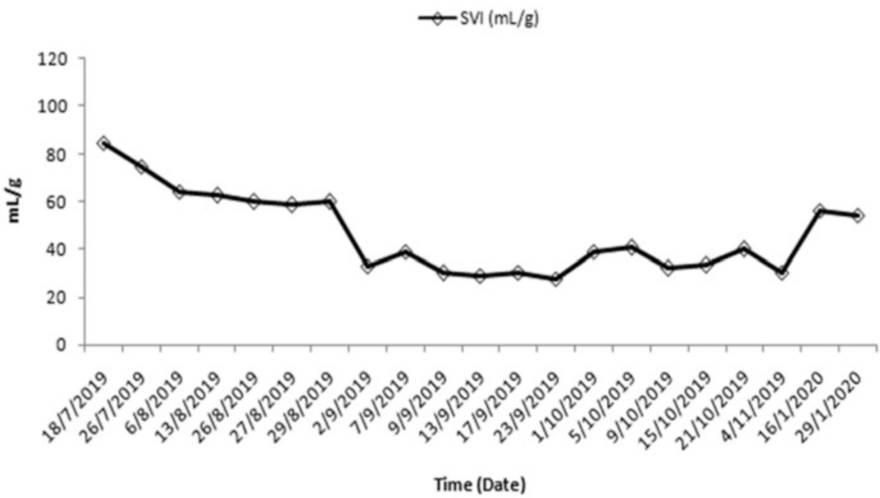

Fig. 29.4 SVI in the 3 MLD SBR plant

Now, the use of selectors has become more common for achieving low SVI values (Albertson 2002). The assessment of the full-scale performance characteristics of activated sludge plants incorporating selectors has high value for future plants' design and operation (Parker et al. 2003). Figure 29.3 illustrates the SVI values with MLSS and MLVSS in the aeration tanks of C-Tech-based SBRs. Figure 29.4 illustrates the SVI values in the aeration tanks of 3 MLD SBR.

29.3.3 Role of Anoxic Selectors in Denitrification and Substrate Removal

Anoxic selectors perform two important roles in nitrogen removal:

29.3.3.1 Denitrification of Nitrates in the Anoxic/Anaerobic Selector

In mixed fill, bacteria biologically degrade the organics and use residual oxygen or nitrates as the electron acceptor. For the SBRs with “anoxic, aerated, and low DO” selectors where DO <0.0–0.3 mg/L, NO3-N ≥ 0.5 mg/L in mixed liquor formed, denitrification may occur under these anoxic conditions (Albertson 2002; USEPA 1999). It has been observed in detail in the plant’s selector performance of 3 MLD SBR, Roorkee in Fig. 29.5, and the physicochemical parameters, as displayed in Table 29.5. The denitrification tendency in the plant is quite variable. It fluctuated as $39.74 \pm 32.15\%$. The inlet nitrate in the selector includes nitrates in the influent (2.65 ± 1.9 kg/day) and nitrates’ rate from the RAS (3.71 ± 1.2 kg/day). The denitrified portion is that portion of nitrates converted into nitrogen gas from the anoxic selector (2.26 ± 1.52 kg/day). The ramping of nitrates and DO due to RAS affects the denitrification in the selectors and causes instability. However, it benefits the performance of SND in the aeration zones by prevailing floc formers.

The retention time in anoxic selectors and RAS % also affect nitrification and PO4-P release. If biological phosphorus removal and nitrogen removal are required, the longer cycle time should be selected to operate with a minimum (sludge) recycle ratio (Artan et al. 2002).

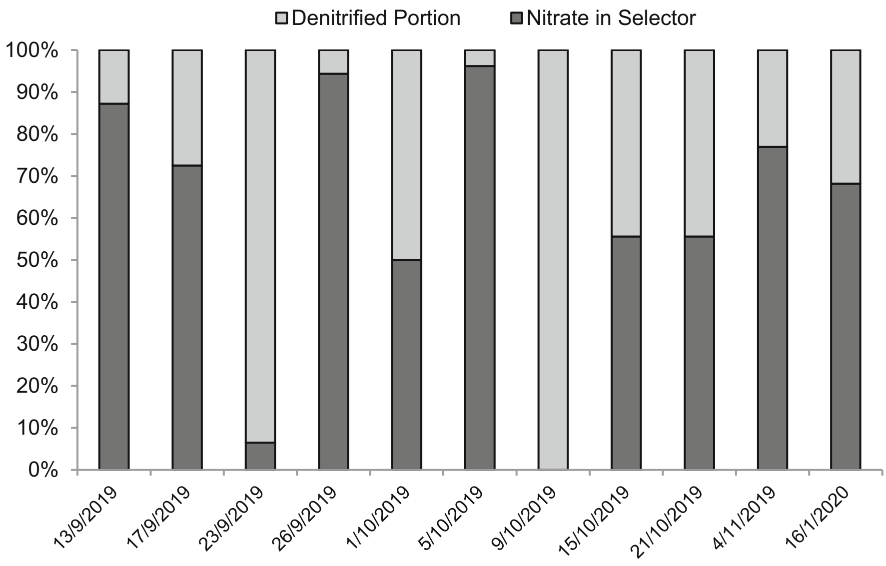

Fig. 29.5 Denitrification of nitrates in the anoxic selector of 3 MLD SBR

Table 29.5 Detailed physicochemical parameter analysis of 3 MLD SBR

Parameter	Influent	Anoxic selector	Aeration tank 1 (during aeration)	Aeration tank 2 (during aeration)	Effluent (after disinfection)
Temperature (°C)	25.41 ± 6.5	25.1 ± 6.3	24.9 ± 5.7	24.78 ± 6.1	24.9 ± 7.02
DO (mg/L)	0.01 ± 0.008	0.14 ± 0.06	1.5 ± 0.98	1.4 ± 0.95	0.08 ± 0.02
pH	7.18 ± 0.35	7.79 ± 0.2	7.53 ± 0.11	7.49 ± 0.14	7.37 ± 0.23
ORP (mV)	10 ± 4.1	−90 ± −24	98 ± 40	105 ± 45	45 ± 22
MLSS (mg/L)	–	–	7189 ± 2903	7518 ± 2852	–
MLVSS (mg/L)	–	–	3087 ± 550	3740 ± 934	–
SVI (mL/g)	–	–	48 ± 17	47 ± 13	–

29.3.3.2 Anoxic Selectors and Substrate Removal (Profiles)

It is reported that the selector provides enough time to remove all soluble readily metabolizable organic matter to selector sizing. Selector's design practice in Czechoslovakia and Austria suggest criteria for COD removal of 80% of the removable organic matter (Parker et al. 2003). It has also been observed that for a fixed period, the anoxic/ anaerobic selectors contribute to the biology to achieve efficient nutrient removal in the actual wastewater of Pune, India (Magdum et al. 2015). Selector phase biology concepts and mass profiles of the COD, nitrogen (ammonical nitrogen and nitrate), and orthophosphates have been represented in Magdum et al. 2015. Similarly, ammonia and nitrate profile in 4 h cycle of 3 MLD SBR, Roorkee is shown in Fig. 29.9. In a detailed study of the Roorkee plant, three compartmented selectors removed the average Total COD of 24.3%. The denitrification of nitrates was on an average of 40% in the selectors, and orthophosphates release was 37%. Characteristic profiles of the nutrient with DO and ORP in the anoxic selector compartments of 3 MLD SBR, Roorkee, Uttarakhand are represented in Fig. 29.6. No constraint in pH was observed in the plant for nutrient removal. The pH range in the 3 MLD SBR selectors is represented in Table 29.5. Table 29.6 shows the removal of contaminants in the plant due to the selectors and effects of wastewater parameters.

29.3.3.3 Proliferation of Floc Formers and Development of Large-Sized Floc Which Causes SND

The bio-selective mechanism is to contact the return activated sludge (RAS) and an internal recycle when employed with the influent wastewater in the biological reactor's initial contact zone (Albertson 2002). The initial contact zone typically consists of three or four zones with a high to lower F/M (food to microorganisms ratio) and having limited or no molecular oxygen present. In these zones, heterotrophs remove the low molecular weight, soluble substrates from the solution. Since the favored substrate (small, soluble organics/molecules) of the filamentous bacteria is limited in the heavily aerated oxic zones following the selectors and

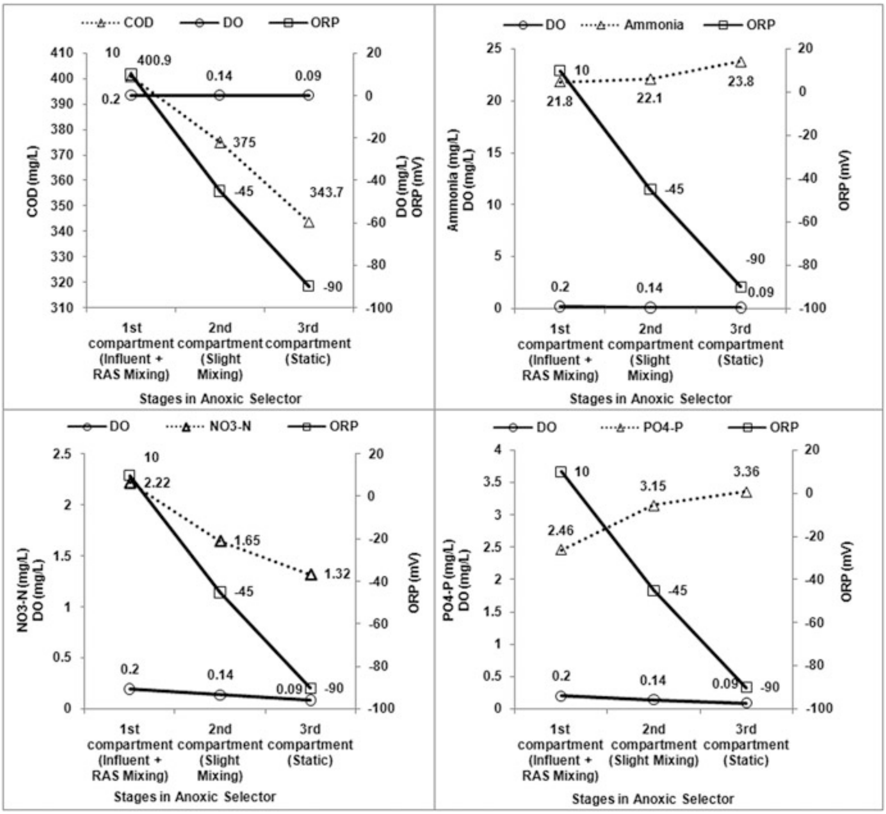

Fig. 29.6 Ammonia, nitrate, orthophosphate, and COD profiles against DO and ORP in anoxic selector zones of 3MLD SBR, Roorkee

anoxic (denitrification) zones (if employed), their growth is inhibited. The presence of a limited quantity of filamentous bacteria is generally desirable (Albertson 2002). They can help produce a more substantial and larger floc structure, which will readily settle and compact well in the secondary clarifier (Albertson 2002). Larger sludge flocs show an increase in SND activities (Pochana et al. 1999). The floc reaction profile allows for the nitrification in the peripheral sections, and denitrification prevails in the floc‘s inner parts (Mustafa et al. 2009). The selector‘s other advantage is higher dissolved oxygen levels can more easily be maintained in the aerobic zones (Parker et al. 2003). However, care must be taken in both design and operation to provide adequate dissolved oxygen (DO) control in the aerobic zones, mainly if the plant is nitrifying. The dissolved oxygen concentration reached near-zero values at depths for larger floc particles (2-mm diameter and more significant) depending on the process operating conditions. A mathematical model based on diffusion of dissolved oxygen, the organic substrate (methanol), ammonia, nitrite,

Table 29.6 SBR plants and nutrient removal efficiencies

Plant capacity and location	COD removal (%)	BOD removal (%)	TN removal (%)	TP removal (%)	TSS removal (%)	SND (%)	rbCOD/ TCOD (%)	Soluble BOD/BOD_5 (%)	Soluble COD/ TCOD (%)	COD/ TN	COD/ TP
3 MLD, Roorkee, Uttarakhand	96	95	67	42	96	78	11	38	35	12.8	62
120 MLD Varanasi, UP	98	94	82	77	97	94	17	27	33	6	**73.1**
7 MLD Karnal, Haryana	97	92	84	19	97	83	16	47	45	7.1	69
10 MLD Karnal, Haryana	98	93	81	16	98	86	17	31	40	5.8	49
27 MLD Haridwar	96	95	77	39	96	80	8	48	29	11	83
60 MLD, Ahmedabad, Gujarat	97	95	75	32	91	80	12	69	47	6.4	50
25 MLD, Abohar, Punjab	96	92	71	39	93	100	14	32	26	38.7	357
70 MLD Rajkot, Gujarat	95	83	56	25	74	57	10	64	69	3.9	23
56 MLD Rajkot, Gujarat	97	90	77	32	88	90	14	63	54	7.6	47
7.4 MLD Dwarka, Gujarat	81	74	55	46	83	80	8	38	39	5.1	83

and nitrate through a spherical floc and consumption of dissolved oxygen by heterotrophs and autotrophs accurately predicted the dissolved oxygen profile (Daigger et al. 2007; Mustafa et al. 2009). Propositions of simultaneous nitrification and denitrification include consideration of the factors that affect floc size and distribution. Table 29.6 shows the values of SND in the SBR plants.

29.3.4 SND and Its Requirements

29.3.4.1 Role of Wastewater Characteristics on SND in the SBR Plants

The presence of appropriate proportions of the macronutrient C and N in municipal wastewater is essential for optimum SBR process performance employing SND. There are many factors responsible for wastewater characterization during biological nutrient removal in WWTPs, especially using SND. First is the length of the sewer. If wastewater remains in the sewers for a short time, there will be no H_2S formation and no volatile fatty acids (VFA) formation and may have a lower NH_4-N/TKN ratio and low PO_4-P/TP ratio. It is observed in 3 MLD SBR taking wastewater from IITR Sewer. Suppose long sewer lines are present before meeting the WWTPs. In that case, anaerobic condition prevails, and there is a high amount of H_2S, VFA, and availability of high NH_4-N/TKN and high PO_4-P/TP in the wastewater. It is observed in 120 MLD Varanasi sewer's influent and in 27 MLD Haridwar Sewer's influent. The second effect is industrial ingress (Dairy waste (experienced in 25 MLD Abohar, Punjab)) and high TDS content observed in 7.4 MLD SBR from Dwarka, Gujarat. In the Abohar plant, the wastewater possessed a high amount of organic matter and high Sulfate, H_2S, and toxic chemicals. The third and vital role was recorded by interception and diversion of drains from the local area (containing storm water contributing to dilution, which results in low BOD and low rbCOD) and sometimes mixing of cow dung and husk deposits from localized rural areas in the drains experienced in case of 7 MLD SBR, Gharaunda, Karnal, Haryana. Finally, the fourth and magnificent factor can be providing a primary settling tank ahead of SBR. In SBR Haridwar 27 MLD, it is observed that PST reduces BOD (suspended) by 20%–40% but not soluble BOD and TN and TP. It dramatically affects the BOD/TN ratio, which further affects SND% or TN removal.

There are various aspects responsible for the SND process to occur (Pochana et al. 1999) where the primarily important factor is carbon supply or source (sCOD, rbCOD, and soluble BOD) and specifically C/N ratio, where SND is an increasing function of rbCOD (Khursheed and Kazmi 2011). COD is estimated to be a more useful parameter than BOD, but from a modeling standpoint, COD cannot differentiate between biodegradable and inert organic matter or between readily and slowly biodegradable fractions. The various fractions of COD are significant (Rossle and Pretorius 2001). There is a correlation between these parameters and STPs' nutrient removal efficiencies, as shown in Table 29.6. Ratios like rbCOD/sbCOD, sbCOD/TCOD, and soluble BOD/total BOD affect a lot during the ease of biodegradation and fulfill the demand of carbon source for denitrification and uptake into the cells for biological phosphorus removal (Khursheed et al. 2018). Knowledge of the share of organic contaminants susceptible to biodegradation and resistant to biodegradation is fundamental in the design of biological removal of nitrogen and phosphorus, as it influences the dynamics of the activated sludge process, e.g., the oxygen demand, maintaining a constant sludge age, and kinetic parameters of the biological reactor operation (Płuciennik-Koropczuk et al. 2017). Particulate form goes into the sludge (Ekama et al. 1986; Khan 2012; Khursheed et al. 2018). The COD fractionation method is not devoid of errors. The share of fractions can be determined in various methods and with varying accuracy. Some of the fractions cannot be determined directly. The total COD of wastewater, divided into fractions, can be calculated in a simplified way:

$$\mathrm{TCOD} = \mathrm{RBCOD}(\mathrm{S_S}) + \mathrm{NBSCOD}(\mathrm{S_I}) + \mathrm{SBDCOD}(\mathrm{X_S}) + \mathrm{NBPCOD}(\mathrm{X_I}),\quad \mathrm{g\,O_2\,m^{-3}}$$

(Płuciennik-Koropczuk et al. 2017).

Acceptance of COD as the main parameter determining the amount of organic carbon in the wastewater and the division of COD into fractions describing different degrees of their biodegradation is, for now, a significant extension of the characteristic of wastewater. One of the COD fractions plays an active component in biochemical transformations. Other factions are, e.g., substrates in metabolism for the biomass. Among them, we distinguish easily biodegradable substrates and those that hydrolyze slowly. The rest of the organic matter is not biodegradable (inert). Figures 29.7 and 29.8 show COD fractions and the correlations between rbCOD% and SND% in the SBR plants analyzed in the study. One hundred percent SND is observed in 25 MLD SBR, STP at Abohar, Punjab at a high COD/TN ratio of 38.7, and rbCOD/ TCOD% of 14% at low DO concentration ranges (0–2.3 mg/L).

Denitrifying bacteria are known to compete for a carbon source with other heterotrophs. A low C/N ratio in the influent results in a rapid carbon deficit, causing unbalanced simultaneous nitrification and denitrification (SND) (Zhao et al. 2008; Phanwilai et al. 2020). Complete denitrification is achieved at TCOD/TKN ratio of 7 (Randall et al. 1992). It was proposed that the COD/TN ratio for denitrification is in the range of 3.5–4.5 g COD/g N (Pochana and Keller 1999).

29.3.4.2 Detailed Wastewater Characterization and Influence on SND in 3 MLD SBR, Roorkee

Detailed wastewater analysis of 3 MLD SBR, IIT Roorkee, was performed to analyze the relationship between rbCOD % and SND%. Excellent relationship was observed in Fig. 29.7a, b at $R^2 = 0.77$ and $R^2 = 0.81$, respectively, for rbCOD/sCOD (%) and rbCOD/TOD (%) with SND% (Fig. 29.10). In many plants, ramping of the operational DO profile occurs from 0 to approximately 2.5 mg/L. The DO profile enables the SND feature to occur where the fractional intra portion of the biomass is about 50%–70% in the biomass. Other anoxic conditions are also developed in the settle and decant air-off sequence to about 20% of the sequence time (Goronszy (1992)). Sensor applications useful in control strategies (such as ORP) have

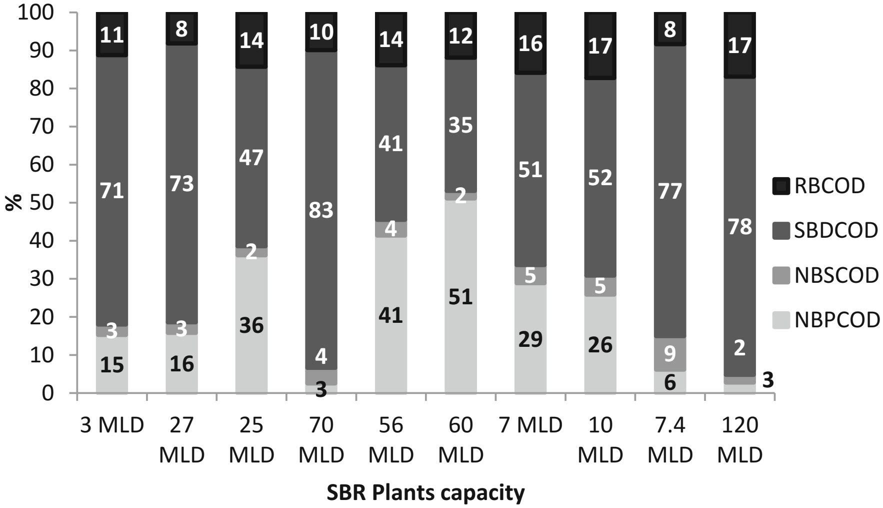

Fig. 29.7 Wastewater characterization based on COD Fractions in the SBR plants. *RBCOD* readily biodegradable COD, *SBCOD* slowly biodegradable COD, *NBSCOD* nonbiodegradable soluble COD, *NBPCOD* nonbiodegradable particulate COD

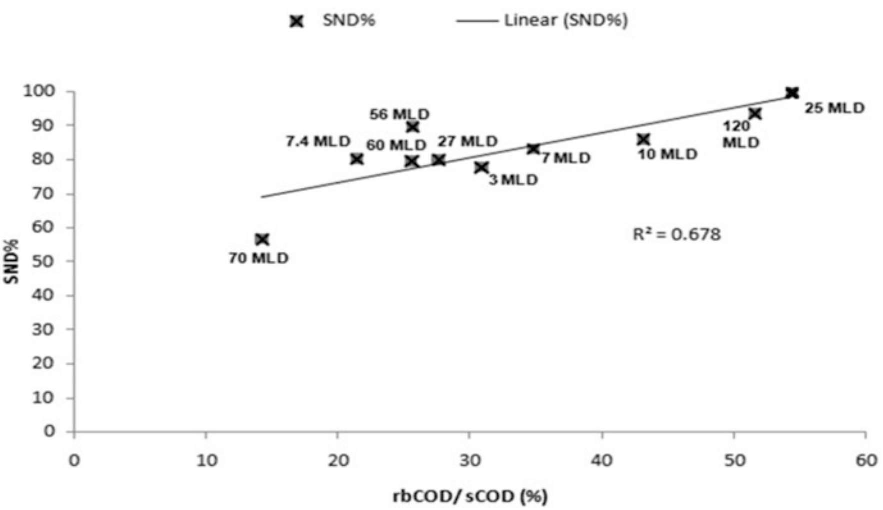

Fig. 29.8 Relationship between rbCOD/ sCOD (%) and simultaneous nitrification and denitrification in the SBR plants

demonstrated their usefulness in discontinuous systems such as sequencing batch reactors. Potential applications are using nitrate or ORP sensors in the anoxic selector zones to adjust the level of internal mixed liquor recycling (Parker et al. 2003). The relationship between DO, ORP, and ammonia removal can be observed

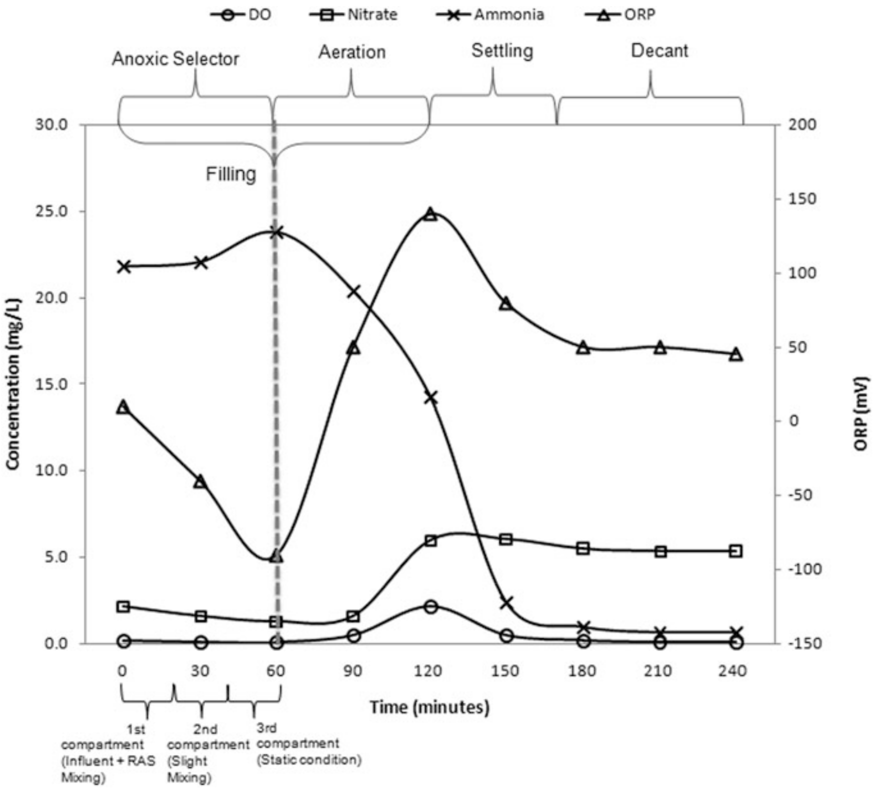

Fig. 29.9 Ammonia and nitrate profile against DO and ORP in 3 MLD SBR, Roorkee—Evaluation of different sequential phases (anoxic-aerobic) in designed 4 h SBR cycle

in Fig. 29.9. The COD/TN ratio in the SBR plant is 12.8 ± 4.8, and TN removal is 67.4 ± 12.3% and achieved the optimum total nitrogen removal of 83% at COD/TN of 9.1. The total phosphorus (TP) removal was 42.03 ± 15.32% at COD/TP and BOD_5/TP ratios of 81.8 ± 37.2 and 33.3 ± 14.5, respectively.

29.3.4.3 Relationship of rbCOD: TN and BOD_5: TKN on Denitrification Rates in SBR Plants

To accomplish denitrification in any process, the availability of readily biodegradable organic carbon is an essential factor (Randall et al. 1992). Operation data from different SBR plants showed the effects of the influent rbCOD/TN and BOD_5/TKN ratios on the effluent nitrate concentrations operating in SND mode (Figs. 29.11 and 29.12). It can be observed that higher rbCOD specifically is needed to achieve denitrification and which strongly influences SND performance (Pochana and Keller 1999; Jimenez et al. 2010).

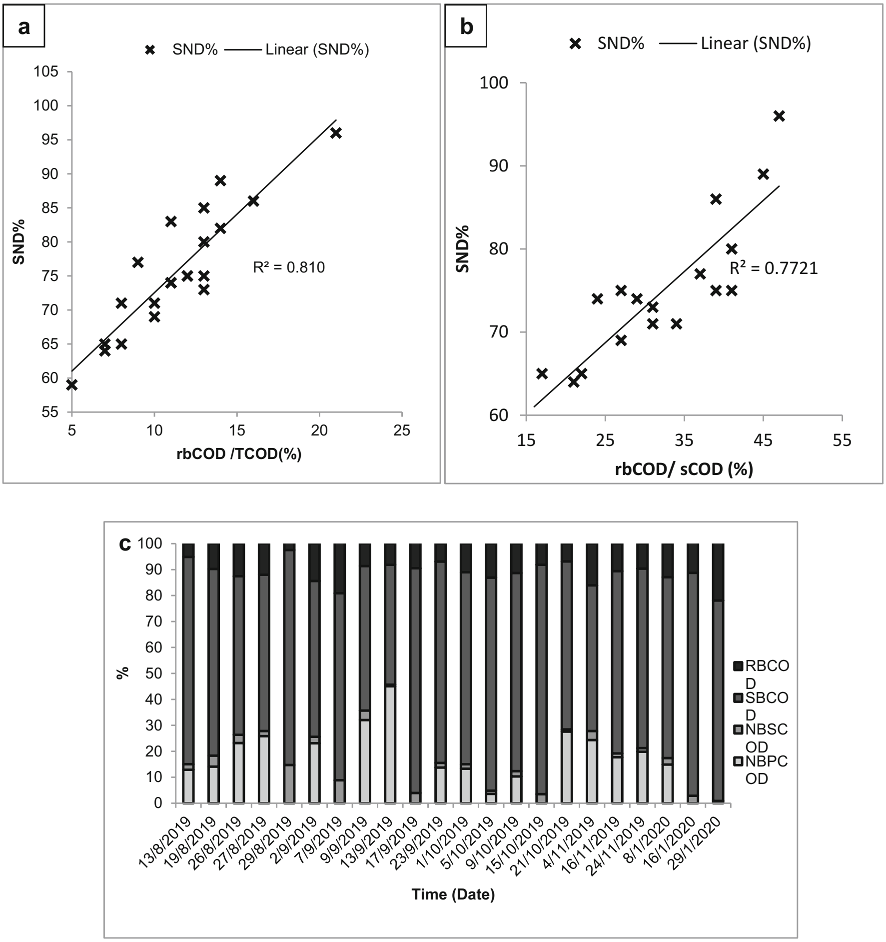

Fig. 29.10 Relationship between (**a**) %SND and rbCOD/TCOD% (**b**) SND% and rbCOD/ sCOD (%), and (**c**) wastewater characterization based on COD fractions in 3 MLD SBR plant, Roorkee (based on Metcalf and Eddy 2003). *TCOD* total COD, *RBCOD* readily biodegradable COD, *SBCOD* slowly biodegradable COD, *NBSCOD* nonbiodegradable soluble COD, *NBPCOD* nonbiodegradable particulate COD

29.3.5 Storage Products for SND

As autotrophic nitrification is generally slow in comparison with heterotrophic metabolism, SND requires a slowly degradable carbon substrate to provide reducing power for denitrification during the nitrification process (Third et al. 2003). When treating complex wastewaters, there is an increased likelihood that slowly degradable or particulate organic material will be available to serve as the electron donor for denitrification. Up to 100% of SND can be achieved when treating such wastewaters at low DO (Pochana and Keller 1999). When the particulate substrate is not

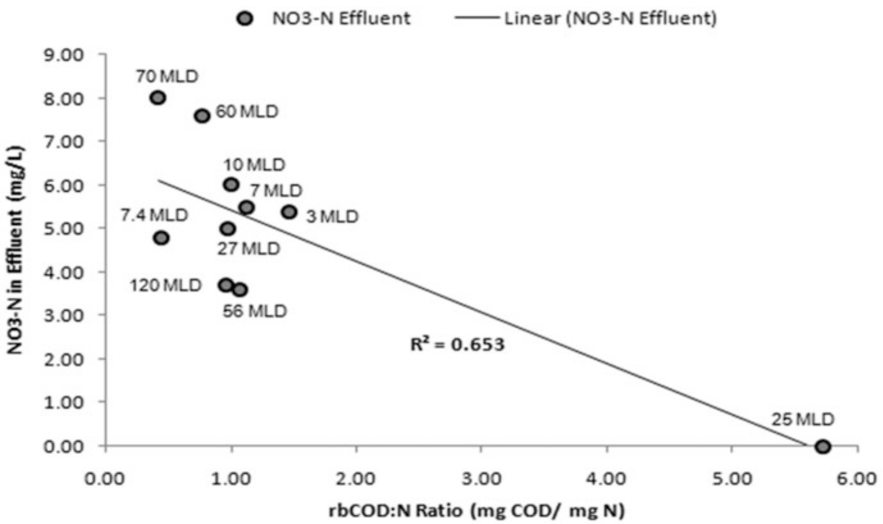

Fig. 29.11 Effect of rbCOD/TN on the denitrification rate

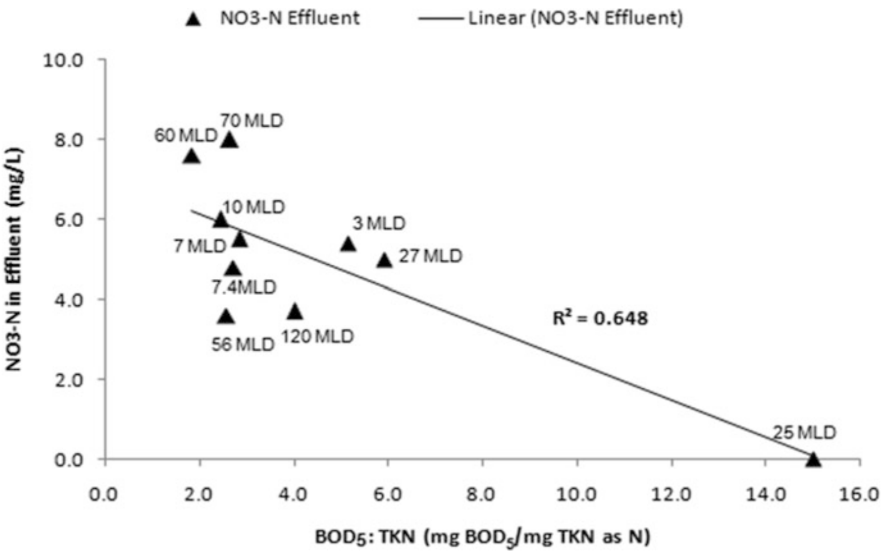

Fig. 29.12 Effect of BOD_5/TKN on the denitrification rate at the 3 MLD SBR

available, the soluble substrate's storage as PHB represents an opportunity to conserve organic carbon as a slowly degradable polymer for denitrification (Jones et al. 1990). Heterotrophs can rapidly remove the soluble substrate and store PHB as a slowly biodegradable polymer to preserve reducing power for denitrification for SND. During the feast period, i.e., acetate present, heterotrophic respiration activity

Table 29.7 Processes undergoing different phases of treatment in bio-selector-based SBR

Phase	Processes undergoing
Anoxic selector/aerobic stage 1	Acetate uptake, PHB synthesis (feast)
Aerobic stage 2—high aeration	Nitrification and PHB degradation
Aerobic stage 3—S/D	Residual PHB degradation and maintenance respiration

was high, and nitrification was prevented due to the inability of nitrifying bacteria to compete with heterotrophs for oxygen (Table 29.7). Once acetate was depleted, the oxidation rate of PHB was up to six times slower than that of soluble acetate, and nitrification could proceed due to the decreased competition for oxygen (Third et al. 2003). The slow nature of PHB degradation means that it is an active substrate for SND (Third et al. 2003; Miao et al. 2015). Granulation of biomass, resulting in superior supernatant-biomass separation, high mixed liquor suspended solids (MLSS) concentrations, and ability to meet high loading rates, has been noticed in many anaerobic processes. However, the mechanism of this phenomenon remains to be incomprehensible.

29.3.5.1 Internal Storage in Anoxic Selector

The described metabolic mechanisms responsible for substrate uptake in the selector are the formation of internal storage products and high rate metabolism. As presented by Jenkins et al. (1993), little amounts of the substrate are oxidized in the selector during the formation of internal storage products. Hence, excessive quantities of the substrate can be removed while reducing only small amounts of the terminal electron acceptor. The internal stores are metabolized in the main biological reactor only when the exogenous substrate has been exhausted (Mangrum 1998). The two processes that enable rapid substrate uptake are high rate metabolism and the production of storage products. High-rate metabolism refers to when the electron donor (the energy source) is used directly for oxidative phosphorylation and biosynthesis. This does not imply; however, that the entire process occurs in the selector zones. Substrate storage occurs through the production of non-nitrogenous products within the cells assimilating the organic substrate. Typical storage products are polyhydroxyalkanoates and carbohydrates (Gaudy Jr and Gaudy 1988). Microorganisms that completely metabolize the substrate remove approximately 8 mg/L of soluble substrate per mg/L of nitrate reduced (Jenkins et al. 1993).

29.3.5.2 Visualization of PHBs in 3 MLD and 120 MLD SBR Plants

PHAs exist in the cell cytoplasm as 0.2–0.5 μm granules surrounded by a membrane. Polyhydroxybutyrate (PHB), a lipid-like polymer of 3-hydroxybutyrate, is the most common PHA stored by bacteria. In 3 MLD SBR, Roorkee, and 120 MLD SBR, Varanasi, some of the PHBs are observed in anoxic selectors and aeration tank's sludge (Fig. 29.13). Qualitative microscopic observations were carried out in a mixed liquor sample of aeration tanks and selectors. One hundred microliter subsamples of sludge were examined at 100X magnifications (with immersion oil) as per the prescribed protocol (USEPA 1987).

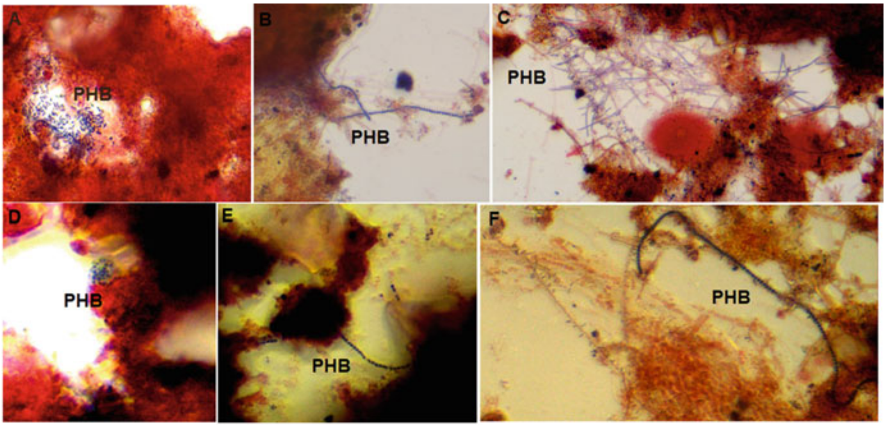

Fig. 29.13 Bright-field micrographs of the biomass samples. Panels **a**, **b**, and **c** (week 28, 3 MLD SBR tank's sludge): all with 100× magnification. Panels **d** and **e** (120 MLD SBR aeration tank's sludge): with 100× magnification. Panel **f** (week 26, 3 MLD SBR sludge): 100× magnification using immersion oil. Panel **a** and **d**: PHB granules present in between the sludge flocs. Panels **b**, **c**, **e**, and **f**: PHB granules present in the filaments. Sudan Black B was used to stain PHB inclusions in samples [blue-black cells: PHB (+), pink cells: PHB (−)]

29.3.6 Protozoa Identification

Protozoans are single-celled organisms ranging in size from 10 μm to over 300 μm. They are easily visible under the microscope at 40× and 100× magnification. Their primary function is the treatment process is to remove non-flocculent bacteria and very small floc that would not settle. Protozoa are responsible for the flocculation process, which results in the biosorption phenomenon of organic particles. These processes are essential in the treatment of conventional pollutants and micro-contaminant degradation. Hence, protozoa's presence or absence indicates the number of bacteria in the sludge and the degree of treatment (Chigusa 1990). Counting microbiota includes protozoa, metazoa, and filamentous organisms carried under a phase-contrast microscope at 20×; 40× magnifications are shown in Table 29.8.

29.4 Conclusions

For exhibiting simultaneous nitrification and denitrification in the SBR plants, bio-selectors (anoxic) play a prominent role in providing the necessary conditions, i.e., carbon source and natural selection floc-formers, which results in larger floc size and lower dissolved oxygen concentration ranges. Plants with high rbCOD fractions show excellent denitrification rates and SND. PHB degradation's slow nature means that it is an active substrate for SND, so polyhydroxybutyrate is the SND's practical storage products. Species like *Arcella*, *Vorticella*, and *Opercularia* are present in

Table 29.8 Microbiota profile and the sludge conditions in aeration tank

S. no.	Microbiota	3 MLD	27 MLD	25 MLD	70 MLD	56 MLD	60 MLD	7 MLD	10 MLD	7.4 MLD	120 MLD	Conditions when they appear
1	*Arcella*	++++	+++	Nil	+++	+	++	+++	++++	+	+++	Low DO (1–2 mg/L), satisfactory water quality, and occurrence of nitrification in the aeration tank
2	*Vorticella*	+++	+++	+	+	++	+	++	++	+	++	Robust against contamination due to the surrounding cilia at its mouth; floc condition is satisfactory, about 500–800 μm thick and dark brown
3	*Peranema*	Nil	Nil	Nil	+	++	+	Nil	Nil	Nil	Nil	The indicator of satisfactory effluent quality appears when the sludge is in a recovering state
3	*Colpidium*	Nil	Nil	+	Nil	++	Nil	Nil	Nil	Nil	Nil	Sludge condition is not satisfactory
4	*Litonotus*	++	+	+	++	++	+	Nil	+	+	Nil	Appears from the time the load was high until the condition becomes good
5	*Aspidisca*	++	+	+	++	++	++	+	+	Nil	+	DO is low, with a small population appearing at such a time and then disappearing when the water quality is excellent and satisfactory
5	*Rotaria*	+	++	Nil	+	+	Nil	+	+	Nil	+	Found in the later stages of our treatment process. It appears when the organic load is low and sufficient dissolved oxygen is present in the system
6	*Opercularia*	++	++	+	Nil	+	Nil	++	+	+	++	Appears when treatment is satisfactory if found in large numbers
7	*Filamentous*	++	+	+++	+++	+++	++	Nil	+	+++	+	Multiplies in the aerobic system, leading to sludge bulking sometime when higher in number, depending upon the sludge and reactor volume

Where (+) = 5–10 protozoans/mL, (++) = 10–100 protozoans/mL, (+++) = 100–1000 protozoans/mL, (++++) = >1000 protozoans/mL (Chigusa 1990; Bhatia et al. 2017) good numbers in the plants' satisfactory treatment. The present study can help make wise for laying out an upgraded, optimized bio-selector-based SBR treatment systems for better performances in Indian contexts. Further assessment of microbial ecology present in selector-based SBRs could open novel dimensions of wastewater treatment.

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Emerging Technique of Enzymatic Biotransformation of Amides to Hydroxamic Acid for Pharmaceutical and Dye Waste Treatment

30

Zainab Syed and Monika Sogani

Abstract

The potential applications of acyltransferases in the synthesis of pharmaceutically significant hydroxamic acids have rendered it substantial industrial importance. Enrichment techniques were used to isolate a thermophilic bacterial strain which is capable of degrading acetamide. This isolate was classified as *Bacillus* sp. on the basis of morphology, biochemical and physiological tests. Based on the 16S ribosomal RNA sequencing, the isolate was designated as *Bacillus megaterium*. Its resting cells containing the active acyltransferase enzyme were immobilized in the gel beads of different matrices as sodium alginate, agar, polyacrylamide and polyvinyl alcohol-alginate. The assay of acyltransferase activity of the immobilized beads was done using iron (III) chloride reagents at high temperature of 55 °C. The best performing sodium alginate beads were optimized for various parameters such as substrate concentration, temperature, pH and the type of buffer. This chapter provides insights on acyltransferase enzyme immobilization technique, its operational stability and its significance in hydroxamic acid synthesis.

Keywords

Bioremediation · Biotransformation · *Bacillus megaterium* · Hydroxamic acid · Immobilization · Acyltransferase

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30.1 Introduction

Enzymatic biotransformation is a proven method for organic compound synthesis. It has been estimated that two-third of the total biotransformation research has been carried out using hydrolases (Ramteke et al. 2013). The most widely used biocatalysts in organic synthesis are hydrolases (hydrolytic enzymes). They are used to generate pesticides and pharmaceutical intermediates and chiral synthons for asymmetric synthesis. In the biotransformation field, enzymes which degrade nitrile such as nitrilase, nitrile hydratase and amidase play significant roles (Veselá et al. 2012). Amidases are of particular interest amongst hydrolases due to their possible applications in bioremediation, microbiology, neurobiochemistry and plant physiology. "Amidases are universal enzymes which differ greatly in their biological activity. Carbon and nitrogen fixation in prokaryotes by amide hydrolysis, Indole-3-acetic acid (IAA) formation in *Agrobacterium*, and neuromodulatory fatty acid amides catabolism in mammals are the biological roles of these enzymes in nature" (D'Oca et al. 2010). These enzymes are also used to biodegrade nitriles and toxic amides from contaminated areas (Coffey et al. 2010). In many manufacturing processes, amide compounds are used extensively. As most of these compounds are extremely toxic and some are mutagenic and carcinogenic as well, their presence in industrial effluents and wastewater contributes to hazardous effects on ecology and environment (Sogani et al. 2014). Therefore, amidases can be used in the treatment of hazardous wastewater or industrial effluent amides. Some earlier papers have reported acetamide hydrolysing activity (AHA) in microbes utilizing acetamide as a source of carbon and/or nitrogen for growth as *Rhodococcus* sp. (Fu et al. 2014), *Klebsiella pneumonia* (Kumar et al. 2015), *Pseudomonas putida* (Li et al. 2016), *Rhodococcus rhodochrous* NHB-2 (Thakur et al. 2015), *Pseudomonas aeruginosa* (Uemura et al. 2016) and *Bacillus* sp. (Li et al. 2015). Amidase catalyses the hydrolysis of amide to free carboxylic acid and ammonia, and in the presence of hydroxyl amine, these amidases also show acyltransferase activity (Santoshkumar et al. 2017; Ruan et al. 2016). In addition, amidase acyltransferase activity (ATA) could be used to synthesize hydroxamic acids which are pharmaceutically active and are identified with antimalarial, anticancerous and anti-HIV properties (Mukram et al. 2016). Rzeznicka et al. (2010) have stated that amidase derived from *R. equi* TG 328 was found to be stable at 50 °C for a period of 8 h, but *Rhodococcus rhodochrous* J1 amidase was incubated in 10 mM phosphate buffer (pH 7.5) for 30 min, and this enzyme was found to be fully stable up to 30 °C but unstable at 55 °C. For the biosynthesis of a large range of hydroxamic acids with a high chelating ability, acyltransferase amidase activity has been used. Amides act as donors of the acyl group and hydroxylamine as acceptors of the acyl group in acyltransferase-catalysed biotransformation (Fig. 30.1). Enzymes are mixed in a solution with substrates in most industrial, analytical and clinical processes and cannot be commercially recovered after the substrates are depleted. When the expense of enzymes is taken into consideration, this single use is clearly very inefficient. Therefore, there occurs an opportunity to utilize the enzyme or cells in an immobilized or insolubilized state such that the subsequently added substrate can

$$\underset{\text{(amide)}}{R\text{-}CONH_2} + \underset{\text{(hydroxylamine)}}{NH_2OH} \xrightarrow{\text{Acyltransferase}} \underset{\text{(hydroxamic acid)}}{R\text{-}CONHOH} + \underset{\text{(ammonia)}}{NH_3}$$

Fig. 30.1 Acyltranferase catalysed biotransformation

be stored in a biochemical reactor to catalyse it further. Using an immobilized enzyme or cells makes it economically feasible to continuously carry out an enzymatic operation.

In a broad range of industries, including the regulation of environmental contamination, immobilized microbial cells create opportunities. Cell immobilization demonstrates many benefits, such as resistance to harmful substances when compared with suspended microorganism technology. The application of immobilized cells technology in the wastewater treatment sector is gaining importance. It is important as it offers many advantages compared with biodegradation by free cells, such as having high levels of biomass, enhancing biocatalyst recovery for reuse, offering better stability, activity, high mechanical strength, high durability to toxic substances, genetic stability and development. The immobilization procedures also solve the problem of removal of cell washout eliminating the unnecessary separation and purification steps. Furthermore, immobilization process has been used both in laboratory and industries in a wide variety of sectors such as food production, biomedical manufacturing, textile and detergent industries, wastewater treatment and biodiesel production.

The current research was conducted to assess the potential of the immobilized strain of *Bacillus* sp. isolated from dye-contaminated soil and pharmaceutical wastewater to biotransform acetamide into acetohydroxamic acid. *Bacillus* sp. wide-spectrum amidase (acetamide amidohydrolase) hydrolyses acetamide into the corresponding acid. In addition, the corresponding hydroxamate was formed when a short-chain amide acyl group was transferred to hydroxylamine. A suitable immobilization method (Jochems et al. 2011) was chosen to ease the handling and improving the stability of amidase. A variety of different matrices, such as agar, alginate and polyacrylamide, have been successfully utilized for immobilization (Choi et al. 2015). For the immobilization of relatively labile enzymes such as amidases, polyvinyl alcohol could be used as a mild cross-linking agent for modifying the alginate system (Sogani et al. 2012a, b; Kumar et al. 2006; Schoevaart et al. 2004). Therefore, this study tested the suitability of PVA alginate system for immobilizing whole cells of *Bacillus*.

30.2 Materials

30.2.1 Chemicals

Acetohydroxamic acid, hydroxylamine and acetamide were purchased from Hi Media. Yeast extract and Peptone were bought from “Suyog Diagnostics Pvt. Ltd. (New Delhi, India)”. The remaining reagents were obtained from Merck, India, and were of high analytical grade.

30.3 Methods

30.3.1 Sample Collection

The pharmaceutical and dye effluent sites of industries situated in Sitapura and Sanganer sites of Jaipur (Rajasthan), India, were selected for collecting the soil samples.

30.3.2 Bacterial Isolation Using Media Enriched with Acetamide

The collected soil samples from the effluent sites were transported and stored in sterile containers at a temperature of 4 °C. Bacteria were isolated by an enrichment culture technique from the soil sample (Eppinger and Stolz 2019). The nitrogen-free minimal media (NFMM) consisting of 2 g of acetamide as a source of nitrogen was used for enrichment (Pandey et al. 2011). Soil suspension was prepared in 10 mL (0.85%) of saline solution by adding 1 g of soil sample. Two millilitres of the suspension aliquot was transferred to 28 mL of minimal media in an Erlenmeyer flask. The cultures were incubated in a rotary shaker incubator at 150 rpm for 5 days at 30 °C. The cultures were then transferred (1:15, v/v) to the new medium. Pure cultures were isolated by further diluting and plating the resulting cultures on the limited medium plates after repeating this process four times. Dilutions of 10^{-3} to 10^{-5} were selected for the inoculation procedures. Aliquots of 0.1 mL were distributed uniformly on NFMM agar with 0.2 g acetamide as nitrogen source per 100 mL of the medium to induce the activity of the amidase/acyltransferase enzyme. For each inoculation, triplicates were performed. The well-established isolated colonies were streaked on a fresh NFMM agar for isolating a single colony and incubated for 48 h at 37 °C. NFMM consisted of 2 g/L acetamide, 4 g/L glycerol, 0.01 g/L $FeSO_4 \cdot 7H_2O$, 1 g/L NaCl, 1 g/L KH_2PO_4, 0.2 g/L $MgSO_4 \cdot 7H_2O$, pH 7.2.

30.3.3 Identification and Characterization of the Isolated Bacterium Strain

The bacterial isolate was biochemically characterized through fermentation reaction test (lactose) and the activity of the enzymes (methyl red, catalase, Vogues Proskauer, citrate, oxidase and urease) was assessed (Poli et al. 2013).

30.3.4 Analysis of 16S rRNA Sequence

The genomic DNA from the bacterial isolate was removed using a Sigma extraction kit (GenElute) for DNA. Amplification of the 16S rRNA of isolated bacteria was carried out using universal primers 8F and 1541R. "The amplification process was performed as discussed in (Sogani et al. 2012a, b) with initial denaturation step for 5 min at 95 °C followed by 10 cycles of 93 °C (1 min), 63 °C (1 min), 71 °C (1.5 min), 20 cycles of 93 °C (1 min), 67 °C (1 min), 71 °C (2 min) and a final extension step for 5 min at 71 °C. By the use of an automated sequencer at National Centre for Cell Science (NCCS), Pune (India), the purified PCR product was sequenced in both directions".

30.3.5 Transmission Electron Microscopy (TEM)

Using the Gram staining method, the cellular morphology of the isolate was examined and the organism was further examined by using transmission electron microscopy. The bacterial cells after washing were suspended in distilled water. On a grid coated with carbon (3 mm i.d., 3000 mesh), the suspension was applied accompanied by air drying. The bacteria that adhered to the grid were negatively stained with 92% (w/v) phosphotungstic acid and studied at an accelerating voltage of 80 kV under a transmission electron microscope.

30.3.6 Preparation of Resting Cells

Resting cells of bacterial culture were prepared as per the process carried out by Sogani et al. (2012a, b) where "*Bacillus* was inoculated in 50 mL of modified nutrient broth and incubated in an incubator shaker at 175 rpm at 30 °C for 24 h (Chand et al. 2004). The 24-h pre-culture was added to 50 mL of the production medium followed by incubation at 30 °C for 24 h at 175 rpm in the incubator shaker. The culture was then centrifuged at 0–4 °C at 5000 × *g* for 15 min. The cell pellets were washed and suspended in a 0.1 M solution phosphate buffer (pH 7.0)". These were known as resting cells and were used as biocatalysts for the assay of amidohydrolase amidotransferase enzyme activity.

30.3.6.1 Dry Weight Measurement of Resting Cells

In pre-weighed Eppendorf tubes, bacterial resting cells of 1 mL volume were taken and centrifuged (10,000 × *g*) at cooling temperature of 4 °C for 15 min. After this, the supernatant was discarded and the individual tubes with pellet were oven dried at a temperature of 80 °C till they reached a constant weight. The measured dry weight was 0.33 mg.

30.3.7 Colorimetric Determination on the Basis of Acyl Transfer Activity Catalysed by Amidase

The method of colorimetric determination relies on hydroxamic acid synthesis catalysed by amidase. The colour turned from light yellow to magenta by adding an acidic solution of ferric chloride to the reaction mixture, suggesting a positive reaction. Similar to the acyl transfer activity assay developed by Sogani et al. (2012a, b), the colorimetric screening method was performed. The reaction medium consisted of aqueous solution of acetamide (2 mL; 400 mM); hydroxylamine hydrochloride (2 mL; 2 M); buffer of sodium phosphate (2 mL; 100 mM); and the resting cells (2 mL). Both reactants and buffer were adjusted to pH 7 with 10 M NaOH before adding in the reaction medium. The reaction was performed at 30 °C. "The quantity of enzyme needed for producing 1 μmol/min of acetohydroxamic acid at 30 °C and pH 7.0 was specified as 1 unit of acyl transfer activity" (Sogani et al. 2012a, b). Acetohydroxamic acid, on the basis of the colorimetric screening of the resulting red brown complex with Fe (III), was analysed using the method developed by Sharma et al. (2012). After every 4 min, 0.5 mL sample of reaction medium was taken out for enzyme assay after the addition of 1 mL $FeCl_3$ solution. A stained complex was acquired at the end of the reaction. At 500 nm, absorbance was measured. Iron (III)/2, 2 dimethyl cyclopropane hydroxamic acid complex has the molar absorptivity of 3.34×10^2 mol^{-1} cm^{-1}. Reaction mixture with no resting cells was taken as a control and assayed.

30.3.8 Immobilization of Resting Cells of Isolated *Bacillus* Strain

Immobilization of the *Bacillus* cells was carried out in four different matrices following the process discussed by Sogani et al. (2012a, b) as shown in Fig. 30.2.

30.3.9 Acyltransferase Assay of Immobilized Cells

"The immobilized cells were incubated with amide and hydroxyl amine and sodium phosphate buffer at 37 °C for 1 h", according to Ruan et al. (2016) and Bhalla et al. (2018). The beads were removed through filtration after incubation. In order to eliminate the fine particles, the filtrate was centrifuged at 10,000 × *g* for 30 min. For acyl transferase activity, the clear supernatant was then assayed. The reusability

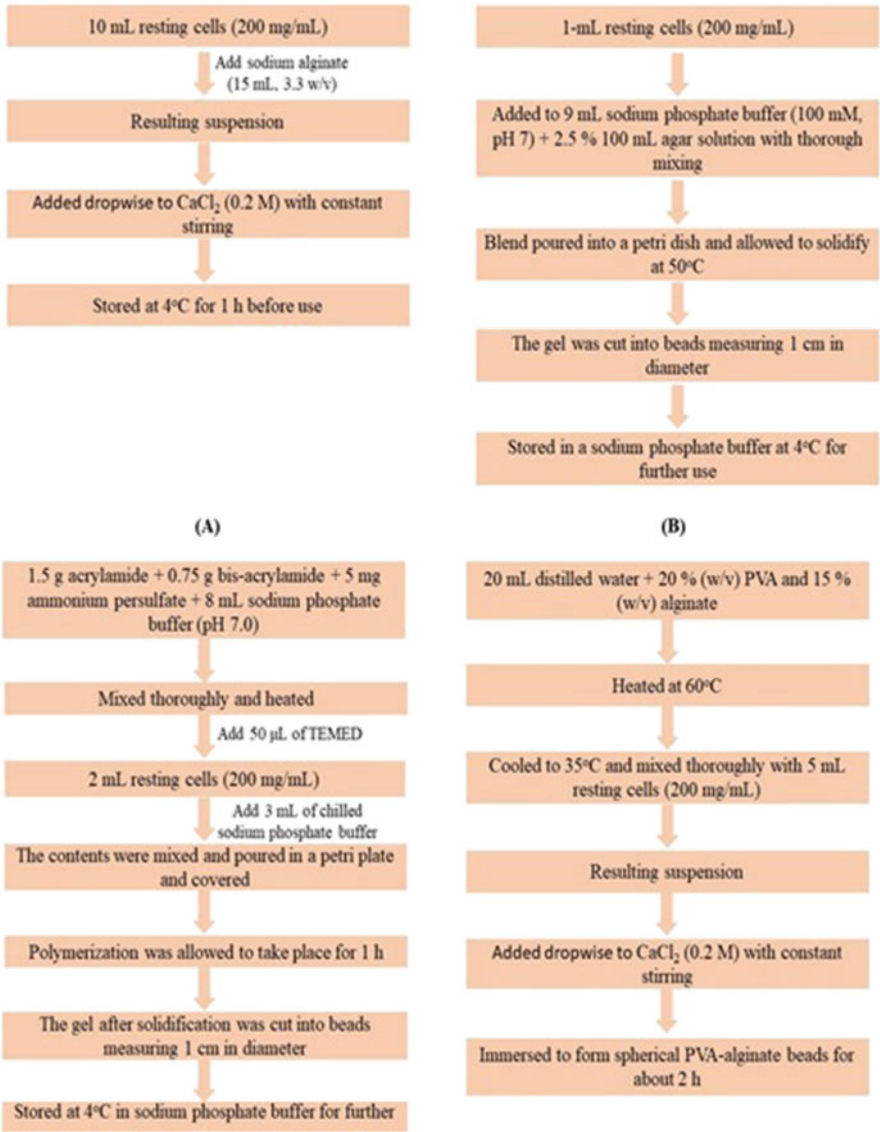


Fig. 30.2 Immobilization of resting cells in different matrices (Sogani et al. 2012a, b). (**a**) Sodium alginate (Raj et al. 2010), (**b**) Agar (Poonkuzhali and Palvannan 2013), (**c**) Polyacrylamide (Poonkuzhali and Palvannan 2013), (**d**) Polyvinyl alcohol-alginate (Pal et al. 2013)

of cells immobilized with agar, alginate, PVA alginate and polyacrylamide was determined for acyl transferase activity. To test their reusability capacity, the beads that were immobilized were used repeatedly in 10 reactions as biocatalysts as discussed by Sogani et al. (2012a, b). "In each reaction mixture, number of beads containing an identical enzyme as present in the given resting cell suspension were added and the removal of beads from the reaction mixture terminate the reaction (Mateo et al. 2004). Retention of the activity of acyl transferase in various matrices by cells which were immobilized in contrast to free resting cells was studied when acetamide and hydroxyl amine were separately incubated in sodium phosphate buffer at 37 °C for 1 h for the cells (400 mg) which were immobilized in different matrices and an equal amount of non-immobilized cells".

30.3.10 Enzyme Assay

AHA and ATA were analysed as per the process discussed by Sogani et al. (2014) as shown in Fig. 30.3. "1 unit of amide hydrolyzing activity (AHA) under assay conditions was described as 1 μmole of ammonia released min^{-1}". Similarly, "1 unit of amidotransferase activity under assay conditions was identified as 1 μmole of acetohydroxamate produced per mL". All tests were conducted in triplicates, and the mean value was taken into account.

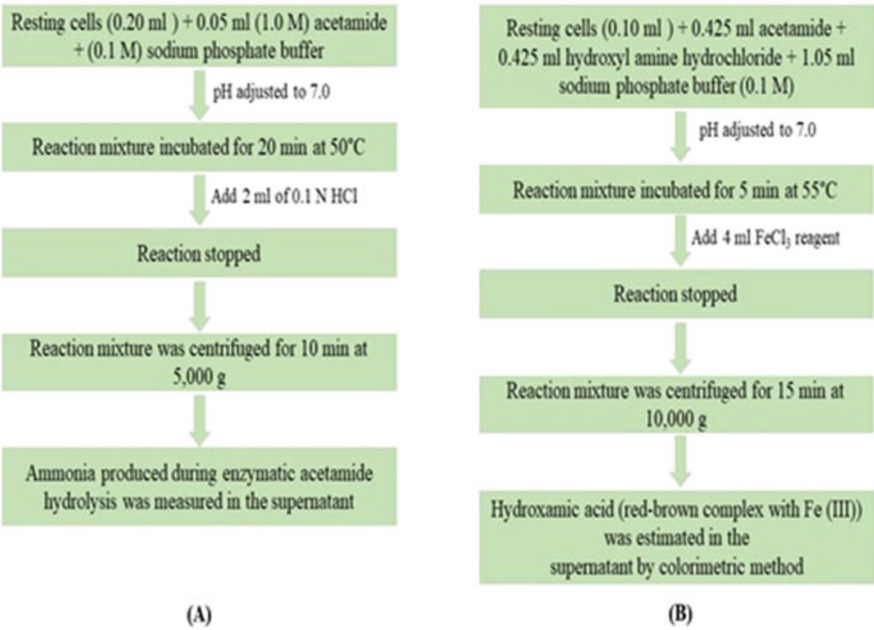


Fig. 30.3 Enzyme assay. (**a**) AHA, (**b**) ATA (Sogani et al. 2014; Offor et al. 2017; Sharma et al. 2012)

30.3.11 AHA Production Under Optimized Culture Conditions in *B. megaterium*

Fourteen media each supplemented with acetonitrile (0.2% v/v) were tested for AHA activity (Sogani et al. 2014). The effect of parameters as temperature (25–55 °C), time of incubation (0–96 h), size of inoculum (1–10% v/v) and pH (5.5–11.5) was studied in the selected medium M5.

30.3.12 Use of *B. megaterium* ATA for Optimizing Reaction Conditions

The effect of parameters as buffer pH, buffer systems, temperature of incubation, time of reaction at different temperatures, quantity of resting cells (in dry cell weight), type and amount of substrate on ATA of *B. megaterium* was studied. Thermal and storage stability on enzyme was also determined (Sogani et al. 2014).

30.3.13 Comparative Study of Acetamide Degradation Pattern Using Free and Immobilized Bacteria

The degradation pattern of acetamide was studied from the reaction mixture containing sodium phosphate buffer (pH 7.0, 100 mM), acetamide (125 mM), hydroxylamine hydrochloride (250 mM) and resting cells (2.65 mL) using free and immobilized cells. The final solution was incubated on a water bath shaker at 55 °C for 5 h. Final estimation of acetohydroxamic acid was done using the HPLC method.

30.3.14 Determination of Amount of Acetohydroxamic Acid Produced

"The amount of the acetohydroxamic acid produced was determined using the HPLC method. The Hewlett Packard Series HPLC system using a C-18 reverse phase column (4.6 × 2.50 mm) with 1 mL min^{-1} as flow rate, temperature (20–25 °C), and 210 nm as wavelength of detection with 25 mM orthophosphoric acid as mobile phase (Sogani et al. 2014; Chand et al. 2004)". After 100 times dilution the 5 μL of sample was injected. The calibration curves were determined for standards of acetohydroxamic acid hydroxylamine hydrochloride and acetamide.

30.4 Results and Discussion

30.4.1 Isolation of Amide Degrading Bacteria

Bacteria that have the capability to utilize acetamide as the sole source of carbon and nitrogen were isolated from the soil receiving pharmaceutical and dye effluents. Out of the 3 isolates, two of them were *Agrobacterium* sp. whereas the third one was a *Bacillus* sp. *Bacillus* sp. was chosen as the most conspicuous user of amide capable of profusely growing on acetamide containing NFMM agar and also depicted improved activity of acyl transferase enzyme as compared with other bacterial isolates and was selected for further analysis. As indicated by Landis et al. (2003), the variation in the activity of enzyme may be because of variations in the metabolism of microbe due to genetic variations and their response to the change in their environment. On the basis of these findings, further experiments with selected bacterial isolate (F-8) have been performed. Bacterial isolate F-8 was a facultatively anaerobic bacterium in the form of a rod that was motile using 1 or 2 flagella. The bacterium gave positive result for catalase enzyme while negative for urease enzyme, and was not able to carry out fermentation of lactose. Morphologically the bacterial colonies obtained were smooth, spherical, creamish-white, whole, opaque and measuring around 2 mm in diameter after incubating for 2 days at 37 °C in selected agar media. On the basis of the morphology and biochemical characteristics, the bacterial isolate F-8 was temporarily identified as *Bacillus* sp. By using the multiple sequence alignment software, the 16S rRNA sequences of the bacterial isolate F-8 were performed at NCCS, Pune, and were then compared with the BLASTN reference sequences from GenBank. By the use of neighbour joining method the phylogenetic tree was built (Ventosa et al. 2014), which revealed 100% similarity with *Bacillus megaterium*. The isolate was redesignated as *B. megaterium* F-8 in this study. The F-8 isolate was deposited into GenBank with the accession number HQ909050. *Pseudomonas* sp. has been used in several experiments on polluted soils undergoing bioremediation (Joshi and Abed 2017), but only limited papers on the function of *Bacillus* sp. have been published for bioremediation of nitriles (Yousefi Kebria et al. 2009). Siyanbola et al. (2015) documented bacterial isolation from different environments like arable land, freshwater, saltwater, woods and compost which can grow on the polymer BAK 1095 which is a polyester amide. Using the selected isolates, the polymer degradation mechanism was studied. The bacteria were found to selectively break the polymer at its ester bonds, releasing low-molecular oligoamides soluble in water. The isolates have also been tested for their ability to breakdown other polymers containing ester, such as linear polyester urethane urea, Degranil W 50. This polymer was also degraded by 8 of the 12 strains (Siyanbola et al. 2015). These isolated microorganisms were primarily from the *Bacillus* genus. The current research also confirms the earlier results that report the tolerance of *Bacillus* sp. to high toxic amide levels because of the presence of resistant endospores.

30.4.2 Identification of Bacteria

The strain was named as *Bacillus* sp. F-8 according to the physiology and biochemical characterization using "Bergey's manual of systematic bacteriology". 16S rRNA sequencing and PCR amplification of the isolate F-8 depicted that it was closely related to *B. megaterium*. The electron micro-photograph of strain F-8 is shown in Fig. 30.4.

30.4.3 Bacterial Cell Immobilization and Study on Reusability of Entrapped Resting Cells

Resting cells were immobilized in four different matrices as alginate, polyacrylamide, agar and PVA alginate by entrapment procedure as described in the method section. No change was observed in pH, buffer, temperature and concentration of the substrate in the reaction with immobilized and free cells, when resting cells having 5 mg dry weight containing 125 enzyme activity units were taken. In case of agar, polyacrylamide and PVA alginate gel immobilization, the recovery level of enzyme activity after cell entrapment was 75%, 65% and 80% respectively, while there was no loss in activity of enzyme with alginate gel. Similarly, in comparison with the free resting cells, the cells entrapped in agar and polyacrylamide displayed lower stability at higher temperatures. Interestingly, PVA alginate and alginate gel entrapped cells displayed higher thermal stability in comparison to free resting cells, presumably due to the inert nature of the alginate. In addition, the gelling reaction was also found to be endothermic. An enhanced enzyme activity was observed in case of agar and polyacrylamide gels on recycling of immobilized cells until the fifth recycle and

Fig. 30.4 Electron micro-photograph of strain F-8. (Adapted from Sogani et al. (2012a, b))

subsequently thereafter activity remained unchanged until tenth recycle. In the beginning, immobilized resting cells displayed residual enzyme activity of approximately 75%, 65% and 80% in agar, polyacrylamide and PVA alginate gels but after the fifth cycle it increased to 92%, 85% and 98% (Sogani et al. 2012a, b). This may be because a certain amount of substrate and product may be retained when the immobilized biocatalyst is used repeatedly, favouring the reaction. However, little protuberance of the matrix gel beads was observed on each recycle which may had promoted the substrate permeability through these compact gel beads. Conclusively, alginate and PVA alginate gel beads demonstrated improved reusability in contrast to agar and acrylamide gel beads.

30.4.4 Assay of Acyl Transferase Activity Under Optimum Reaction Conditions

Acyltransferase assay using entrapped cells revealed no substantial change in the buffer form, pH, temperature and concentration of the substrate as compared with the non-immobilized cells. The optimum pH for the free cells was 7.7, whereas pH optima for alginate entrapped cells was pH 7.0 (Fig. 30.5).

The effect of the matrix may be responsible for this small change of the optimum pH to a little lower pH value. The matrix restricts the diffusion of reaction products, i.e. acetohydroxamic acid which changes the pH within the matrix. With sodium phosphate buffer, immobilised cells showed greatest enzyme activity, whereas free cells showed maximum enzyme activity with potassium phosphate buffer (Fig. 30.6).

Potassium phosphate buffer was not found to be suitable with alginate immobilization process because of the favourable interchange of calcium ions in the potassium phosphate buffer from alginate to phosphate. Consequently, the resulting

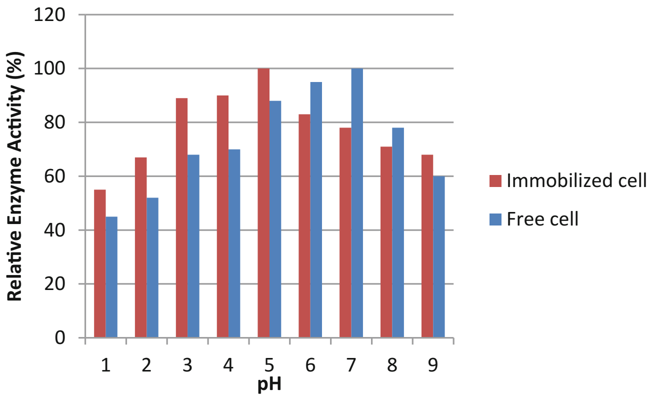


Fig. 30.5 Relative acyltransferase activity of immobilized and free *B. megaterium* F-8 cells affected by pH

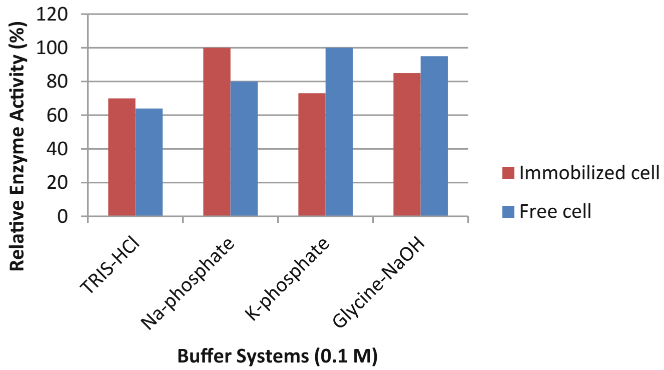


Fig. 30.6 Relative acyltransferase activity of immobilized and free *B. megaterium* F-8 cells affected by various buffers

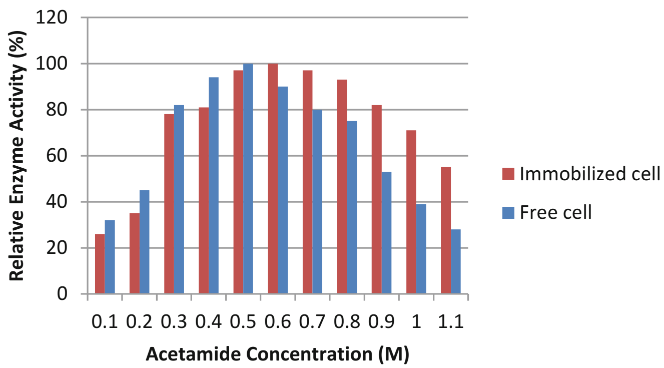


Fig. 30.7 Relative acyltransferase activity of immobilized and free *B. megaterium* F-8 cells affected by the concentration of acetamide

potassium alginate was extremely water soluble after the exchange reaction. In contrast to the free cells that depicted maximum enzyme activity at lower acetamide concentration, immobilized cells showed the highest activity at acetamide concentration of 600–700 mM, as higher concentrations completely inhibited the growth of free cells (Fig. 30.7).

Due to minimal diffusion in the matrix, the sudden decrease in enzyme activity was due to the inhibition of product caused at higher acetamide concentration of 700 mM. Diffusion restrictions, however, did not seem to be of significance at lower concentrations of acetamide because the utilization of acetamide by free cells was almost identical to that of the immobilized cells. The acyl transferase activity for both free and immobilized cells was maximum at temperatures ranging from 45 to 55 °C (Fig. 30.8).

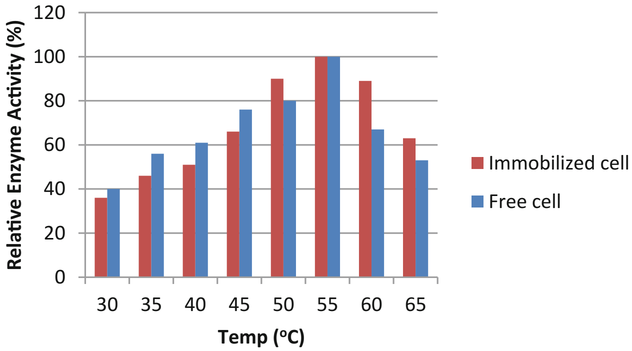


Fig. 30.8 Relative acyltransferase activity of immobilized and free *B. megaterium* F-8 cells affected by different temperatures

However, highest thermal stability was exhibited by alginate immobilized cells, possibly because of the inert nature of alginate, relative to free cells, and the gelling reaction was also endothermic.

30.4.5 AHA Production in *B. megaterium* F-8 Under Optimized Culture Conditions

30.4.5.1 Media

Maximum AHA activity (0.442 U/mL) was obtained in the M5 media which was modified nutrient broth supplemented with 0.5% sodium chloride, 3% tryptone and 1.5% yeast extract, whereas a slightly lower AHA activity (0.318 U/mL) was obtained in MY medium (M9) amongst the 14 media [M1-M14] that were studied. In modified nutrient broth, aliphatic nitrile/amide hydrolysing behaviour was also noted for *Nocardia globerula* NHB-2 (Kumar et al. 2006). For the development of amidase enzymes from Alcaligenes, a synthetic basal medium was used (Pandey et al. 2011). But *B. megaterium* F-8, on the contrary, produced lowest amidase activity in synthetic basal medium. A comparative study about the media composition used in the present research revealed that AHA activity in *B. megaterium* F-8 could have been improved by using both sodium chloride and yeast extract in the M5 medium.

30.4.5.2 Temperatures

Maximum AHA activity (0.442 U/mL) was obtained at 45 °C. This has shown the thermophilic nature of AHA produced by bacterial isolate F-8. The majority of species which degrade amide were increasing and the enzymes which hydrolyse amides work at about 30 °C (Gong et al. 2012). For cultivating and producing AHA by the isolated, thermophilic *Bacillus* sp., optimum temperature was 55 °C. At 30, 35 and 40 °C, comparable growth of the microorganism was observed, whereas at 20 and 25 °C slower growth of the microorganism was observed.

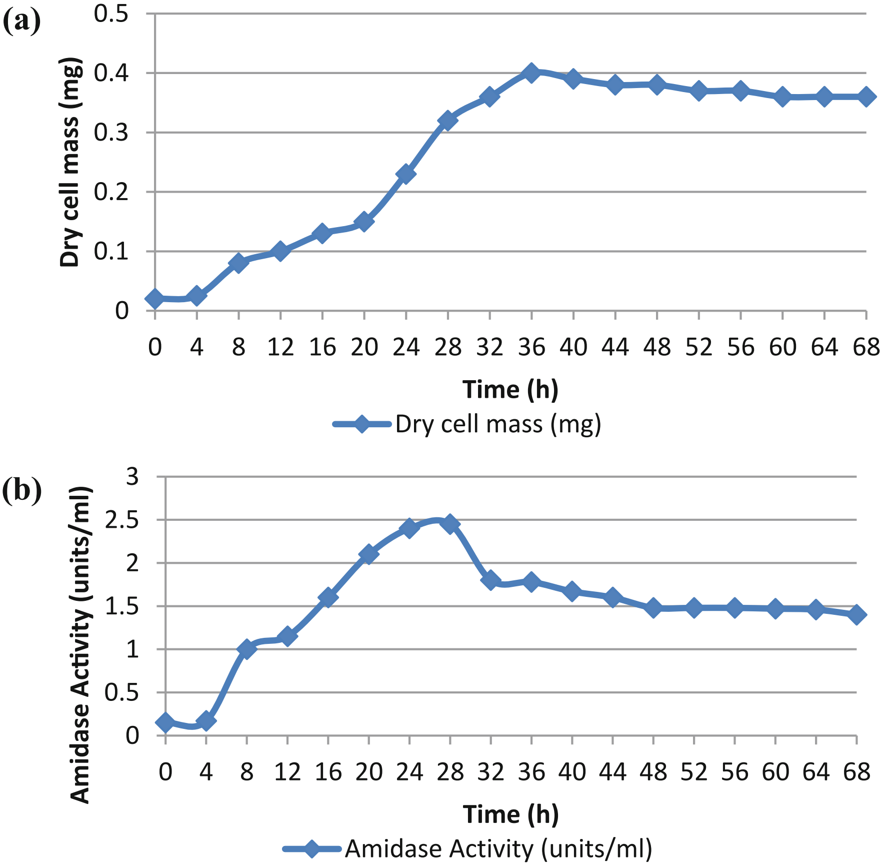


Fig. 30.9 (**a**) Incubation period effect on dry cell mass by isolated bacterium F-8. (**b**) Incubation period effect on amidase activity of isolated bacterium F-8

30.4.5.3 Time Course of Activity of Enzymes

During the lag period of bacterial growth (i.e. from 0 to 8 h) the AHA activity was low in *B. megaterium* F-8, which increased speedily during the log/exponential phase and the maximum activity (2.456 units/mL) was reached in 28 h during the mid-exponential phase. Subsequently, a sudden decrease in the AHA activity was observed during the stationary phase after 36 h and it became almost constant after an incubation period of 48 h (Fig. 30.9a, b).

Intracellular protease is formed during extended incubation period which reduces the activity of the enzyme, and AHA becomes vulnerable to proteolysis. There has been a similar pattern recorded in *N. Globerula* NHB-2 by Kumar et al. (2006), where maximal activity was observed (0.08 U/mL) at 21 h in the mid-exponential period. Subsequently, in the stationary phase (i.e. 28 h onwards), a sudden decrease in the enzyme activity was reported.

30.4.5.4 Inoculum Size

Optimum AHA production (0.474 units mL^{-1}) was estimated at 8% of the inoculum. A rapid decrease in the activity of enzyme was seen above 8% (v/v) inoculum, and around 77% of enzyme activity was reported at 10% (v/v) inoculum compared with the control (8% of the inoculum). Sogani et al. (2014) explained that "the decline in the activity of enzyme at higher levels of inoculum may be due to a comparatively greater abundance of cells that had entered late log or stationary growth phase due to early nutrient depletion". Bhatia et al. (2013) also reported maximal amidase activity at 8% inoculum of *Rhodococcus* sp. NHB-2. The difference in inoculum size relies on the microorganism type present in the inoculum and the number of viable cells.

30.4.5.5 pH

B. megaterium F-8 was cultivated at pH 5–11.5 in the medium (M5), where the detection of AHA was only observed from pH 5.5–9.5. At pH 7.5, major AHA activity (0.40 U/mL) was observed. Around 50% of enzyme activity was observed in the pH range 8.5–9.5, relative to optimum. When the pH of the medium was below 5.5 and above 10, AHA was not produced. Similarly, Nader (2012) observed maximum growth and amidase activity at pH 7.2 for *P. aeruginosa*, while optimal amidase activity was seen at pH 7.0 for *Brevibacterium* sp. R312 (Jiang et al. 2019). Nevertheless, *B. megaterium* F-8 upon cultivation at pH 7.5 expressed maximum amidase activity. It showed that the optimum pH was around neutral or slightly alkaline for the production of AHA.

30.4.5.6 Impact of Different Nitriles and Amides

The (M5) media which was supplemented with acetonitrile showed maximum relative amidase activity; similar activity was observed in case of acetamide, acrylamide, caprolactam, propionitrile and propionamide. Nevertheless, choosing acetonitrile as one of the strongest amidase inducing agents of *B. megaterium* F-8 shows that other nitrile compounds used in our study such as acetonitrile could also be degraded by the organism. Similarly, in *Bacillus pallidus* Dac 521 (Dennett and Blamey 2016) acetonitrile was reported as the inducer, and *P. aeruginosa* reported *N*-ethylacetamide as the inducer (Nader 2012). It also indicates that there are two enzymes in the microorganism (nitrile hydratase and amidase): nitrile hydratase, which catalyses the hydrolytic pathway of acetonitrile, and amidase, which catalyses hydrolysis of acetamide. The effect of different amides and nitriles on AHA is shown in Fig. 30.10.

30.4.5.7 Concentration of Acetonitrile

In the present analysis, the maximum AHA production by *B. megaterium* F-8 isolate was observed with 0.2% of acetonitrile in M5 media (2.456 units mL^{-1}). With increase in the acetonitrile (0.1–0.65 v/v) concentration in M5 media, there was a gradual decrease in AHA. When acetonitrile was used as the sole source of nitrogen and carbon in M5 media, it induced the amidase enzyme expression in *B. megaterium* F-8, which is responsible for degrading acetamide.

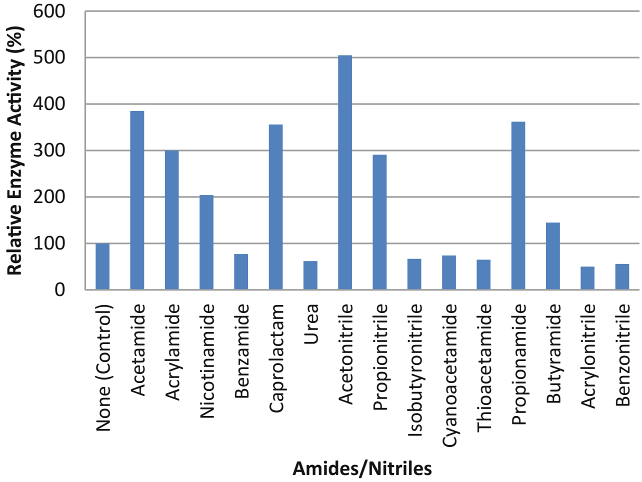


Fig. 30.10 Relative AHA affected by different nitriles and amides

30.4.6 ATA Under Optimum Reaction Conditions

30.4.6.1 pH Buffer

AHA was exhibited by isolated bacterium F-8 resting cells in pH ranging from 5.5 to 9.5 while maximum activity (0.40 U/mL) was observed at pH 7.5 when incubated for 5 min at 55 °C. ATA was observed in the pH range of 5–11; however, pH 7.5–8.0 (0.411 U/mL) was found to be the optimum pH for acyltransferase activity. The apparent involvement of two separate enzymes in acetamide hydrolysis was further confirmed by adding another substrate (Sogani et al. 2014).

30.4.6.2 Buffer System

The four buffers [glycine-NaOH, tris-HCl, sodium phosphate and potassium phosphate (0.1 M) pH 8.0] were measured in an enzyme assay for 5 min at 55 °C, where potassium phosphate buffer showed the maximum ATA (0.41 U/mL). However, with glycine-NaOH, tris-HCl and sodium phosphate the ATA was 0.326, 0.242 and 0.348 U/mL, respectively. The lower activity of acyltransferase in the tris-HCl (Tris (hydroxymethyl) aminomethane) buffer may potentially be because of some enzyme system inhibition by its reactive primary amine that interacts with the enzyme protein and inhibits it (Mikkelsen and Cortï 2016). Similar to our findings, maximum acyltransferase activity for *Brevibacterium* sp. R312 was reported with potassium phosphate buffer (Wu et al. 2017). On the other hand, in another finding using *Rhodococcus* sp. R312 maximum acyltransferase activity was obtained with sodium phosphate buffer (Sogani et al. 2012a, b).

30.4.6.3 Temperature of Incubation and Thermostability

Reaction for acyltransferase assay was carried out for 120 min at various temperature ranges varying from 25 to 60 °C and the activity of enzyme was observed at (5, 10, 15, 20, 40, 60, 80, 100, and 120) min. "After incubating the reaction mixture for 120 min, there was a gradual increase in the ATA from 0.3 to 2.5 U/mL at temperature ranges of 25 and 55 °C respectively. After 120 min of reaction, the maximum enzyme was obtained at 55 °C. The activity of the enzymes steadily increased from 25 to 55 °C; there was a loss of activity above this temperature" (Sogani et al. 2014). It may be because of the enzyme's thermal inactivation. "In the mesophilic range (23–37 °C), the majority of acyl transferase enzymes optimally catalyse acetamide degradation, but the Bacillus strain's acyl transferase activity is thermophilic in nature" (Dennett and Blamey 2016). This is close to our incubation temperature finding for *B. megaterium* F-8 maximum activity of acyltransferase. "Resting cells of *B. megaterium* F-8 have been suspended in 100 mM of sodium phosphate buffer for 120 min at varying temperatures ranging from 35 to 85 °C in order to study the thermostability of ATA by pre incubation. This activity was stable upto 55 °C and subsequently, with the increase in the temperature, the activity of the enzyme decreased" (Sogani et al. 2014). At 65, 75 and 85 °C, the enzyme activity remaining after 120 min of pre-incubation was 26%, 14% and 6%, respectively.

30.4.6.4 Storage Stability

Acyltransferase of *B. megaterium* was studied for its storage stability by storing resting cells for 30 days both at 4 °C and at room temperature (Fig. 30.11). After 30 days the residual activity reported was 92% and 83% at 4 °C and at room temperature, respectively. The bacterium acyltransferase was extremely stable at 4 °C without any noticeable loss of activity for a period of 30 days.

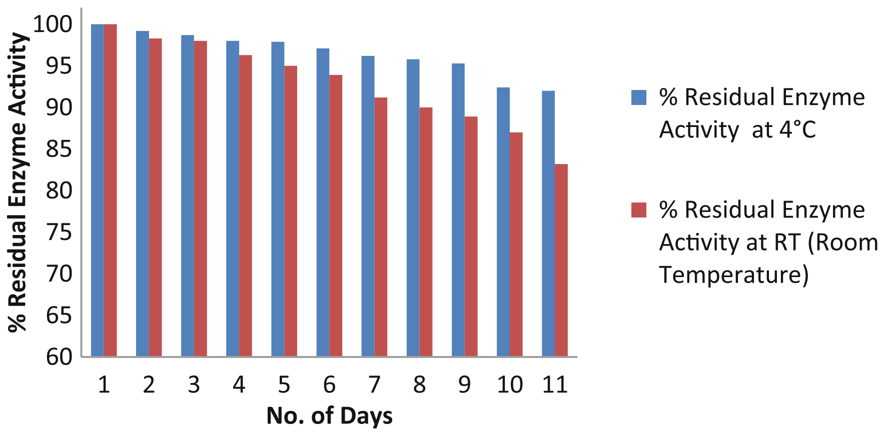
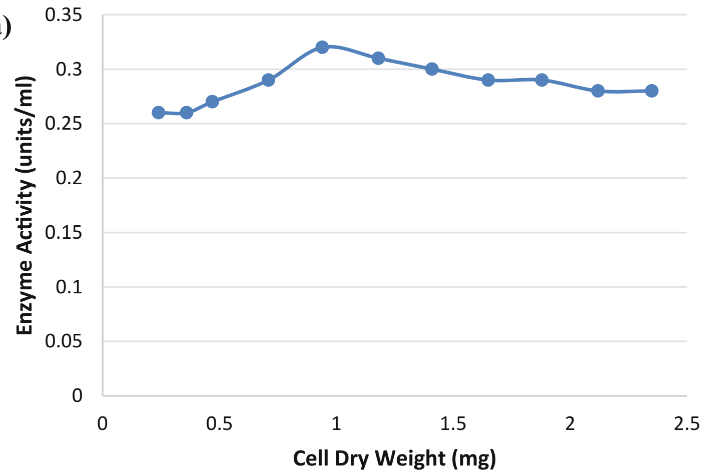


Fig. 30.11 Storage temperature affecting the relative acyltransferase activity of isolated bacterium F-8

30.4.6.5 Concentration of Biocatalyst

The production of hydroxamic acid increased linearly when there was an increase in the resting cells (ATA-containing biocatalyst) from 0.235 to 2.35 mg cells (dry cell weight) in the reaction medium. A steady increase in the activity of acyltransferase was observed up to 0.94 mg cells (0.322 U/mL) and sudden decrease was reported thereafter (Fig. 30.12a, b). This decline was likely because of the decrease in the ratio of substrate enzyme with increase in the biocatalyst concentration, so the enzyme activity reached a maximum point at a concentration of 0.94 mg cells, and then decreased slowly. There was also an uninterrupted increase in the production of acetohydroxamic acid with the increase in the activity of enzyme from 0.235 to

(a)



(b)

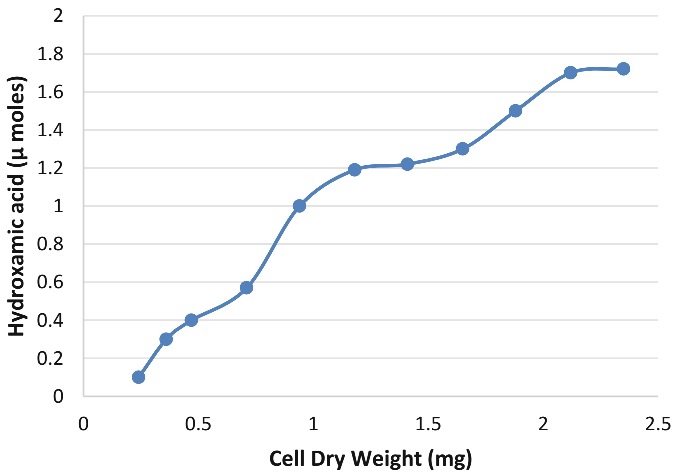


Fig. 30.12 (**a**) Acyltransferase activity affected by the concentration of biocatalyst. (**b**) Production of hydroxamic acid affected by the concentration of biocatalyst

0.94 mg cells, and the enzyme activity decreased steadily from 0.94 to 2.35 mg cells, but the product accumulation continued to increase, although it occurred at a slower pace as the enzyme level was retained in the medium with increase in the concentration of biocatalyst favouring the slower conversion of amide to hydroxamic acid.

30.4.6.6 Concentration of the Substrate and Affinity

The acetamide concentration ranged from 0.1 to 1.8 mmol in the reaction mixture. At a substrate concentration of 1.8 mmol acetamide, approximately 70% acyltransferase activity was observed. When 0.850 mmol of acetamide was present, the reported acyltransferase activity was maximum. There was no increase in the product above this concentration, possibly due to a limitation of the enzyme quantity relative to the substrate. In view of the possible hydroxamic acid applications (Li et al. 2007), amidase of isolated bacterium F-8 has been explored for its acyl transferase activity in order to achieve the optimum amide to hydroxamic acid transformation. Large substrate specificity was demonstrated by ATA of the isolated bacterium as shown in Fig. 30.13.

Acetamide, followed by acrylamide, lactamide and propionamide were the best donor for the acyl group. The acyltransferase affinity of this isolate was greater for aliphatic amides relative to aromatic amides. The ability to transfer acyl group of acetamide as a donor to hydroxylamine as an acceptor was employed for acetohydroxamic acid production. Acetohydroxamic acid production was measured using two approaches, in one spectrophotometric method where a reddish brown stained complex was formed due to the formation of a complex of hydroxamic acid with ferric chloride reagent. In other HPLC method first the concentration of standard acid was obtained by measuring absorbance at 500 nm and then a standard curve was plotted (Sogani et al. 2014). Next, quantitative determination of acid was done by the corresponding peaks and peak areas in the HPLC chromatogram.

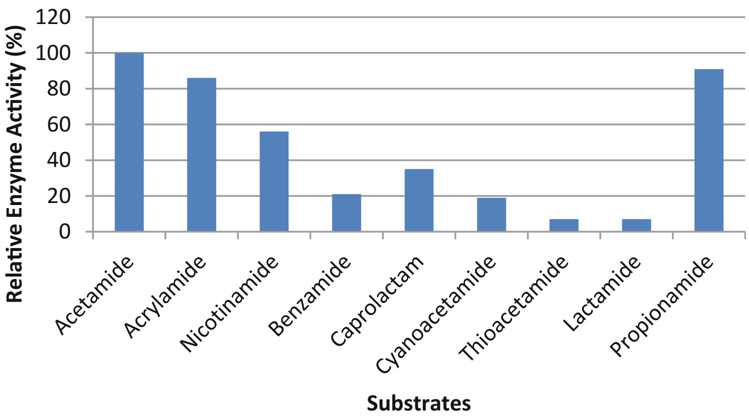


Fig. 30.13 Substrate specificity demonstrated by ATA of isolated bacterium F-8

30.4.7 Acetamide Degradation Pattern

The rate of synthesis of hydroxamic acid steadily increased with incubation time in both free and immobilized cells. After only 5 h of incubation at 55 °C, entrapped cells were able to degrade about 90% of the xenobiotic, while free cells develop poorly at 55 °C and degrade less than 60% of the xenobiotic. This data showed that immobilized *Bacillus* cells degrade acetamide to acetohydroxamic acid faster than free cells, and this sudden change is attributable to the fact that "immobilized cells have a large concentration of cells at their peak catabolic activity, whereas the free cells take longer to multiply. The matrices of the immobilized cells also protect the cells from the toxicity of the substrate and provide a defensive barrier against severe physical conditions" (Sogani et al. 2012a, b). The immobilized cells of PVA alginate were able to degrade 60% of the xenobiotic at 55 °C after an incubation period of 5 h. This method of PVA alginate is therefore acceptable and can be effectively extended to the immobilization of *Bacillus* amidase.

In F-8 maximum ATA was observed at 55 °C when incubated for 20 min. Enzyme activity increased steadily from 30 to 55 °C, and there was a loss of activity above this temperature. The majority of acyltransferase enzymes catalyse the degradation of acetamide in the mesophilic range (23–37 °C), but Dennett and Blamey (2016) indicated that the Bacillus strain's acyltransferase activity could be seen at higher temperatures. This is comparable to our current findings of optimum temperature of incubation for *B. megaterium* F-8 for maximum acyltransferase activity. For any bioprocess, biocatalyst thermostability is of vital significance and for synthesizing acetohydroxamic acid, the calculated bioconversion yield accounted to 90% in the current process, which is greater than earlier reported 86% synthesis using *Rhodococcus* sp. R312 (Sogani et al. 2012a, b).

30.5 Conclusions

It is concluded that the newly isolated, identified and characterized bacterium, *B. megaterium* F-8, degraded the toxic amides up to 90% and exhibited thermophilic nature. Therefore, it has a huge potential for biodegradation of toxic amides from textile and pharmaceutical industries in extreme environmental conditions. Furthermore, immobilization procedure shows the expression of acyltransferase enzyme expressing thermostable activity and can therefore be used for hydroxamic acid synthesis. In comparison to chemical methods, biotransformation on a commercial scale has a huge potential for the synthesis of hydroxamic acid, which is the major compound in medicine, analytical chemistry, phytochemistry, agronomy and studies in nuclear technology and wastewater disposal. The chapter successfully reports the bioconversion of amides to useful acids via whole cells and immobilized cells using a simple, thermostable, low cost, easy to apply enzymatic process for the treatment of toxic amides. More application-based investigations are needed in order to understand the biotechnological prospective of such a multifaceted biocatalyst in the light of such wide-ranging uses of hydroxamic acid. Via the latest smart technical design and support materials, immobilization technology will allow a fascinating rapid development in many manufacturing applications by decreasing the cost but increasing the yield. In reality, immobilization can be improved for manufacturing molecules with industrial significance. Immobilized cells can be useful for the treatment of waste in order to turn the toxicant through its intermediates into nutrients, biomass and CO_2 through biodegradation. In immobilized cells, an improved biodegradation rate was observed due to the absence of internal and external mass transfer resistance. Immobilized microbial systems offer different advantages over free systems at the moment. Using this technology to minimize environmental emissions through the biodegradation of several harmful compounds is one of the most promising areas of study. The use of immobilization technology in the environmental sector is in its preliminary stages, but the findings seen so far are encouraging.

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31 Biopolymer-Based Nanocomposites for Removal of Hazardous Dyes from Water Bodies

Aisha Zaman, Mir Sahidul Ali, Jonathan Tersur Orasugh, Priya Banerjee, and Dipankar Chattopadhyay

Abstract

The present world is confronted with perils of freshwater resources and hence contamination of water bodies in specific has raised genuine concerns. The possible application of biopolymers and its composites for removal of hazardous dyes has been widely investigated in recent studies. Polymers derived from biological sources, such as polysaccharides, lignin, alginate, and bacterial polymers, are the major polymeric materials adopted for water purification systems. Driven by the potential applications of such biopolymers for wastewater remediation, researchers take a great deal of interest in developing biomaterials

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that can perform specific functions in response to a given set of environmental conditions and parameters. One way to design such materials is to create composite functional structures, which can adsorb or bind with a specific type of contaminant present in water bodies. Composites are highly advantageous for adsorption applications as they possess desired properties of both parent materials and hence limitations of individual components can be overcome. In this vein, nano-sized bio-composites can present superior adsorption capacities in comparison with their micro-sized counterparts. This chapter focuses on the principles of designing and preparation of bio nanocomposites particularly from polysaccharides, which offer advanced applications in highly functionalized and molecule specific adsorption and super adsorption of hazardous dyes. Such emerging technologies and recent advancements in the application of polysaccharides as composite nanostructures, for water purification, have been thoroughly discussed. Along with recent advancements, the chapter also outlines the developmental history, current status, future prospects, and significance of exploring and utilizing biopolymer nanocomposites in this domain.

Keywords

Adsorption · Biopolymers · Biosorption · Cellulose · Chitin · Chitosan · Hazardous dyes · Nanocomposite · Polysaccharides · Starch · Wastewater treatment

31.1 Introduction

Growth and civilization and science and technology have been paralleled by overexploitation and pollution of natural resources. One of the major challenges to be overcome in this aspect is security of freshwater sources, which is of critical importance to both natural ecosystem and human development. Many industries like textiles, paper, leather tanning, printing, and plastics consume substantial volume of water and generate equally large quantity of wastewater laden with hazardous substances such as organic compounds, toxic metal ions, colored pigments, and dyes. The textile industries in particular generate a huge amount of colored wastewater due to their inefficient techniques of dying textile fibers. The quality of natural water bodies has thus been deteriorating at a rapid pace due to such irresponsible discharge of waste materials and undesirable chemicals, which are often toxic and intractable in nature. The inefficiency of traditional water treatment methods has pushed the researchers to design simple, effective, and economic as well as environment-friendly techniques. Adsorption as a tool for water remediation has proven its worth in the recent times, and the use of biomaterials as adsorbents is being extensively explored. In fact, scientists are now aiming to develop composite structures so as to combine desired properties of different materials and hence achieve the best possible approach toward wastewater remediation. This chapter encompasses the properties of biopolymers for water treatment with special emphasis on the use of polysaccharides and its nanocomposites, preparation techniques, and subsequent application as bio-sorbents for removal of dyes from waters bodies.

31.2 Methods of Water and Wastewater Treatment

Among the various water pollutants, hazardous dyes tend to raise a larger concern because they are not only mutagenic and carcinogenic, but they also possess a high degree of visibility even at very minute concentrations. Based on their solubility, chemical properties, and molecular structure and sometime based on application, dyes can be broadly classified as- disperse dyes, reactive dyes, basic dyes, and direct or acid dyes (Zaman et al. 2016). It is the chromophore group like an azo, nitro, nitroso, sulfo, and keto that are present in the dyes that are responsible for imparting color. Presence of such chromophores, high solubility, and hydrophilic nature of the dyes renders them recalcitrant and nonbiodegradable (Lellis et al. 2019). Hence, conventional water treatment methods such as sedimentation, coagulation, flocculation, membrane separation, reverse osmosis, ion exchange, and even microbial degradation often fail to efficiently remove such complex natured hazardous dyes (Das et al. 2016). All such techniques involved for the treatment of water and particularly for the removal of dye from effluents have their own merits and demerits. Chemical precipitation is based on consumption of several chemicals and is yet inefficient in removing contaminants present in trace amount. Simple methods like coagulation and flocculation often result in toxic solid waste (Zia et al. 2020). Microbial degradation of toxic dyes also results in undesired secondary metabolic waste in lesser yet toxic forms and generates biological sludge (Crini and Lichtfouse 2019). The old school methods of water treatment often generate solid waste or sludge, disposal of which raises another environmental challenge. Many of these limitations are overcome by adsorption techniques, which have attracted a lot of attention due to its inexpensiveness and ease of operation. Also, an innumerable range of materials including forest waste, agricultural wastes or by-products and other industrials wastes, and electronic wastes can be effectively employed as adsorbents for the removal of almost all kinds of water pollutants (Popa and Visa 2017; Goswami et al. 2017; El Essawy et al. 2017; Roy et al. 2016). In a previous study, metal oxide and graphite were recovered from the cathode and the anode of spent lithium ion batteries, respectively (Natarajan and Bajaj 2016). Metal oxide was obtained in the form of $LiMn_2O_4$, and the recovered graphite was converted to graphene oxide by modified Hummer's method. Both the constituents were then separately employed for the adsorption of Methylene blue and Congo red yielding an exceptional result of 100% removal of MB and CR, up to 1000 mg/L in just 5mins due to electrostatic interaction of oppositely charged adsorbent–adsorbate species (Natarajan and Bajaj 2016). A very recent study made use of forest waste to prepare activated biomass charcoal (BCA) (Popa and Visa 2017). Raw charcoal was obtained from waste tree branches by dry distillation at 150–350 °C followed by carbonization at 550 °C. The BCA so obtained treated with NaOH and for further enhancement of photocatalytic properties TiO_2 was added. While the alkali treatment added hydroxyl groups, the TiO_2 boosted the photocatalytic activity of the prepared composite for fruitful synergistic adsorption of methylene blue and cadmium ions (Popa and Visa 2017). Several studies in the past have shown that adsorption techniques are often useful for dye removal. In fact, some researchers have also reported that the dyes adsorbed on the adsorbent material can be desorbed, regenerated, and processed for reuse.

Adsorption process is such a technique, which can be adapted to any water treatment format (Crini and Lichtfouse 2019). Carbon-based materials like activated carbon, graphene oxide, or waste materials like fly ash, raw agricultural solid waste and byproducts, jute waste, sugarcane bagasse, rice husk, shells of several fruits even eggs, sawdust, and bark from forest industries, clay, dead microbial biomass, and practically any kind of waste or by-product can be successfully employed as an adsorbent. Success in the area of adsorptive removal of water pollutants has led scientist to explore different biomaterials, which are environment-friendly and economical and can be used as efficient adsorbents either individually or in composite forms. Composites have an advantage over individual materials, as they possess properties of both parent materials and the limitations of the individual component can be overcome at same time. In this aspect biopolymers such as polysaccharides, lignin, and alginate are inexpensive and readily available stand out as excellent candidates for adsorption or biosorption of dye molecules (Zia et al. 2020). Moreover, the advancement in nanoscience and nanotechnology has inspired researchers to develop highly functionalized nanocomposites based on biopolymers for the adsorptive removal of toxic dyes.

31.3 Biopolymers as Adsorbents

31.3.1 Classification of Biopolymers

In these times when mankind is moving rapidly toward green technology and sustainability, biopolymers have revolutionized the world of material science. The term biopolymer itself indicates that these are derived from biological sources such as plants, animals, or microbial biomass (Kanmani et al. 2017). Biopolymers can also be obtained from fossil fuels, which were once-living materials. Based on the source of origin material and degradability, biopolymers can broadly be classified into three categories:

31.3.1.1 Biodegradable Biopolymers

These are made from renewable bio-based raw materials like plants, animals, and microbes or can be chemically synthesized from organic parent materials such as starch, sugar corn. For example, chitin and chitosan are obtained from the shells of crustaceans, insects, and some fungi. Brown seaweed is the natural source of alginate. Bacterial cellulose can be obtained from bacterial biomass, and there are many such biodegradable polymers that can be derived from a wide variety of living materials (Khademian et al. 2021).

31.3.1.2 Nonbiodegradable Biopolymers

These are made from renewable bio-based raw materials but are not biodegradable. Polymer products such as polyamides and bio-polyethylene are derived from natural renewable feedstocks but are nondegradable, such as PVC derived from sugarcane, whereas polymers like poly lactic acid, poly-hydroxyalkanoates, and thermoplastic starches are obtained from renewable feedstock but are partially biodegradable that is they can be biodegraded only under specific conditions (Lackener et al. 2021). Some naturally occurring biopolymers like rubber or amber are also nonbiodegradable (Khademian et al. 2021).

31.3.1.3 Biodegradable Fossil Fuel-Based Biopolymers

Unlike the first two categories, these are derived fossil fuels and are biodegradable, such as aliphatic polyesters procured from crude oil and natural gas (Khademian et al. 2021). Although the use of traditional plastics, synthetic- and petroleum-based polymer products are essential in our daily life, yet their impact on the environment and especially aquatic ecosystem raise great concern. Hence, degradable biopolymers derived from renewable sources have great advantage over the synthetic ones derived from nonrenewable resources and are most valuable in terms of inexpensiveness, easy economical and eco-friendly production, and varied applications (Dassanayake et al. 2018). The degradable biopolymers are mostly generated from either agricultural wastes/by-product, textile wastes, fishery industries, etc. or directly obtained from plants, animals' bacteria, yeast, etc. With growing pollution and scarcity of resources, researchers and even industrialists are adopting the idea of "best out of waste," hence biopolymers obtained from waste or by-products are being extensively explored. In the next section of this chapter, the structure and properties of biodegradable bio-based biopolymers have been comprehensively discussed with special reference to polysaccharides obtained from plants, animals, and waste sources and their adsorption application.

31.3.2 Polysaccharides: Structure and Adsorptive Properties

Among the three major types of polymers that are polynucleotides, polypeptides, and polysaccharides, polysaccharides are the most abundant, naturally occurring biopolymer that consists of monomers of sugars held together by *O*-glycosidic linkages. A polysaccharide, also called a glycan, can be defined as a large molecule consisting of several smaller monosaccharides, which are simple sugars, like glucose. The individual monomer units also called residues are linked together through glycosidic bonds, thus creating long chains of sugar polymers or polysaccharides. Polysaccharides can be a *homopolysaccharide*—consisting of only one type of monosaccharide or heteropolysaccharide—those which consist of different kind of sugars linked together into a repeating unit to form a monomer of the polymeric chain. Polysaccharides can be linear with a straight chain, which forms a rigid polymer such as cellulose, or it can be branched with arms and turns, often forming soluble polysaccharides such as gum (Dassanayake et al. 2018). Hundreds of polysaccharides have been identified and employed for a wide range of commercial, industrial, medical, and environmental applications. Polysaccharides such as cellulose, chitin, chitosan, guar gum, and xanthan have been extensively applied for adsorptive removal of various pollutants. To be a good adsorbent, any material needs to have some essential properties like insolubility in water, relatively large surface area, porous structure, and small pore size and high selectivity. Apart from these basic eligibility criteria, an adsorbent must also have high abrasion resistance, high thermal stability, non-toxicity, biodegradability, etc. In the field of adsorption of pollutants from aqueous medium, cellulose, starch, chitin, and chitosan are most commonly used for a wide range pollutant. In the following sections, we thoroughly discuss the properties and role of these polysaccharides in the removal of dyes from water bodies. This chapter further discusses various techniques involved in the preparation of biopolymer-based composites and nanocomposites and their derivatives via modification and functionalization.

31.3.2.1 Cellulose

Cellulose a natural polymer is the most abundant, renewable organic material on our planet. It is the major structural component of the plant cell walls; around 33% of all plant materials consist of cellulose (Khademian et al. 2021). Not just plants, most bacteria and some fungi and algae can also biosynthesize cellulose (Wang et al. 2016a, b). It has a global annual production of about 1000 tons (Peng et al. 2020). Cellulose is regenerable, biodegradable, hydrophilic, and insoluble in water. Cellulose is a homopolymer where hundreds of β-D glucose monomers are covalently linked together by a glycosidic bond to form a long linear chain. This is a result of condensation reaction between the hydroxyl groups at the first carbon of a glucose unit and the fourth carbon of the neighboring glucose unit. Since monomeric glucose units are oriented at an angle of 180° to its neighboring unit, along the linear axis, the dimer of glucose also called cellobiose is considered as the repeating unit of cellulose polymer. Some of the physical properties of cellulose such as a high degree of polymerization, (DP—approximately 14,000), high degree of crystallinity (89%), and high young's modulus (114 GPa) have made it an attention-grabbing material. Apart from the aforementioned properties, cellulose also has some other physical and chemical properties that make it highly suitable to be an adsorbent material. Cellulose has a high specific surface area equal to 37 $m^2\ g^{-1}$. Its high molecular weight, toughness, fibrous nature, and insolubility in water and other common solvents make it favorable for adsorption of organic and inorganic pollutants from water bodies. The presence of abundant hydroxyl groups in cellulose can allow active interaction with the surface functional groups present on the dye molecules, thereby leading monolayer surface adsorption (Zaman et al. 2020). Moreover, these active hydroxyl groups also allow easy chemical modification and functionalization of the cellulosic materials by introduction of desired functional groups for enhanced

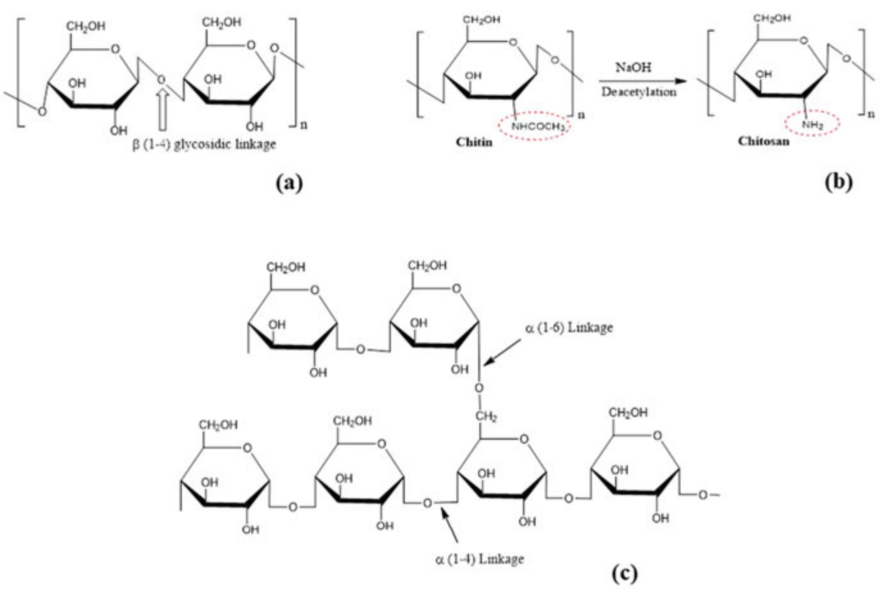

Fig. 31.1 Structure of different polysaccharides (**a**–**c** showing cellulose, chitosan from chitin and starch, respectively)

and selective adsorption of specific dyes and other organic pollutants (Yue et al. 2019). Schematic representation of the structure of cellulose has been shown in Fig. 31.1a.

31.3.2.2 Chitin and Chitosan

Chitin is the second most abundant bio-polymer after cellulose (Pérez-Calderón et al. 2020; Sarkar et al. 2017). It was first observed in mushrooms in 1811 and then also found in insects later in 1823 (Olivera et al. 2016). Today we know that chitin is a widely distributed material in nature; it is present as a major component in the exoskeleton of the crustaceans such as crab, shrimp, and lobsters and can also be derived from the cuticles of insects and cell walls of fungi and some bacteria (Muinde et al. 2020). The waste from seafood industry is the major commercial source of chitin. It is a nitrogenous polysaccharide and is tough and semi-transparent. The structure of chitin is similar to that of cellulose; the only difference is the N-acetyl and the hydroxyl functional group present at the second carbon position of chitin and cellulose, respectively. Chitin is used as a starting material for the production of chitosan, which is a de-acetylated product of chitin (Qamar et al. 2020). Chitosan is a hetero-polysaccharide comprising of β-(1–4)-D-glucosamine (deacetylated units) and N-acetyl-D-glucosamine (Pérez-Calderón et al. 2020). Like cellulose, chitosan exhibits some attractive properties such as hydrophilicity, insolubility in water and most other organic solvents, biodegradability, non-toxicity, biocompatibility, and even antibacterial properties. Its hydrophilic nature gives it the ability to form gels at acidic pH. Hence, it has wide application in agriculture, biomedical, and wastewater remediation. It also has substantial amount of primary and secondary hydroxyl groups, which can offer electrostatic interaction and therefore present excellent adsorption capacity for removal of various organic pollutants including a wide range dyes except for basic dyes. Chitosan acts a natural chelating polymer for many transitional metal ions. Chitosan consists of abundant free reactive amino groups. In acidic media, it behaves as a cationic polysaccharide due to protonation of free amino groups and hence serves as a site for coordinate interactions. Schematic representation of the structure of chitosan has been shown in Fig. 31.1b. This cationic nature and chelation capacity of chitosan is highly advantageous for applications in wastewater treatment for the removal of metal ions, organic compounds, pesticides, and synthetic dyes.

31.3.2.3 Starch

Starch is a natural carbohydrate polysaccharide occurring in green plants as energy store. Starch granules are made up of two kinds of D-glucose units, namely, amylase and amylopectin, the monomers being linked together through α-D-(1–4) and α-D-(1–6) linkages (Sarmah and Karak 2020). The proportions of amylase and amylopectin may vary from 10% to 20% and 80% to 90%, respectively, depending on the source (Mandal and Ray 2015). Amylose is a rather linear α-glucan with 1% α(1–6) and 99% α(1–4) linkages. Amylopectin on the other hand has an extremely branched structure having around 5% of α(1–6) and 95% α(1–4) linkages (Haroon et al. 2016). Starch naturally occurs in granular form due to the presence of short branched amylopectin chains, which can form a helical structure through crystallization (Mandal and Ray 2015). Small fractions of lipids and proteins are also found in starch. Schematic representation of the structure of starch has been shown in Fig. 31.1c. Just like the other polysaccharides, starch is also biodegradable, biocompatible, non-toxic, and very hydrophilic in nature. It is fairly insoluble in water at room temperature but becomes readily soluble at temperatures around 50–70 °C. Starch exhibits good swelling properties in aqueous media and can form stable hydrogels. Numerous starch-based hydrogels are reported for the removal of dyes and metals (Starch in its natural state has limited industrial applications; however, the presence of large number of reactive primary hydroxyl groups at the 2-, 3-, and 6-positions in the glucose unit makes it highly reactive chemical species. These reactive hydroxyl groups allow direct substitution reactions; thus starch can be easily oxidized or reduced (Mandal and Ray 2015). These groups can also actively take part in ether, ester, and hydrogen bond formation. The functionality of starch can be easily modified or enhanced by a several ways such as chemical modification, physical modification, even genetic modification, thereby yielding different derivatives for specific domains of application. The most vital application of the chemically modified starch is in the form of an adsorbent for the removal of synthetic textile dyes and heavy metal ions from effluents and contaminated water bodies. Abundant literature is present on the preparation and characterization of starch derivatives and starch-based biosorbents for removal of toxic dyes and metals.

31.4 Biopolymer Composites as Adsorbents

Recent studies have shown that biopolymers like cellulose, chitin, or starch can be combined with another one or more biopolymers or carbonaceous materials like graphene oxide, activated carbon, or fly ash to create a nanocomposite or even a tri-composite structure (Orasugh et al. 2018a, b, c, 2020). In a very recent study modified hummers' method was utilized for the direct in situ synthesis of cellulose–graphene oxide nanocomposite in a single pot reaction. There was absolutely no need of synthesizing GO and nanocellulose separately. The prepared nanocomposite was successfully applied for the adsorptive removal of methylene blue dye with an adsorption capacity as high as 751.88 mg g^{-1} (Zaman et al. 2020). Another study shows the development of a biocomposite adsorbent consisting of graphene oxide cross-linked with potato starch and applied for removal of methylene blue (Bhattacharyya et al. 2018). Development of such nanocomposites help to achieve desired properties of different biopolymers and other materials in a single unit system and provides high functionality, specific binding affinity, and hence efficient selective removal of specific type of dye.

31.5 Biopolymer Nanocomposites as Adsorbents

A composite can be defined as an assembly containing at least two or more nonmiscible materials forming a novel material with new properties that are quite different from those of individual materials. In recent years, biopolymer nanocomposites fascinated the mind of many researchers because their enhanced functionality due to the combination desired properties are potentially able to fulfill the requisite requirements. The composite matrix or host material can be a synthetic or biopolymer, while the guest component (filler or reinforcement) material may be particles, fibers, ribbons, flakes, sheets, platelets, or tubes. Biopolymer-based novel functional composite materials have been largely used for several industrial applications like energy, sporting goods, water treatment, automotive, biomedical, defense, and infrastructure. Owing to their combined properties such as biodegradability, high strength, high durability, simple processability, and high flexibility, biopolymer hybrid products greatly encourage extensive use in water purification systems (Olivera et al. 2016). Polymer matrix containing fillers are known as polymer matrix composites. Biocomposites are composite materials comprising one or more phase(s) derived from a biological origin (Orasugh et al. 2020). Qi et al. (2017) reported the preparation of composite materials using graphene oxide and three different natural polysaccharides—inulin, xylan, or k-carrageenan. GO was synthesized by modified Hummer's method and subjected to ultrasonic treatment followed by treatment with epichlorohydrin in N_2 atmosphere and then combined with each polysaccharide separately. The PS/GO composites were applied for removal of four dyes including both cationic and anionic organic dyes. Results indicated that PS/GO composites were more efficient than activated carbon-based substance prepared from GO. The epoxy activation of hydroxyl-containing GO was to a great extent responsible for the improved interaction of GO with polysaccharides (Qi et al. 2017).

Nano-biocomposites are referred to as those composites, which are made of nano-forms of biologically derived substances often combined with metal nanoparticles or other inorganic nanomaterials or even carbon-based nanoproducts. The term nano is used to define nanometer-scale items (10^{-9} m). A nanometer is, therefore, equivalent to the billionth of a meter, or 80,000 times thinner than a human hair. The nanometer range covers sizes bigger than several atoms but smaller than the wavelength range of white light. However, bio-nanocomposite materials have been extensively studied for only about the past 20 years.

The components of a bionanocomposite material can be constituted with at least one material of biological origin. Actually, they represent an emerging group of nanostructured hybrid materials. With growing concept of biocomposites and nanostructured hybrid materials, bionanocomposites (BNCMs) can be categorized into two groups. One consists of nanocomposite materials made from renewable nanoparticles like biopolymers obtained from plants, animals, and agricultural waste and by-products, such as nanocellulose or cellulose nanocrystals (CNC), nanofibrillated cellulose (NFC), bacterial cellulose (BC), polylactic acid (PLA), and polyhydroxyalkanoates (PHA). Other include nanocomposites made from petroleum-derived polymers like PP, PE, and epoxies. Nevertheless, nanocomposites derived from biopolymers (e.g., PLA and PHA) and synthetic or inorganic nanofillers (e.g., carbon nanotubes and nanoclay) also come under bionanocomposites. Researchers are keenly attempting to overcome the drawbacks of macro- or microsystem composites by developing high-performance multifunctional bionanocomposites (BNCMs) that are commercially feasible, biologically safe as in nontoxic, and biocompatible, state of the art and that satisfies all the attributes required for the dye adsorption applications for water treatment. Schematic representation of polymer-based nanocomposite preparation has been shown in Fig. 31.2.

Various organic and inorganic materials have been compounded with biopolymers, particularly with different polysaccharides for dye adsorption and water purification. Several studies have reported the successful incorporation of magnetic particles into nanocellulose matrix simply by application of external magnetic field (Olivera et al. 2016). Combination of metal nanoparticles with biopolymers for development of bionanocomposite adsorbents has proven to be very fruitful for hazardous dye adsorption. This new generation of emerging nanostructured organic/inorganic or organic/organic materials constitutes a new class of materials, which can be used extensively in industries.

31.6 Preparation Techniques of Biopolymer Nanocomposites

The most commonly used preparation methods of polymer composites especially for application in toxic dye removal include the following:

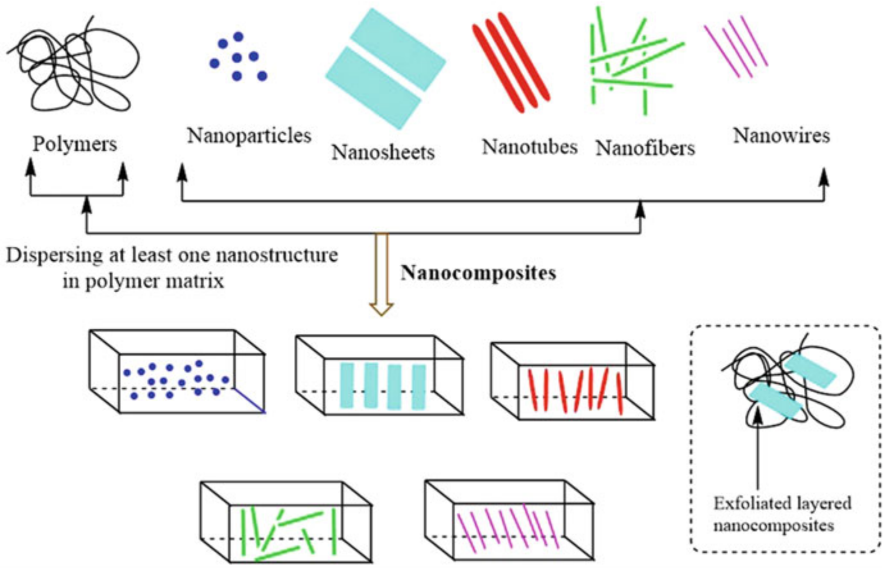

Fig. 31.2 Schematic representation of steps for nanocomposites preparation

31.6.1 In Situ Synthesis

In situ polymerization eventuates between mixtures of the filler and the biopolymers in the beginning, which is succeeded by the incorporation and then the reaction starts. From this approach, we can achieve a homogeneous dispersion of filler and host polymer matrix and their molecular or bonding interactions being strong. It is a decisive method for synthesizing thermally unstable and insoluble polymers that cannot be processed via melt or solution blending approach and is a simple technique that is well suitable for synthesis of graphene derivatives (such as GO, reduced GO (rGO), and graphene nanoplatelets (GNPs)) polymer composites, etc. Zaman et al. reported in situ synthesis of novel graphene oxide-cellulose nanocrystals nanocomposite in a single reaction vessel by modified Hummers' method as shown in Fig. 31.3 (Zaman et al. 2020).

31.6.1.1 Solution Blending or Solvent Casting

The solution blending approach includes the dispersion of conductive filler into the appropriate solvent via ultrasonication process. The filler–solvent mixture is mixed with the polymer–solvent mixture, and finally, this solvent is evaporated via thermal treatment or precipitation or distillation, to form filler-polymer composite films. This approach is especially suitable for synthesizing graphene-based polymer composites. The advantages of this approach include lower viscosities and homogeneous dispersion. The main drawback of this approach includes the need to use a large number of solvents and the difficulty involved in evaporating them. This

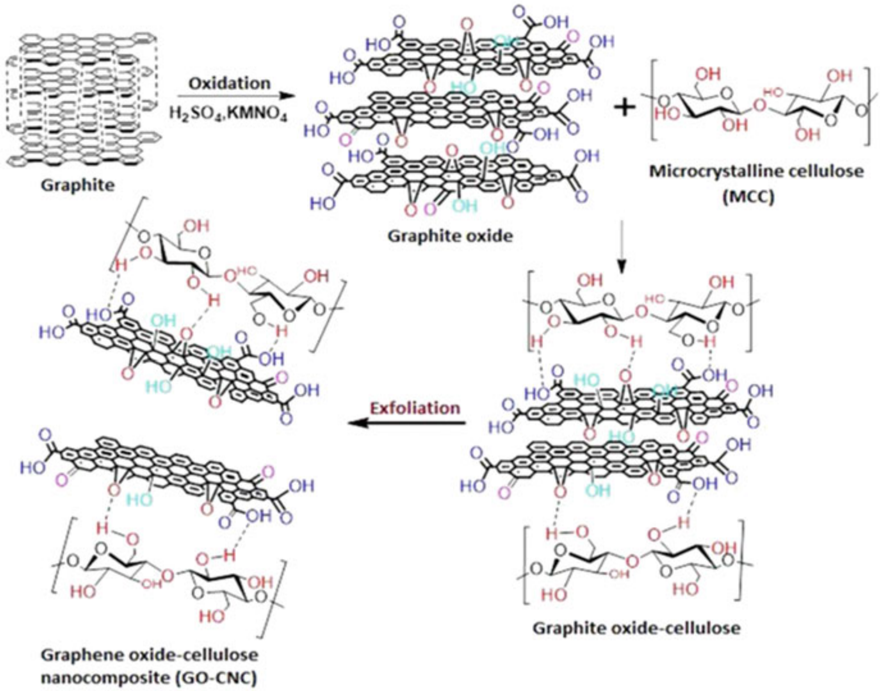

Fig. 31.3 Schematic representation of the plausible mechanism for in situ formation of graphene oxide–cellulose nanocomposite (GO–CNC). (Reproduced with permission from Zaman et al. 2020 © Elsevier)

method is the most commonly used, and three systems can be distinguished depending on the polymer used as the matrix, i.e., water-soluble polymers, polymer emulsions, and nonhydrosoluble polymers.

31.6.1.2 Extrusion with Freeze-Dried Nanoparticles

Two routes can be envisaged in order to obtain non-flocculated dispersions of cellulose nanocrystals in an appropriated organic medium. The first involves coating of the surface of the cellulose nanocrystals with surfactants having polar heads and long hydrophobic tails. The second is grafting of hydrophobic chains at the surface of cellulose nanocrystals. These procedures allow the preparation of polymer nanocomposites by mixing the nanoparticle suspensions in organic medium with a solution of polymer.

31.6.1.3 Melting Compounding Technique (Extrusion Method)

This is one of the most recent methods used to prepare such kind of nanocomposites, and not many studies have been carried out in this field yet. Therefore, it goes without saying that the possibility to scale-up the laboratory work to industrial scale starts to be more realistic. In this case, the main issue is to work with nanocellulose in the dry state. Indeed, as soon as these polysaccharides nanoparticles (NPs) are dried, strong bonding like hydrogen bonding (H bond) establish and most of the time aggregates are obtained limiting the nanosized reinforcement. It was demonstrated that nanocomposites prepared by casting and evaporating a mixture of cellulose whiskers and synthetic latex presented better mechanical properties than nanocomposites of the same mixture prepared by freeze drying and hot pressing. A very large reinforcing effect reported for cast and evaporated materials was recognized to the formation of a rigid whisker/whisker network, probably linked by hydrogen bonds. It was suggested that the formation of this bonding network is more predominant in the evaporated films due to lower processing times.

31.6.1.4 Melt Blending

Melt blending, when compared with the other techniques for preparation of BNCMs are largely used for industrial applications owing to their simplicity and low cost that facilities mass-scale production. This approach basically is accomplished by melting of polymers for obtaining viscous liquid and followed by using high shear force for dispersing the nanofillers. Thus, it does not need any solvent for dispersing filler and polymer matrix. It is an environmentally friendly and green method as it encompasses commercially available polymers and also the traditional blending instruments such as twin-screw extrusion. The simplicity and versatility of this method have commercially gained the attention for processing polymer composites when compared with the other preparation methods. The main disadvantage of this approach is the low dispersion ability of the graphene, making it a rarely used synthesis approach for graphene-based polymer composites (Deshmukh et al. 2016; Joshi and Datar 2015; Li and Zhai 2015; Mohanapriya et al. 2016).

31.6.1.5 Cross-Linking Method

Recent days, there is a nonstop sustained demand for the improvement of recent adsorbents experiencing high adsorption limit and fast adsorption rate for wastewater treatment (Pandey et al. 2020). Among various adsorbents, hydrogels and biopolymer-based composite hydrogel (BCH) gained noticeably attention recently. Polymeric hydrogels, specifically the ones involving naturally origin polysaccharides, undergo attracted special attention as effective adsorbent materials for dye removal due to their environmental-friendly nature. Cross-linked hydrogel is synthesized by free-radical and condensation method by UV radiation or thermal condition. Hydrogels have different functional groups. Hydrogels are made out of hydrophilic homopolymer or copolymer network and can swell within the presence of water. The hydrophilicity of the network is due to the presence of hydrophilic groups such $-NH_2$, $-COOH$, $-OH$, $-CONH_2$, $-CONH-$, and $-SO_3H$. Hydrogel bionanocomposite materials are of remarkable interest because of their promising applications, as an example, heavy metal adsorption, wound dressing, superabsorbent for dye removal, and drug-delivery systems (Pandey et al. 2020). Pandey et al. (2020) have reported the synthesis of natural locust bean gum-based LBG-cl-Poly (DMAA) hydrogel for efficient removal of water-soluble cationic dye-Brilliant green (BG). Another recent study has reported the synthesis of chitosan and acrylic acid

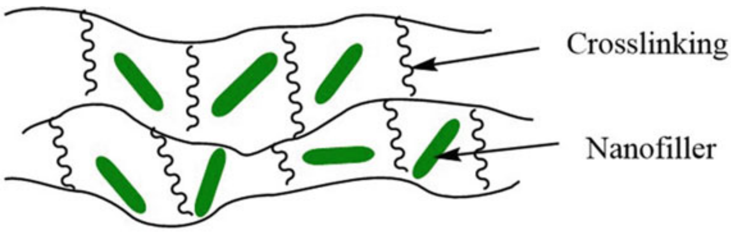

Fig. 31.4 Schematic representation of structure of cross-linked hydrogel nanocomposite

monomer-based BCH by cross-linking method for removal of synthetic dye rhodamine 6G (Rh6G) (Bhullar et al. 2018). Schematic representation of cross-linked hydrogel nanocomposites has been shown in Fig. 31.4.

31.7 Functionalization of Biopolymer Nanocomposites

Novel functional materials based on biopolymers are of huge interest for water treatment as the hybrid materials offer attractive properties such as flexibility, low density, toughness, active surface, and high adsorption capability. BNCMs are also biocompatible, biodegradable, and environmental-friendly and can be easily handle. The development of BNCMs/polymer adsorbent systems is highly encouraged nowadays because of their large removal efficiency and the spontaneous decontamination of water pollutants. Various methods are employed for the surface functionalization modification of the biopolymers. Cellulose, chitosan, and starch nanocomposites become attractive choice of materials due to large surface area, chemical accessibility, and ease of functionalization (Suman Kardam et al. 2015). Nanocelluloses contain large number of hydroxyl groups by virtue of their chemical structure. Extreme number of hydroxyl functionalities can exert adverse effects and limit their applications. However, these reactive -OH groups will impart functional flexibility to the host and hence facilitate the modification of NCs so as to improve their adsorption behavior in wastewater treatment. Functionalized NCs have shown excellent adsorption performance toward water contaminants. Schematic representation of the common techniques involved in polymer composite formation and their functionalization for waste removal applications has been shown in Fig. 31.5.

31.7.1 Application of Biopolymer Composites as Dye Adsorbents

Recent studies reporting biopolymer nanocomposites as hazardous dye adsorbents have been enlisted in Table 31.1.

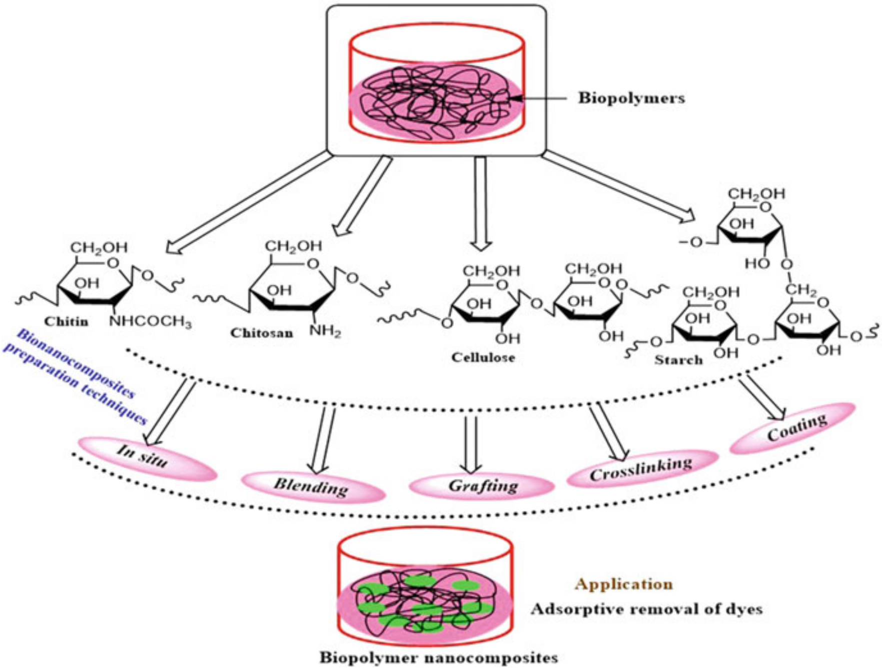

Fig. 31.5 Schematic representation of the common techniques involved in polymer composite formation and their functionalization for waste removal applications

31.8 Patents Related to Biopolymer Composite for Adsorption of Hazardous Dyes

The drive toward industrial acceptability and field application of biopolymer-based composites targeted at the adsorptive removal of hazardous dyes from wastewater calls for patenting of novel and well-established adsorbents. Few researchers have patented their research work based on novel biopolymeric-based composites along with the findings for the remediation of wastewater contaminants, especially hazardous dyes (Alshehri et al. 2016; Al-Ahmed et al. 2018; Haider et al. 2016).

31.9 Challenges and Future Perspectives

At present, the major challenge hindering the application of diverse biopolymers and their nanocomposites for adsorption on an industrial scale, is the complete separation (100%) of the adsorbent particles from the treated wastewater. Technical hitches with regard to scale-up and amalgamation into a relevant technology, cost-efficacy (not all biopolymers are cheap), and energy correlated issues have also challenged and delayed marketing of some biopolymeric nanocomposite adsorbent-based

Table 31.1 Literature reports on studies done in the field of biopolymer-based composite formation for the removal of dyes from water

Cellulose-based nanocomposites and modified cellulose for dye removal: Quite a few publications disclose the synthesis of cellulose-based composites and nanocomposites with different magnetic and nonmagnetic materials such as graphene oxide, metal nanoparticles and other polymers and biopolymers. Some scholars even attempt to derive cellulose from waste materials of different industries. A few studies in the recent literature where such composites have been synthesized using advanced technologies and eventually applied for the successful adsorption of toxic dyes have been presented below

Biopolymer composite/ nanocomposite	Nature of the work and application	Reference
Cellulose–attapulgite nanocomposite hydrogel	This study has used a very interesting material called attapulgite. Attapulgite is a kind of silicate and has permanent negatively charged sorption sites (Si-O^-) and nano-rod like morphology. Cellulose was cross-linked with sodium alginate in the presence of attapulgite to formulate attapulgite incorporated cellulose nanocomposite hydrogel. It showed high adsorption capacity for selective removal of methylene blue dye from a mixture of methylene blue and methyl orange	Chen et al. (2019)
Chemically modified cellulose-NH_2-HBP and β-CD grafted activated cotton fiber	Amino-terminated hyperbranched polymer (NH_2-HBP) has abundant functional groups, and β-cyclodextrin is a torus-shaped cyclic oligosaccharide with a remarkable capacity to form inclusion complexes. Cellulose in activated cotton fiber was grafted with these polymers to achieve highly functionalized adsorbent. The resultant adsorbent showed high adsorption capacities for both cationic methylene blue dye and anionic Congo red dye	Yue et al. (2019)
Cellulose–zinc oxide nanocomposite	In this recent study, cellulose nanocrystals (CNC) derived from sawdust was incorporated with ZnO ultrasonicated solution. CNC was added dropwise and then stirred for 24 h. The resulting CNC/ZnO nanocomposite showed both spherical and rod-like morphologies and enhanced crystallinity due to the presence of ZnO nanoparticles. It was used to removal of methylene blue dye with maximum adsorption capacity of 64.93 mg g^{-1}	Oyewo et al. (2020)
In situ cellulose–graphene oxide nanocomposite	In a very recent study modified Hummers' method was for the first time directly utilized for the in situ synthesis of cellulose–graphene oxide nanocomposite in a single pot reaction. There was	Zaman et al. (2020)

(continued)

Table 31.1 (continued)

Cellulose-based nanocomposites and modified cellulose for dye removal: Quite a few publications disclose the synthesis of cellulose-based composites and nanocomposites with different magnetic and nonmagnetic materials such as graphene oxide, metal nanoparticles and other polymers and biopolymers. Some scholars even attempt to derive cellulose from waste materials of different industries. A few studies in the recent literature where such composites have been synthesized using advanced technologies and eventually applied for the successful adsorption of toxic dyes have been presented below

Biopolymer composite/ nanocomposite	Nature of the work and application	Reference
	absolutely no need of synthesizing GO and nanocellulose separately. Moreover, the cellulose used in the study was derived from jute waste. The prepared nanocomposite was successfully applied for the adsorptive removal of methylene blue dye with adsorption capacity as high as 751.88 mg g^{-1}	

Chitin–/chitosan-based composites for dye removal: In the past and recent times, plenty of work has been done to prove the admirable efficiency of chitin and its derivate chitosan as a low-cost alternative adsorbent for various dyes, metal ions, and other inorganic and organic pollutants. This low cost, easily available biopolymer has been combined with various organic and inorganic species to develop suitable composite biosorbents. Some examples of such studies have been showcased here

Biopolymer composite/ nanocomposite	Nature of the work and application	Reference
TiO_2/chitosan/rGO composite	A very interesting combination of three different materials—TiO_2, reduced graphene oxide, and chitosan was used to prepare highly aligned macroporous cross-linked composite by freeze cast method. Desired lamellar pore size was achieved by altering the weight ratio of TiO_2. Hence, obtained TiO_2/chitosan/rGO composites were effectively applied for photocatalytic degradation of methyl orange. Subsequent irradiation studies confirmed the substantial oxidative degradation of MO exhibited particularly by the scaffold sections of the trio composite	Chen et al. (2017)
Poly(vinyl alcohol)/ chitin nanofiber/Fe(III) complex	A tri-composite was synthesized by incorporating chitin nanofibers on Fe(III) cross-linked polyvinyl alcohol (PVA). The composite was utilized for adsorptive removal of methyl orange dye. Results indicated that the adsorption efficiency of PVA/Fe(III) was enhanced due to chitin nanofibers loading, adsorption capacity reaching as high as 810.4 mg/g	Ghourbanpour et al. (2019)
Cross-linked chitosan–oxalic acid hydrogel beads	Chitosan hydrogel beads were synthesized by cross-linking with oxalic acid in bulk. FTIR-ATR results showed electrostatic	Pérez-Calderón et al. (2020)

(continued)

Table 31.1 (continued)

Chitin−/chitosan-based composites for dye removal: In the past and recent times, plenty of work has been done to prove the admirable efficiency of chitin and its derivate chitosan as a low-cost alternative adsorbent for various dyes, metal ions, and other inorganic and organic pollutants. This low cost, easily available biopolymer has been combined with various organic and inorganic species to develop suitable composite biosorbents. Some examples of such studies have been showcased here		
Biopolymer composite/ nanocomposite	Nature of the work and application	Reference
	interactions between oxalate ions and the protonated amino groups of chitosan. Oxalic acid, which is a natural cross-linking agent, reduced swelling potential and increased the chemical stability of cross-linked chitosan. The synthesized biomaterial was effectively used for the adsorption of reactive red 195-azo dye, achieving a maximum adsorption capacity of 110.7 mg g^{-1}	
Starch-based composites and their derivatives for dye removal: Starch in its natural state is not readily usable; however, abundant literature is available that shows the use of starch derivatives that are produced by chemical or physical modification, for various industrial applications. Development of suitable adsorbents based on starch derivatives for water remediation application is a field that has been extensively explored. Some studies, which employ advanced modification techniques along with nanotechnology, are discussed below		
Biopolymer composite/ nanocomposite	Nature of the work and application	Reference
Starch incorporated acrylic gels	Several starches incorporated acrylic gels were prepared by polymerizing acrylic acid (AA), sodium acrylate, and AA/hydroxyethyl methacrylate in an aqueous solution of starch. The result was employed for adsorption of safranine T and brilliant cresyl blue dyes from water. Among the different kind of gels prepared, starch incorporated sodium polyacrylate gel showed the highest adsorption swelling and adsorption properties	Mandal and Ray (2015)
Starch/poly (alginic acid-*cl*-acrylamide) nanohydrogel	Alginic acid cross-linked with acrylamide was grafted onto starch gel by the co-graft polymerization method. The prepared nanohydrogel had a smooth surface with particle size ranging from 30 to 80 nm. It was effectively used for the removal of Comassie brilliant blue R-250 dye through spontaneous adsorption	Sharma et al. (2017)
Starch–polyacrylic acid-based amphoteric hydrogel	Starch and polyacrylic acid were cross-linked with triethylamine to form a double-network hydrophobic-hydrogel. Epichlorohydrin (ECH) and N,N-methylenebisacrylamide were used as cross-linkers in the one-pot polymerization technique. The synthesized amphoteric hydrogel possessed both positive and negative charges due to interaction between the triethylamine epoxy	Sarmah and Karak (2020)

(continued)

Table 31.1 (continued)

Biopolymer composite/ nanocomposite	Nature of the work and application	Reference
Starch-based composites and their derivatives for dye removal: Starch in its natural state is not readily usable; however, abundant literature is available that shows the use of starch derivatives that are produced by chemical or physical modification, for various industrial applications. Development of suitable adsorbents based on starch derivatives for water remediation application is a field that has been extensively explored. Some studies, which employ advanced modification techniques along with nanotechnology, are discussed below		
	ring of ECH rendering positive charge on the hydrogel and presence of anionic carboxylate ion of polyacrylic acid. Hence, it was able to remove both cationic and anionic dyes	
Other polysaccharides: Apart from chitin/chitosan, starch, and cellulosic materials, some other polysaccharides have also been successfully applied for water purification systems in the composite adsorbents		
GO–inulin, GO–xylan, and GO–k-carrageenan composites	Qi et al. reported the preparation of composite materials using graphene oxide and three different natural polysaccharides—Inulin, xylan, or k-carrageenan. GO was synthesized by modified Hummer's method and subjected to ultrasonic treatment followed by treatment with epichlorohydrin in N_2 atmosphere and then combined with each polysaccharide separately. The PS/GO composites were applied for removal of four dyes including both cationic and anionic organic dyes. Results indicated that PS/GO composites were more efficient than activated carbon-based substance prepared from GO. The epoxy activation of hydroxyl-containing GO was to a great extent responsible for the improved interaction of the latter with the polysaccharides	Qi et al. (2017)
Graphene oxide–xanthan gum composite	Liu et al. developed a quick and easy economical method for large scale production of high-performance graphene oxide–xanthan gum composites for water purification. Xanthan gum was combined with GO at different weight ratios and freeze dried for 8–12 h to obtain XG/GO aerogels. Further study revealed high values of adsorption capacity for methylene blue, rhodamine B, and Cu II ions. The author suggested that the highly porous structure of the prepared hybrid aerogels provided stability and enhanced the diffusion of adsorbates, thereby facilitating the adsorption process	Liu et al. (2017)

products. The drawn-out improvement of worldwide water remediation is firmly associated with the development of the total populace and worldwide environmental change. The interest in new water remediation tools/techniques is significantly on the rise. Lately, the utilization of nanomaterial-based biopolymeric composites in water remediation has expanded impressively. The nanomaterials have novel size-subordinate properties and permit the improvement of novel cutting-edge materials for productive water, what's more, wastewater treatment measures, specifically films, adsorption materials, nanocatalysts, functionalized surfaces, coatings, and reagents. On account of agglomeration and precariousness, nanocomposites especially biopolymeric nanocomposites are presently being favored over nanomaterials. The potential of nanocomposites in different areas of examination and application is promising and pulling in expanding speculation from governments and businesses in numerous pieces of the world. While there are some specialty applications where nanotechnology has entered the market, the significant effect will be at any rate 10 years away. There are sure attractive biopolymer nanocomposites that have extraordinary highlights. Biodegradable polymer-based nanocomposites have a lot of future guarantees for likely applications as elite biodegradable materials. These are new kinds of materials dependent on plant and natural materials. Despite of a lot of points of interest in biopolymer nanocomposites in water remediation, there are as yet a few downsides that must be arranged. Materials functionalized with nanoparticles fused or kept on their surface have hazard potential, since nanoparticles may bring and discharge to the climate where they can amass for significant stretches of time. Accessible data in the writing has uncovered that few nanomaterials that may adversely affect the climate and human well-being. By the by, norms for evaluating the harmfulness of nanomaterials are generally lacking at present, though many researchers are currently looking into this aspect. Subsequently, far-reaching assessment of the harmfulness of nanomaterials utilized in the fabrication of novel composites based on biopolymeric materials for adsorption of hazardous dyes is in pressing need to guarantee their genuine applications, to make polymer nanocomposites (Khan and Lo 2016). These call for further research into the design and development of new and cost-effective biopolymeric hybrid nontoxic composite systems for the remediation of wastewater bodies.

31.10 Conclusion

The rise in demand paralleled with scarcity for clean water arising from many industrial applications calls for novel and sustainable technologies for the elimination of contaminants like hazardous dyes or other harmful materials from wastewater. The insufficient supply of fresh clean water is the utmost persistent problematic thing affecting people globally. All-embracing research has consequently been devoted to resolve this global water pollution problem. Even though numerous wastewater treatment approaches have been established, adsorption has been extensively accepted as a facile and most multipurpose technique available for the remediation of organic and inorganic pollutants from the wastewater. This chapter has thus presented an up to date record of the recent knowledge as per available literature on the application of biopolymer composites and nanocomposites for the adsorptive removal of hazardous dye contaminants present in wastewater with a focus on selected polysaccharides. These bio-nanocomposites, being environment-friendly materials, have great worth in the removal of numerous hazardous dyes from the wastewater. Adsorption targeted and engineered polysaccharide-based biopolymeric nanocomposites that demonstrate outstanding properties such as non-toxicity, sustainability, biodegradability, biocompatibility, and reusability, making them the most suitable and ideal green composites for wastewater remediation and numerous applications. The form of nanomaterials within its host biopolymeric matrix and changes in the architecture and properties of the nanomaterials and host matrices could be among the significant concerns in the field of biopolymer composites in wastewater remediation. In order to avoid the hazards of the recovered pollutants, researchers have fabricated biopolymer-based composite adsorbents that are not just eco-friendly and cost-effective but also reusable. The adsorbent and pollutant can be separated and recovered later (Yousefi et al. 2018). However, there is a need for collaborative efforts between researchers and industrialists for developing simple, cost-effective techniques for the synthesis of biopolymer-based composites and analysis of their efficiency along with subsequent implementation for improved waste management on large-scale in real life.

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32 Arbuscular Mycorrhizal Fungi-Assisted Bioremediation of Heavy Metals: A Revaluation

Sakshi Patel, Ameeta Sharma, and Neha Gheek Batra

Abstract

Generation of wastes by anthropogenic activities of human has now become a major threat in developing countries. In the last two decades, discharge of heavy metal contaminants has increased at an expeditious rate leading to deterioration of both organisms and environment. Due to the mobile nature of these heavy metals (HMs), they have now become an extended part of the food chain and affect human health. Due to an urgent need to remediate these toxic metals from the soil, bioremediation is used as a technique, and it has proved too beneficial to remediate the heavy metal contaminants. The use of arbuscular mycorrhizal fungi (AMF) as an approach for bioremediation of heavy metals is now widely used. Arbuscular mycorrhizal fungi are commonly known as biofertilizers and are considered as the keystone mutualists in terrestrial ecosystem because they form a link between biotic and abiotic components in soil. These remain in symbiotic association with the host plant, helping it in nutrient uptake, toxicity removal, increasing growth, and development, and in turn, these derive the photosynthetic products produced by plants. AMF can also be used in conjugation with soil organisms like earthworms and other fungi to increase the efficiency of bioremediation, thereby leading to an eco-friendly and sustainable environmental condition. The present chapter focuses on the bioremediation strategies for metal pollution used by AMF in symbiosis with the host plant and its influence on host plant growth and development.

Keywords

AMF (arbuscular mycorrhizal fungi) · Bioremediation · Heavy metals

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32.1 Introduction

Soil pollution has nowadays become a major global threat across the world (Alves et al. 2020). The increasing rate of industrialization and urbanization along with the improper waste disposal is nowadays the main source of heavy metal concentration in the surrounding environment (Devi and Kumar 2020). Several human activities have discharged more and more wastes including gases, wastewater, and residues among which heavy metals are the major ones having a greater impact on the soil profile affecting its physical, chemical, and biological properties (Gong and Tian 2019). In the last two decades of the twentieth century, the worldwide discharge of metal contaminants has reached a high peak because of human activities like mining, agriculture, and domestic wastes. Mining can transform fertile, cultivated land into waste land as mine wastes deposit at the surface and occupy a huge area of land, which causes lot of toxicity in the environment. To overcome all these problems caused by heavy metals, scientists have used soil remediation as an essential practice to create a healthy environment and also to increase the food demand of the growing population of the world. Various soil remediation methods have been used since then, such as the physical excavation and transport of polluted soil to landfills for disposal, use of solvent extraction techniques, electrokinetic separation, chemical oxidation, soil solidification, and bioremediation. All these methods have their own advantages and disadvantages, depending on the type of pollutant to be remediated (Chibuike 2013). A more common method of soil remediation is the use of chemicals like ozone, $KMnO_4$, H_2O_2, and Fenton's reagent. However, this method is expensive to use, and it also hampers with the soil's ability to support plant growth. Apart from these physical and chemical methods, biological methods, which include the use of plants and microorganisms, have proved to be innovative and beneficial as it is cost-effective, easy to implement, and maintains the natural properties of the soil.

Bioremediation technology has emerged as a potent way to tackle the pollutants of the soil. The types of bioremediation are microbial bioremediation, phytoremediation, and mycoremediation. In the natural ecosystem, plants interact with the large number of microorganisms in the rhizosphere and develop a beneficial mutual relationship with each other. Arbuscular mycorrhizal fungi (AMF) maintain a beneficial symbiotic relationship with the plant roots where it helps the plant by enhancing its nutrient uptake, protecting it from heavy metal toxicity, increasing the biomass accumulation, and improving photosynthesis, and in turn, the photosynthetic products produced by plants are received by fungus (Dhalaria et al. 2020). AMF is commonly divided into ectomycorrhiza in which the hypha of fungi does not penetrate individual cells within the root and endomycorrhiza in which the hypha of fungi penetrates the cell wall and invaginate the cell membrane. Mycorrhiza cannot exists without a plant support; therefore, using them can be described as the modified form of phytoremediation, which exploits the beneficial effects of mycorrhiza for remediation of plant and the soil (Chibuike 2013). The association of AMF with plants is known to be as old as the evolution of angiosperms millions of years back (Arya et al. 2018). It has become the core of research to remediate soil and guarantee clean agriculture using ternary remediation system composed of plants, AMF, and microorganisms (Gong and Tian 2019).

32.2 Pollution by Heavy Metals

A heavy metal is any element that has high density and is toxic to organisms even in low concentration (Morkunas et al. 2018). Heavy metals are nonbiodegradable, persistent inorganic chemicals, which have high atomic mass and possess cytotoxic, genotoxic, and mutagenic effects on humans, animals, and plants. These can only be transformed from one state to another (Khan 2020). In soil, they occurs as free metal ions; exchangeable metal ions; soluble metal complexes, which are sequestered to ligands; precipitated or insoluble compounds such as oxides, carbonate, and hydroxides; and organically bound metals, or they may form part of the silicate structure. Some heavy metals such as Cu, Mn, Fe, Co, and Zn are required for the normal growth, development, and reproduction of plants. These are considered as essential elements when these are present in low concentration, but when their concentration increases, these lead to toxic effects because of the formation of reactive oxygen species, which is known to be harmful (Ferrol et al. 2016). Various human activities like mining, discharge of industrial effluents, sewage sludge, polluted water, and fertilizers, are all responsible for addition of heavy metals in soil, which are both phytotoxic and nonessential (Devi and Kumar 2020). Some of the examples of these heavy metals are lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), copper (Cu), nickel (Ni), zinc (Zn), and chromium (Cr), which cause environmental contamination and health risks (Clemens 2006). These are collectively known as "problematic heavy metals" or "toxic heavy metals" (Rahman and Singh 2020). The heavy metals have become contaminants in the soil environment because (1) these are generated at a rapid rate by humans relative to the natural ones, (2) they have been transferred from mines directly to the location or environment where the peril of acquaintance is high, (3) the concentration of metals in discarded product is rather high compared to those in the receiving environment, and (4) the chemical form in which the metal is found in the environment render it more bioavailable. Heavy metal in soil can enter food chain through various ways and produce heavy metal contaminated foods like poisonous vegetables and cadmium rice, which is dangerous for human health (Gong and Tian 2019). The absorption of heavy metals does not remain uniform along the root of plants; it differs from the root apices to the other parts of root (Dhalaria et al. 2020).

The origin and impact of different HMs on plant growth and environment vary from place to place like:

1. Arsenic, which is a naturally occurring metal pose threat to human health across the globe. It is usually originated from volcanic eruptions, mining, erosion of rocks, smelting, and application of pesticides. The arsenic seeps into groundwater and enters into drinking and irrigation water, which leads to its accumulation in crops like carrot, lettuce, rice, and spinach. The toxic form of arsenic present in

the environment are inorganic arsenate As (V) and arsenite As(III); these are regarded as the major environmental pollutants. Both of them are responsible for generation of reactive oxygen species and interfering with the energy metabolism process.

2. Cadmium, which is considered as one of the most phytotoxic heavy metal because of its solubility in water due to which it is easily taken up by the plant. It is reported that it is released from various industrial processes and farming practices into the arable soil. It is classified as potent human carcinogen by The International Agency for Research on Cancer. In plants, it alters the enzymatic activity of Calvin cycle, carbohydrate and phosphorus metabolism, and CO_2 fixation, which results in stunted growth, inhibition of photosynthesis, chlorosis, induction of lipid peroxidation, disruption of antioxidant mechanism, modification in chloroplast structure, pollen germination inhibition, and change in nitrogen and sulfur metabolism.
3. Lead, which is the most widely and evenly distributed trace element in the environment. It is released from industrial sites, leaded fuels, dust, orchard sites, and old lead plumbing pipes. Its long-term exposure can be harmful to both plants and animals. In plants, it impairs with the biological process such as chlorophyll biosynthesis, root elongation, seed germination, cell division, and transpiration. It also affects the cell membrane permeability in plants.
4. Mercury, which is a natural component of earth's crust, gets accumulated in land and water ecosystem because of mining and various industrial processes. In environment, it exists in elemental (Hg^0) and inorganic (Hg^{2+}) form. It is highly phytotoxic to plant cells and causes visible injuries and physiological disorders, and it also interferes with the mitochondrial activity in plants (Tiwari and Lata 2018).

In plants, there are various pathways through which the heavy metals enter the roots of plants; these include (1) H^+ ATPase/pumps, which maintain the negative potential across the epidermal membrane of the roots, (2)ion channels through which the HMs present in soil gain entry into the plant epidermis, or (3) as organic compounds which is the result of chelation and enters the root epidermal layer as metal–ligand complexes (Dhalaria et al. 2020). The levels of heavy metal contamination may vary from organism to organism. In plants, these obstruct the normal functioning of the plant and metabolic processes in a variety of ways, like the formation of bonds between the HM and sulfhydryl group that displaces the building blocks of protein structure, stimulation of free radicals and reactive oxygen species, results in oxidative stress, inactivation and denaturation of enzymes, proteins that prevent germination and development of seedlings, substitution of essential metal ions from functional cellular units and biomolecules, blocking of functional groups, and modification and disruption of membrane integrity, which leads to inhibition of metabolic processes like photosynthesis and respiration (Hossain et al. 2012). These also lead to aging of the root tip cells of plants, making their appearance wilted, withered, and yellow (Gong and Tian 2019). The HMs get transferred to the human body through interactions between the soil and plant, contaminated drinking water and through inhalation from dust. It is reported that workers in factories where heavy metal work is done, like chromium, develop nasal irritation, nasal ulceration, perforated eardrum, and irritated skin due to its continuous exposure. Metal toxicity in plant leads to the production of free radicals like reactive oxygen species that are formed during the cellular metabolic processes, and overproduction of these species lead to oxidative stress. Free radicals react with cell organelles, membranes, and biomolecules like lipids, proteins, and nucleic acids of the cell reducing its normal functioning and causing abnormality. On the basis of oxidation states, heavy metals can be highly reactive and lead to toxicity of plant cells in many ways (Hossain et al. 2012).

Different HMs have different impacts on human health like:

1. Lead, from automobile plant, can cause high blood pressure and less heme biosynthesis; from vegetables, it can cause neurological and immunological effects (Wilberforce and Nwabue 2013).
2. Chromium, from groundwater, can cause gastrointestinal and dermatological complaints and abnormal hematological functions; from vegetables, it can cause respiratory problems, lung cancer, and skin rashes (Wilberforce and Nwabue 2013).
3. Arsenic, from drinking water, can cause increase in stillbirths and skin lesions on woman; from soil and vegetables, it can cause liver damage, gastrointestinal effects, lung cancer, and skin lesions (Wilberforce and Nwabue 2013).
4. Copper, from drinking water, can cause nausea, abdominal pain, or vomiting (Sarvestani and Aghasi 2019); from vegetables, it can cause kidney damage or tumors (Islam et al. 2015).
5. Cadmium, from soil, can cause high prevalence of renal dysfunction, bone mineral loss, hypertension, and urinary stones; from smelting, it can cause renal dysfunction.
6. Mercury, from mines, can cause finger and eyelid tremor, gingivitis, and typical dark line on gums (Li et al. 2008).

32.3 Response of Plants to Heavy Metal Stress

The exposure of plants to toxic heavy metals leads to triggering of many physiological and metabolic alterations. Different HMs have different site of action in plants, and its effect depends on its concentration. The most widespread visual evidence of HM toxicity is the reduction in plant growth because of necrosis, turgor loss, leaf chlorosis, decrease in rate of seed germination, and ultimately leading to plant death. The generation of reactive oxygen species such as superoxide anion radical ($*O_2^-$), H_2O_2, and hydroxyl radical (*OH) due to heavy metal toxicity leads to attack on all types of biomolecules. The *OH molecule is one of the most reactive species known because of its ability to initiate radical chain reactions responsible of irreversible chemical modifications of various cellular components. The second one is the involvement of $*O_2^-$ in lipid peroxidation. The presence of HMs leads to molecular, biochemical, and ultrastructural changes in the tissues and cells of plants. Moreover, it also influences the homeostatic events such as nutrient metabolism, transpiration, water uptake, transport, and interferes with the uptake of various important nutrients. Promutagenic damage such as inter- and intramolecular cross-linking of DNA and proteins, DNA base modification, rearrangement and depurination, and DNA strand breaks are also caused by binding of some heavy metals to the nucleus of the cell. HMs also alters the cell cycle and cell division by affecting the microtubule assembly–disassembly. It is also observed that under stressful conditions the level of ethylene increases in plants, which lead to various other responses and senescence (Hossain et al. 2012).

32.4 Heavy Metal Signaling and Tolerance in Plants

There are various genes that are induced in heavy metal stress such as transcription factors bHLH, bZIP, AP2/ERF, and DREB in *Arabidopsis, Brassica,* and *Lycopersicum.* It is reported that the use of various proteomics techniques such as MALDI-TOF, 2-D electrophoresis, and LC-MS has led to the discovery of target proteins, which take part in detoxification of heavy metal in several plants like *Zea mays, Oryza sativa, Populus,* and *Arabidopsis* species. It is also reported that various metabolites such as amines, amino acids, phenol, α-tocopherol, and glutathione are also involved during heavy metal tolerance response. Several phosphatases and kinases generate stress signals, which lead to gene expression of several TFs and synthesis of metal detoxifying peptides. Activation of various pathways also takes place during HM stress like ROS signaling, calcium-dependent signaling, mitogen-activated protein kinase signaling, and hormone signaling, which enhance the expression of TFs and stress responsive genes. There is also the presence of various Ca^{2+} sensors in plants such as calmodulins (CaMs), CaM-like proteins, calcineurin B-like proteins (CBLs), and Ca^{2+}-dependent protein kinases (CDPKs), which sense, decode, and convey the alterations in cytosolic Ca^{2+} concentration for the stress response (Tiwari and Lata 2018). It is also reported that cellular exclusion of HMs is an important adaptive strategy for HM tolerance in plants. The HM transporter proteins play an important role in exclusion of toxic HMs from symplastic to apoplastic space found in the roots of plants. The formation of peptide metal-binding ligands such as phytochelatins (PC) and metallothioneins (MTs), which acts as chelators for heavy metals, is also produced by plants. Apart from these some organic acids, amino acids and phosphate derivatives also play an important role in chelation of heavy metals. These include histidine, citrate, nicotianamine, oxalate, malate, and phytate that are all responsible for tolerance and detoxification of HMs. Other proteins responsible for protection and repair of plants during HM stress are the heat shock proteins (HSPs). These acts as molecular chaperones and their accumulation occur during stress conditions (Hossain et al. 2012).

32.5 Role of Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi is one of the most efficient biofertilizer. It provides a direct physical link between the soil and the plant roots and is known as the rhizospheric microorganism (Yu et al. 2005). It is widely believed that AMF can be used to provide tolerance to the host plants against stressful conditions like heat, drought, salinity, extreme temperatures, and heavy metals. The subphylum *Glomeromycotina* of the phylum *Mucoromycota* has majority of the AMF species (Spatafora et al. 2016). AMF plays an important role in soil phytoremediation and enhances the biomass concentration and mineral uptake. AMF is known to alter the water relationships of plants by improving their resistance in drought conditions. AMF in metal contaminated soil reduces the shoot uptake of metals and protects the plant against harmful effects of metal concentration (Yu et al. 2005).

AMF in the host plant regulates the tolerance mechanism and prevents the downregulation of key metabolic pathways. Approximately 90% of the plant species including the flowering plants, bryophytes, and ferns can develop interdependent relationship with the AMF. These are believed to improve the characteristics of soil and encourage plant development. AMF forms vesicles, arbuscules, hyphae in roots, and spores in the rhizosphere. It is believed that fungi store metals in their spores, which is only possible in a single colony culture (Gong and Tian 2019). The development of arbuscules takes place between the cell wall and the plasma membrane of root cortical cells, and it gets differentiated from plant plasma membrane by the periarbuscular membrane. They are obligate biotrophs that ingest photosynthetic products of plant and lipids to carry out their life cycle (Jiang et al. 2017). In host plants, AMF improves water and mineral nutrient uptake from the adjoining soil and also safeguards the plants from fungal pathogens (Smith and Read 2008; Jung et al. 2012).

AMF are known to be of key importance for sustainable development of crop and agriculture ecosystem. There exists a symbiotic association between the AMF and the host plant (Kumar and Dubey 2020). The bidirectional exchange of resources between AMF and host plant is a hallmark and also a functional necessity in mycorrhizal symbiosis (Riaz et al. 2020).The main morphological structure of AM fungi is arbuscules, vesicles, auxiliary cells, hyphae, and spores, which are responsible for performing various functions. Extraradical hyphae as a root extension provide large surface area for the absorption of water and nutrients from soil solution. The nutrients and water absorbed are transported to the intraradical hyphae and plants in exchange with photosynthetic fixed carbon (Parihar et al. 2020). The use of chemical fertilizers can be lowered down by approximately 50% by the incorporation of AMF on the fields for best agriculture production (Begum et al. 2019). AMF are also known to degrade polycyclic aromatic hydrocarbons (PAH) through enhanced production of extracellular peroxidases. AMF are known to increase the effectiveness of absorbing capability of surface host roots up to ten times. The hyphae of mycorrhiza are also known to increase the effectiveness of immobile elements up to 60 times. It is also believed that growth of the plant can be enhanced by incorporation of AMF in the rhizospheric soil, which causes the dilution of metal ions in plant tissues (Begum et al. 2019).

AMF are considered to be the keystone mutualists in terrestrial ecosystem because they form a link between biotic and abiotic components, which are transferred between host plant and fungi in the soil. AMF are a type of endomycorrhizal association, characterized by the formation of intracellular structures such as arbuscules. AMF have ability to alter the water relationship of plants, thereby improving plant resistance to drought conditions. Furthermore, several studies have also reported an increase in plant water use efficiency, which was induced by mycorrhiza. AMF also contribute to carbon storage in soil by altering the quality and quantity of organic matter present in soil. The tolerance of plants to various stressful conditions is improved by AMF colonization, which brings about several changes in plants morphological and physiological traits. It has also been reported that the symbiotic relation between host plant and AMF have beneficial effects on many physiological parameters under saline conditions like photosynthetic rate, stomatal conductance and leaf water potential (Ait-El-Mokhtar et al. 2019), gas exchange rate, chlorophyll content (Elhindi et al. 2017), leaf area index, fresh and dry biomass (Borde et al. 2010), and nitrogen concentration of shoots and roots. It is reported that inoculation of AMF under saline stress results in alleviation of salt stress and increase in chlorophyll content, photosynthetic rate, transpiration rate, water use efficiency, gas exchange, stomatal conductance, and relative water content (Chandrasekaran et al. 2019).

AMF also act as suitable buffer to reduce the heavy metal stress on plants by influencing the chemical properties, absorption, and movement of heavy metals on plant growth (Okon et al. 2018). It promotes plant extraction, helping the plants in absorbing more metal elements for its growth when the concentration of metal is low in the soil, but when the metal concentration in soil increases, it facilitates the fixation of these metal elements by plants accumulating the most of the toxic metal elements in plant root thereby avoiding their transportation to the aboveground parts of the plant (Gong and Tian 2019). It also colonizes a number of tropical plants including vegetables and some crops like carrot and tomatoes and soils of cultivated cereal crops and medicinal plants.

AMF are believed to strengthen the defense mechanism in host plants to support its growth under heavy metal stress condition (Begum et al. 2019). These are also believed to regulate the uptake and accumulation of some inorganic elements like uptake of Si by spores and hyphae and restoration of Cd in mycelium. It supports plant growth by combating both heat and cold stress. It is reported that AMF used in combination with compost has the potential to reduce the heavy stress; increase biomass production, stomatal conductance, and total chlorophyll content; and improve relative water content in *Medicago sativa* plants (Raklami et al. 2020). It is observed that application of AMF at places where there was chromium stressed resulted in mitigation of stress by increase in the average leaf area, which helped the plant to survive in harsh conditions (Devi and Kumar 2020). It was reported that the effect of AMF on soil remediation played a significant role when *Trifolium repens* (a legume), and two native plants, *Coreopsis drummondii* and *Pteris vittata*, were

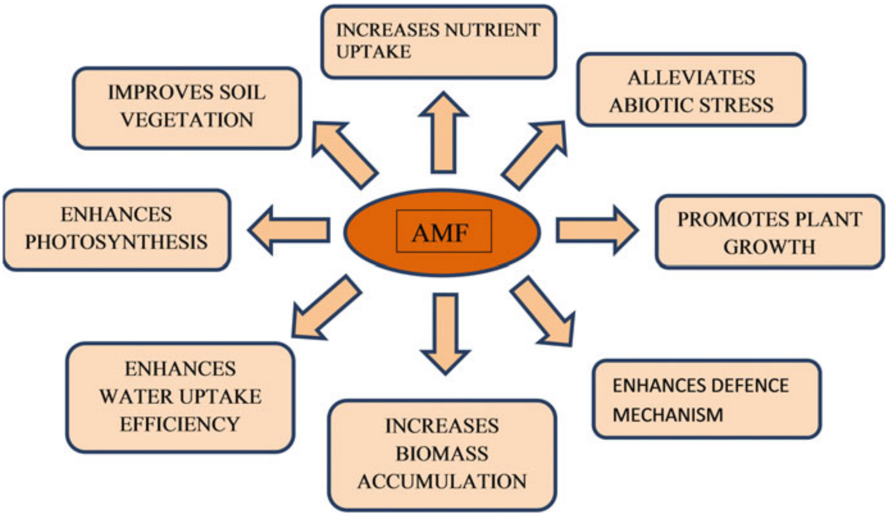

Fig. 32.1 A diagrammatic representation of beneficial roles of arbuscular mycorrhizal fungi in soil remediation technique

planted on soil having high concentration of copper (Chibuike 2013). The use of AMF is believed to increase the secondary value of plants used for phytoremediation as these plants can be used to check the soil erosion of the remediated soil and the fungi spores, which remain in the soil can also be used for colonization and support for the growth of the crop planted in the soil after its remediation. Studies have also shown that the use of other remediation methods such as the addition of soil amendments like organic materials and phosphate rocks with the AMF can enhances soil remediation contaminated with heavy metals (Chibuike 2013).

AMF is also known to enhance the dietary quality of crops by affecting and production of carotenoids and certain volatile compounds and increased content of sugar, organic acids, vitamin C, flavonoids, and minerals (Begum et al. 2019). It is also reported that root colonization by AMF can provide protection from parasites and nematodes and can also increase the root growth of infected plants and cause no change in xylem pressure.

AMF is also considered as a key indicator for soil pollution or soil quality because (Fig. 32.1):

1. These are ubiquitous in nature.
2. They provide a direct link between the plant roots and soil.
3. Many plant species highly depend on them for their growth.
4. They are involved in the remediation of many pollutants.

32.6 Bioremediation

Different environmental conditions and contaminants present in the soil can be removed by approaching different types of remediation processes. These can be (1) physical remediation which includes excavation and removal, construction of barrier system, stabilization and soil washing, (2) chemical remediation which includes the use of combustible substances such as solvents, petroleum, coal derived hydrocarbons, BTEX, aromatics, PAHs, highly volatile CFCs, chlorinated herbicides and pesticides and (3) biological remediation, also known as bioremediation which involves the use of living organisms for the treatment of contaminated soil.

The bioremediation can be divided into three types on the basis of the organism used:

1. Microbial remediation, which uses microorganism to either degrade organic contaminants or to bind heavy metals in more inert and less bioavailable forms.
2. Phytoremediation in which plants are used to repair and regenerate soil, groundwater and surface water. There are six different ways in which the plant deals with the contaminated soils—phytoextraction, phytodegradation, phytovolatization, rhizodegradation, rhizofiltration, and phytostabilization. There are plant species which are known as phytoaccumulator or phytotolerant, which can grow on contaminated soils and reduce toxicity by preventing translocation or by sequestration of heavy metals (Khan 2020).
3. Mycoremediation, which involves the use of fungi to overcome the soil contamination. Out of all these remediation techniques, bioremediation is now widely used for removal of soil contaminants because of its low cost, highly effective removal, less toxicity, environmentally friendly, and increased yield of plants. It uses the noteworthy capabilities of microorganism associated with roots to degrade the organic contaminants and transform the toxic metal elements (Kumar and Fulekar 2018).

Bioremediation of heavy metals can affected by both abiotic and biotic factors such as pH, temperature, bioavailability of pollutants, biological competitiveness, interactions between pollutants and the biological state (Zhang et al. 2020). Bioremediation with the help of microbes converts the toxic form of metal into bioavailable and soluble form without any lethal effects (Rana et al. 2020).

32.7 Bioremediation of Heavy Metal by AMF

AMF play an important role in enhancing the efficiency of the ecosystem by producing fungal structures like arbuscules, vacuoles, and aid in the exchange of inorganic compounds and nutrients required for plant growth. These also act as biological filters for the heavy metals present in soil (Birhane et al. 2012). AMF has greatest impact on elements such as heavy metals and phosphorus with narrow diffusion zone around plant roots. AMF-assisted alleviation of heavy metals is a cost-effective and eco-friendly strategy (Riaz et al. 2020). It ensures rapid removal of toxic heavy metals from soil and rapid vegetation of remediated soils. There are many factors on which the specific role of AMF in the host plants on exposure to different heavy metals depends such as (1) plant species and its ecotype; (2) fungal species and its ecotype; (3) soil edaphic conditions including soil fertility, root density, and light intensity; and (4) metal and its availability (Tiwari and Lata 2018). Various isolating spores of AMF are found in association with plants in places contaminated with heavy metals. Once a HM enters the soil, it can have a long-term effect on the plant, but this long-term effect can be reduced by inoculating the plant with the AMF. It is observed that the HMs get immobilized in the fungal hyphae living in symbiotic association with plants and are retained in the cell wall or vacuole by chelation, thereby reducing the availability of HMs to plants and decreasing the metal toxicity. Various researchers have observed different strategies used by the AMF to uptake, translocate, and accumulate HMs and also increase the growth, productivity, and tolerance to plants toward the HM stress (Smith and Read 2008).

These strategies include various steps:

1. *Retention of HMs in mycorrhizal roots and external hyphae of AMF*: The HM is retained in the roots and the external mycelium, which then prevents their mobilization to aerial plant tissues. The fungal vesicles in mycorrhizal plants are analogous to the plant vacuoles, which accumulate the toxic compounds. The external mycelium has a wider surface area because of which the absorption of HMs become more convenient. This retention mechanism ultimately protects the leaf tissues of plant from injury, therefore contributing to phytostabilization. Moreover, it is observed that as the concentration of metal ions in the soil increases, there is an increase in production of fungal vesicles (Yang et al. 2015).
2. *AMF promotes nutrient absorption in plants*: Under stressful conditions, the nutrients are absorbed by the fungal hyphae from the soil and are directed to the interface between the fungus and the plant where exchange takes place (Janeeshma and Puthur 2020). It is observed that only phosphate transporter is present in the fungal mycorrhiza, which is responsible for direct transport of inorganic phosphate from soil to plants (Johri et al. 2015). The absorption of metals in plants is related to their degree of dissolution in soil because AMF secretes phosphatase, which hydrolyses the insoluble phosphate in soil and make it mineralized therefore increasing the soluble phosphate content. The organic acids secreted by AMF such as oxalic acids, succinate, and propionic acid also play an important role in activating the insoluble phosphate making it easier for the plant in absorbing and transporting of phosphorus even in harsh conditions (Gong and Tian 2019). Apart from this, there is also an increase in the uptake of nutrients like nitrogen and phosphorus in host plants subjected to HM stress in the presence of AMF (Elhindi et al. 2018).

3. *Sequestration of HMs in vacuoles by AMF*: Once the HM reaches their target site, the sequestration of metal takes place by polyphosphate granules present in the fungal vacuoles (Shi et al. 2019). This sequestration allows the concentration of a large amount of HMs in the aboveground parts without any phytotoxic effects on the plants and also limits the harmful effects of HM.
4. *AMF Assist in HM Binding on the Fungal Cell Walls:* The fungal cell wall is made up of chitin and polysaccharides that acts as a barricade for the HM ions due to which the HM ions get adsorbed on the fungal cell wall. There are various functional groups present on the cell wall such as amino group, imidazole carboxyl groups, and free hydroxyl group, which provide binding sites for HMs and formation of negatively charged structures takes place which adsorb the metal ions present in soil, thereby restricting its movement in the plants. AMF also induces the biosynthesis of cell wall in the roots of the host plant due to which the thickness of the cell wall increases, which results in the increase of surface area for better metal absorption (Zhang et al. 2019).
5. *AMF inoculation enhances the photosynthesis capacity and rectifies the gas exchange capacity:* It is observed that inoculation of AMF increases the chlorophyll content especially "chlorophyll a," which is important for photosynthesis of host plant (Elhindi et al. 2018). It also improves the gas exchange capacity by stomata opening.
6. *AMF enhances the production of antioxidants and reduces the ROS production in host plant:* Under HM stress, the host plants produce antioxidant enzymes, which acts as a defense system and detoxifies the reactive oxygen species. The AMF promotes the synthesis of antioxidants and increase their activity of ROS scavenging and reduces the ROS production (Yang et al. 2015).
7. *Chelation of HMs by AMF*: The chelate mechanism of the hyphae secretion to HMs relies on the extracellular glycoprotein. Glycoprotein—metal chelate—enters the cell, thereby reducing the metal content outside in the soil (Gong and Tian 2019). The chelation of HMs occurs with ligands having high affinity for metal ions in the cytosol by AMF. The chelating compounds are produced when the concentration of chelating compounds increase above the threshold value inside the cells. Due to chelation, the HMs become less available for the cytoplasm and also their solubility and reactivity decreases. The chelating agents present in AMF and plants are metallothioneins, organic acids, phytochelatins, and amino acids, which play a crucial role in tolerance to HMs in plants.
8. *Soil metal complexes induced by glomalin:* Glomalin, a glycoprotein formed by AMF, is homologous to heat shock protein 60 (hsp 60) and plays a vital role in immobilization of HMs. Glomalin is known to have 30%–40% carbon content, and it also safe guards soil from desiccation by enhancing the soil water holding capacity (Begum et al. 2019). The Glomalin-related soil proteins (GRSP) are produced in the inner layer of the cell wall of mycorrhiza, and its production depends upon the level of metal ions present in the soil (Singh et al. 2012). The release of glomalin in soil performs several functions such as retaining the water

content, reducing soil erosion, improving the stability of soil aggregates, enhancing soil quality, forming protein-metal complexes, increasing the development of plant, and decreasing the amount of metal ions in the soil leading the plant and AMF to withstand the HM stress. With different levels of metal contaminants in soil, the proportion of glomalin also differs indicating that the quantity of glomalin depends on the quantity of metal contaminants in soil (Gong and Tian 2019).

9. *Phytoremediation of HMs by AMF:* Phytoremediation is a technique in which plant species known as hyper-accumulators are used for growing in HM-contaminated soil (Kumar and Saxena 2019). Some hyper-accumulator plants like *Pteris vittata, P. cretica, P. biaurita, Sesbania drummondii, Helianthus annuus, Sedum alfredii, Atriplex halimus, Amanita muscaria*, and *Polygonum aviculare* are capable of growing in high concentration of metals absorbing them with the help of their roots and translocating them to the aerial parts of plants without any toxicity (Dhalaria et al. 2020). The absorption of metal ions takes place through the roots of plant and are translocated in plants with minimal toxicity. By inoculating the phytoremediator plants with AMF, the efficiency of phytoremediation can be increased. Phytostabilization and phytoextraction contribute to phytoremediation by immobilizing the HM in the roots, therefore decreasing its mobility and bioavailability and identifying the HM for its uptake and transporting it to the aboveground parts of the plant. The combined action of both AMF and hyper-accumulator plants improves the absorption of trace elements like nickel, iron, cobalt, and molybdenum, and it also activates insoluble phosphorus in soil, improving the resistance physiology of host plants (Gong and Tian 2019).
10. *Presence of regulatory genes in AMF*: It has been found that there are four genes present in AMF, responsible for resisting metal stress and maintaining homeostasis in the cells. These are:
    (a) The GrosMT1 gene in Gigaspora rosea.
    (b) Zinc transporter GinZnT1 in Glomus intraradices.
    (c) GmarMT1 gene in *Gigaspora margarita*, which can encode metallothioneins and regulate the oxidative reduction potential of fungi, thereby protecting the plant from oxidative stress caused by metals.
    (d) GintABC1 can encode a polypeptide of 434 amino acids and participate in the detoxification mechanism of copper and zinc actively (Azcón et al. 2013).

The regulation of all these four genes is mainly induced by the presence of metals, and these play an important role in accumulation, absorption, transportation, and tolerance of heavy metals.

32.8 Bioremediation of HMs Through Interaction Between AMF and Various Organisms

1. *AMF and earthworms:* Earthworms are important soil organisms, which contribute to the maintenance of soil properties. These are known to survive in soils with high concentration of HMs as these are capable of accumulating the HMs in their tissues. They have the ability to increase metal availability in soil and also improve the efficiency of phytoremediation. Rapid remediation of heavy metal contaminated soils was observed through interaction between the earthworm and AMF (Yu et al. 2005). It was predicted that the production of phytohormones by earthworms stimulates the mycorrhizal infection and the effective dispersal of fungi propagules is done by earthworm through its feeding habits. The cast of earthworm was observed, which contained more number of infective mycorrhizal propagules in the soil. Earthworms are also responsible for disconnecting the mycorrhizal fungi from the roots of host plant by their action of feeding and burrowing through the soil. The combined effect of earthworm and mycorrhiza on soil remediation is complex and depends on the plant species colonized by fungi. It has been revealed that use of earthworm with the AMF increases the metal availability in soil through burrowing and casting and plays a potential role in modifying the efficiency of bioremediation (Yu et al. 2005).
2. *AMF and Microorganisms: Fusarium concolor* and *Trichoderma koningii* were the two saprobes that were used in conjugation with the AMF for the remediation of soil polluted with heavy metals. These studies showed that the combined effect of these two saprobes with AMF resulted in removal of large amount of heavy metal pollutants. The quantity of pollutants removed depends on the species of both mycorrhizal fungi and saprobe used for remediation (Arriagada et al. 2007).
3. *AMF and plants:* AMF can be used in conjugation with nonfood bioenergy crops such as *Vetiver zizanoides* and *Cannabis sativa* as these plants are capable of producing high biomass and tolerating heavy metal contamination (Khan 2020). AMF with plants is used to increase the efficiency of slope ecological restoration, which includes three elements soil, plants, and soil microbes (Yan et al. 2020).

32.9 Advantages

Some of the advantages of using arbuscula mycorrhizal fungi for the soil remediation are:

1. It is used to enhance the vegetation of the soil after the removal of heavy metal contaminants from soil.
2. Remediation is done in situ, thereby eliminating the risk involved in transporting the polluted soil from one place to other for treatment.
3. It is achieved through natural process and thus is environmental friendly approach toward remediation.

4. After remediation the fungal spore remains in the soil prove to be beneficial for the growth of other plant species later cultivated in that soil.
5. It is cheaper, less toxic, and relatively easier to accomplish than any other remediation methods.
6. Apart from soil remediation, it is also known to improve growth of plant by increasing its nutrient uptake and enhancing its photosynthesis capacity, transpiration rate, stomatal conductance, water uptake efficiency, and defense mechanism.
7. It can be safely combined with other remediation techniques to accomplish greater and improved results.

32.10 Potential Domains of AMF Application

1. *Agriculture:* AMF plays an important role in ensuring food security. It colonizes within the root of plants and ensures its high yield. It also plays an important role in resisting abiotic and biotic stress when in symbiotic association with the plant. The crop, which is naturally grown under agro-ecosystem, largely relies on the association of mycorrhizae for nutrient supply in required amount for normal plant growth. It provides nutritional benefit in terms of P and N and different micronutrients like Zn and Cu, to the associated plant under poor soil fertility conditions. Due to high immobile nature of phosphorus, it is difficult for the plants to access it, but because of presence of mycorrhizal associations, its accessibility increases in plants. It is also reported that the use of fertilizers per unit area also decreased with the increase in use of AMF. The application of AMF has proved to be very beneficial for agriculture purposes. The AMF can be applied to large areas of field by spore coating over seed or by the use of indigenous strains of AMF to promote plant growth and soil reclamation.
2. *Forestry:* This sector has always served as a major renewable source contributing to the basic need of the world. There is an increase in productivity, profitability, and sustainability with use of AMF in forestry sector. The trees in the forest mostly show mycorrhizal association due to stable nature of ecosystem and long-term association within the soil. Wide variety of species of AMF can be found on the forest land. Due to the increased root surface area with the help of AMF, there is reduction in the transplantation injury to saplings and improvement of water and nutrient uptake particularly in phosphorus deficient soil. It has also been reported that the use of AMF increases *Rhizobium* nodulation in various leguminous trees by supplying adequate amount of phosphorus to the plant, thus reducing the additional use of chemical fertilizer in leguminous forest trees. It also provides resistance to soil borne pathogens such as *Phytophthora, Pythium*, and *Fusarium species*, which cause root rot to the newly planted saplings.
3. *Horticulture crops*: the horticulture crops such as vegetables, fruits, and ornamentals are largely affected by the action of certain harmful soilborne pathogens. The attack by soilborne pathogens on these crops can be reduced by the use of AMF on the seedlings. It also leads to the reduction in the use of

chemical fertilizers and cost cultivation. It is also observed that the application of AMF in horticulture crops leads to the increase in quality of the crop by increasing nutrient uptake, plant growth, plant biomass, and providing resistance toward the environmental stresses. Therefore it can be said that the treatment of plant with AMF acts as an amour for them.

4. *Wastel and management:* the recovery of soil from the pollutants such as heavy metals, which are released through various anthropogenic activities of humans, has become the need of the hour. To overcome the problems caused by the heavy metals AMF is used for the recovery of disturbed lands and reclamation of wastelands because of its great potential. It also acts as a stress alleviator by bioremediation of soil polluted with heavy metals. AMF exists in symbiotic association with the vascular plants, thereby enhancing the soil fertility by producing glomalin that promotes stability of soil and growth of microorganism. The incorporation of AMF on wasteland showed improvement by revegetation and increase in plant growth. Therefore AMF is an important component for soil conservation and sustainable environment (Parihar et al. 2020).

32.11 Future Prospects

The present review focuses on the use of AMF for bioremediation of HMs present in the soil. It is observed that the inoculation of AMF with the host plants leads to a symbiotic association between the two of them, which aid the plant to deal with the HM stress and also increase its nutrient uptake. The technique of using AMF in bioremediation of HM is of great importance and has led to a better sustainable environment. Future researchers should focus on different strategies used by AMF to detoxify the HM contaminants and also use various other microorganisms present in soil in conjugation with AMF to observe their beneficial effects in bioremediation of HMs, to identify the genes and the gene products controlling the AMF-mediated growth and development under stressful conditions. Encouragement of AMF usage with different plants and organisms is of enormous significance for contemporary worldwide agricultural structures for their reliable sustainability. Use of hyper-accumulator plants with high biomass production should be encouraged and enhanced through genetic engineering to extract heavy metals easily from contaminated soil. Researchers must focus on exploring AMF at all levels to look over for its role as a bio-fertilizer in nature for good agriculture production, and also there should be a standardized technique using AMF as a bioassay, and this should be made easy since commercial AMF inoculum is now available.

32.12 Conclusion

The anthropogenic activities of man have led to an increase in the heavy metal contamination of soil leading to the deterioration of soil properties, under developed quality crops, and accumulation of toxic elements in food. All these activities have led to deterioration of the agriculture and environment. To overcome this situation, various remediation techniques were used. Among all those techniques of remediation, bioremediation is the most beneficial and promising one because of its low cost, less toxicity, high yield, and eco-friendly way. In bioremediation, mycoremediation that is the use of AMF has proved to be the most benefitted one, as AMF can be used in conjugation with both phytoremediation and microorganism remediation to improve agriculture yield and reduce the toxic effects of heavy metals. A few research reports have already documented the beneficial role of AMF in improving plant growth under stressed conditions. Therefore, in this review, the existing information related to the role of AMF has been combined in a coherent way for understanding of AMF symbiotic relationship with the host plant under stress environments. It has been clearly depicted now that the plants inoculated with AMF can effectively combat various stressful environmental conditions like salinity, high temperature, drought, cold stress, and nutrient limitation and also increase its growth and development, thereby increasing its yield. The use of AMF for agricultural improvements can significantly reduce the use of other chemical fertilizers, which will ultimately promote healthy and eco-friendly environment. The use of AMF for agriculture enhancement can be beneficial to overcome the demand of food in the increasing population and would also reduce the negative environmental impact. It is hence proved that the plants colonized with AMF are better able to obtain their nourishment from soil and resist the environmental stress. Thus AMF is a very useful component for the remediation of soil and play a key role as a biofertilizer.

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33 Application of Biotechnology for Providing Alternative of Fossil Fuel to Protect Environment

Lalit Kumar Singh, Garima Awasthi, and Mangalam Bajpai

Abstract

The world is currently facing a huge crisis of fossil fuel depletion and environmental degradation. Fossil fuel consists of coal, crude oil, and natural gases which are formed from buried plants and animals for millions of years. This fossil fuel has high carbon content which is the cause of environmental pollution.The whole world is looking for another generation of fuels, which will be environment-friendly and useful for sustainable development and energy conservation. The research trends are moving toward biotechnology to meet the existing problem related to fossil fuels. Biofuels could be a feasible solution for the worldwide crisis. Biofuel is considered as the future of the fossil fuel industry, such as bioethanol and biodiesel. India has announced a new policy on biofuels which targets the 20% combination of bioethanol in petrol and 5% combination of biodiesel in diesel and is proposed by 2030. The contribution of biotechnology in the energy industry by its technical advancement will cut down the prices and will protect the reservoirs. The various substrates are used as unprocessed materials for the making of biofuels such as various parts of crops and unused vegetable materials. The use of crops for food versus fuel production is a matter of concern; this leads to search more possibility to explore sources for the invention of biofuel. Biotechnology is playing a major role in the making of such third-generation biofuel. The biotechnological tools are very effective for

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the upcoming biofuel generations and have a great impact on environmental protection issues.

Keywords

Biodiesel · Bioethanol · Biofuel · Biotechnology · Environmental protection · Fossil fuel

33.1 Introduction

Most of the countries are characterized as developing or developed based on fossil fuels (Sugiawan and Managi 2019; Caetano et al. 2017). Fossil fuel is one of the important sources for mankind. Fossil fuel is the basic need for automobiles, thermal stations, etc. The whole world is currently facing a huge crisis in fossil fuel and is also worried about this crisis. Fossil fuel is formed through buried plants and animals for millions of years. Fossil fuel consists of carbon which is the reason for environmental degradation too. Whenever fossil fuel is burned, it releases carbon contained in the form of carbon dioxide and many more which cause great damage to the environment and living beings on the earth. Fossil fuels consist of coal, oil, and natural gases. These are considered primary energy sources. So, fossil fuel is considered a limited source of energy. Problems exist, such as environmental degradation as mentioned below, scarcity, supply risk, and unstable price; thus we are looking forward to low economical and environment-friendly solution or sources, that is, biofuel (Jamwal et al. 2020). Various countries like Pakistan have a strong connection between fossil fuel usage, air pollution, and natural resource depletion (Heng et al. 2015), whereas China is also facing a lot of problems regarding fossil fuel (Ghag et al. 2019) along with the USA, UK, India, etc.

Coal is a concrete, weighty rock of carbon that is present in four varieties, that is, lignite, subbituminous, bituminous, and anthracite. The coal is extracted by two methods. The first one is the removal of an entire layer of soil where deep underground deposition of coal is present which is called surface mining. Thus surface mining is also known as destructive strip mining and pollutes the entire ecosystem. Whereas, another method of extraction is performed with the help of heavy machinery to extract deep down underground coal.

Crude oil is the liquid form of fossil fuel which is made of a lot of cargo chains with hydrogen. Oil and petroleum form subversive reservoirs. These are present mainly in cracks, crevices, and pores of sedimentary rocks. They are utilized by the penetrating on the land or by strip mining on account of tar sands, oil, and oil shale. Once extracted, oil is shipped to processing plants through supertanker, train, truck, or pipeline to be changed into usable powers, for example, gas, propane, lamp oil, and stream fuel—just as items, for example, plastics and paint. Natural gases are composed of methane, and they are generally considered either conventional or unconventional, depending on where it's found underground. Traditional flammable gas is situated in permeable and penetrable stone beds or blended into oil supplies and can be gotten to through standard boring. Offbeat gaseous petrol is any type of gas that is excessively troublesome or costly to extricate employing normal penetrating and requiring a unique incitement strategy, for example, deep oil drilling.

33.2 Role of Fossil Fuel as Environmental Pollution

Pollution is defined as the contamination of natural resources like air, water, and soil. They are polluted for many reasons. One of which is the burning of fossil fuels which emit carbon sources in the large amount. By this action, it interferes with individual health, the quality of life, and the function of the ecosystem. The release of the pollutant into the environment the fossil fuel is becoming a great concern to the living habitat in the ecosystem. On one side it is limited in an amount which is causing a crisis and on other hand its negative effect on the environment. So while burning coal or any fossil fuel as a source of energy they converted into oxide when they react with the oxygen, while fuel burns completely to form carbon dioxide as a slightly acidic, but when there is very less amount of oxygen present it forms carbon monoxide which is very poisonous gas for human health and can lead to death (Jamwal et al. 2020; Ghag et al. 2019). When coal is burned in power plants, it releases carbon dioxide which causes global warming and degradation of the environment, while carbon monoxide is the gas that is produced as the by-product of fuel flaming, which is the major cause of ozone layer depletion. When CO is inhaled by the individual, it causes several health issues like headache, stress to heart diseases, and sometimes death. Ozone depletion can cause numerous health troubles. Ozone depletion can lead to more common asthma attacks in people and can cause sore throats, cough, and breathing difficulty. It may even lead to premature death (Ghag et al. 2019). When the ozone in the stratosphere is smashed, people are bare to more emission from the sun (ultraviolet radiation). This can lead to skin cancer and eye problems. Higher UV radiation can also destruct plants and animals. Not only carbon dioxide or carbon monoxide but also harmful gases are released; there is the release of nitrogen oxide (NOx) by the protein content of the fossil fuel and sulfur dioxide ($SO_{2)}$ is also released which causes acid rain and environmental mishandling. Together nitrogen oxide and sulfur dioxide cause acid rain which returns to the earth and causes lakes and water bodies to be acidic resulting in damage to animals (Fig. 33.1).

Fossil fuel is affecting the environment badly, so scientists moved toward the biological bend of the fuel generation, i.e., "Biofuel." Biofuel is the future of fossil fuel to generate energy which can lead to sources of environment-friendly and human-friendly resources. This is a great opportunity for scientists and researchers to work on the field of bioenergy as an alternative to fossil fuel.

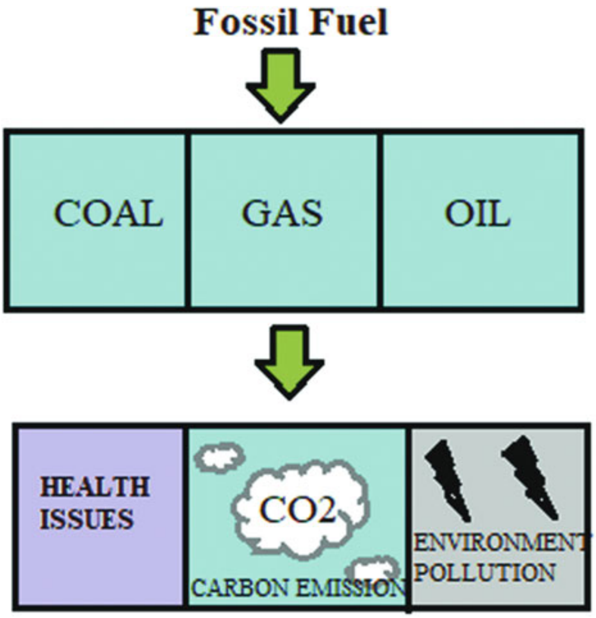
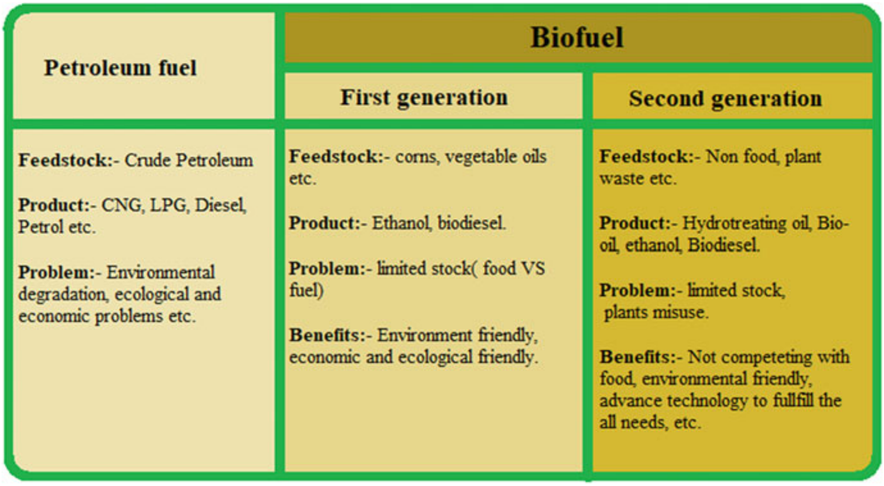


Fig. 33.1 Different types of fossil fuel and their effects

33.3 Biofuel Is a Source of Green Energy

Biofuels are formed from renewable resources which facilitate the reduction of the use of fossil fuel flaming and CO_2 construction. They are produced from biological matters like vegetation or natural wastes and help them to renew. Biofuels can reduce CO_2 emission because plants use CO_2 as they grow (Mabee et al. 2005; Osamu and Carl 1989). Since biofuel is derived from plants, microorganisms, animals, and wastes, its basic concept is to be a source of renewable energy. Biofuel works on the present-day photosynthetic conversion of solar energy to chemical energy, to reduce the use of fossil fuels (Jamwal et al. 2020). The line connecting renewable biofuels and non-renewable fossil fuels is from time to time interchangeable, and only complete life-cycle analyses in the future will reveal which feedstocks are truly renewable to be used in biofuel manufacture (Naqvi et al. 2012) (Fig. 33.2).

From ancient times use of biological material as a source of energy has been recorded like wood which is burned as a souce of heat and light to cook food, illuminate night, and bring warmth and safety. There are many pieces of evidence on the use of biological material in place of fossil fuel to generate energy. Energy production is a basic key role in humans nowadays (Ghag et al. 2019). By these pieces of evidence, we should follow the route of biofuel as an alternative source of fossil fuel and lead to the production of biofuel in large quantity to fill the void of fuel from generation to generation (Jamwal et al. 2020).



Petroleum fuel	Biofuel: First generation	Biofuel: Second generation
Feedstock:- Crude Petroleum **Product:-** CNG, LPG, Diesel, Petrol etc. **Problem:-** Environmental degradation, ecological and economic problems etc.	**Feedstock:-** corns, vegetable oils etc. **Product:-** Ethanol, biodiesel. **Problem:-** limited stock(food VS fuel) **Benefits:-** Environment friendly, economic and ecological friendly.	**Feedstock:-** Non food, plant waste etc. **Product:-** Hydrotreating oil, Bio-oil, ethanol, Biodiesel. **Problem:-** limited stock, plants misuse. **Benefits:-** Not competeting with food, environmental friendly, advance technology to fullfill the all needs, etc.

Fig. 33.2 The difference between fossil fuel and biofuel (Naik et al. 2010)

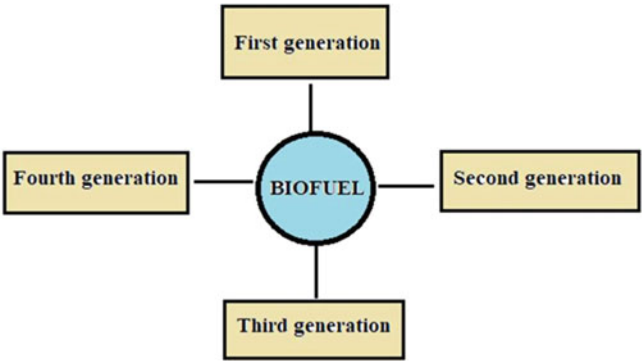


Fig. 33.3 Different generations of biofuel

The two most commont types of biofuel in use today in terms of petroleum are bioethanol and biodiesel. These are mixed with petrol and diesel for more effectiveness and they are less pollution-causing. Biofuels are classified as first-, second-, third-, and fourth-generation biofuel (Fig. 33.3).

These generations mean the formation of biofuel concerning satisfying humans, ecosystems, and others. Since each generation has limitations, researchers moved to another level of generation for more betterment. The generation is divided as per the source of production of biofuel each time and better performance to replace fossil fuels. Biofuels could be a feasible solution for the worldwide crisis. Biofuel is considered as the future of the fossil fuel industry, such as bioethanol and biodiesel. The research and development organizations are promoting biofuel research to explore renewable resource that can provide clean and renewable energy for transportation and can protect the environment. India has announced the new policy on biofuels which targets the 20% combination of bioethanol in petrol and 5% combination of biodiesel in diesel and is proposed by 2030 (Jamwal et al. 2020). The contribution of biotechnology in the energy industry by its technical advancement will cut down the prices and will protect the reservoirs of fossil fuel.

33.3.1 First-Generation Biofuel

The first-generation of biofuel means fuel is produced by the crops, which contain starch, sugar, and vegetable oil and fats (Azapagic and Stichnothe 2011). The organization of biofuel remains identical between dissimilar generations of biofuel, but the categorization is based on the source from which the biofuel is consequent. The most familiar feedstocks used for the creation of first-generation biofuel are wheat, corn, and sugarcane (Stevens and Verhe 2004). Sugar crops, such as sugarcane and sugar beet, contain widespread amounts of sucrose that is capable of being extracted and fermented to bioethanol (Singh et al. 2010). From the economic and environmental perspective, sugarcane and corn starch are a chosen feedstock for ethanol production but inhibited in certain regions because of weather and soil circumstances (Mohanty and Swain 2019). This section presents the major types of first-generation biofuels derived from a variety of feedstocks (Table 33.1).

There is a dramatic increase in oil prices in the most recent decades which has enabled liquid biofuels to become cost-competitive with petroleum-based transportation fuels, and this has led to a surge in research and production around the world. The three main types of first-generation biofuels used commercially are biodiesel, ethanol, and biogas of which world broad large quantities have been produced so far and for which the production progression is considered “conventional technology.” Biodiesel is a substitute for diesel and is produced through transesterification of vegetable oils and residual oils and fats, with minor engine modifications; it can serve as a full substitute as well. Bioethanol is a substitute for gasoline and it is a full substitute for gasoline in so-called flexi-fuel vehicles. It is derived from sugar or starch through fermentation. Bioethanol can also serve as feedstock for ethyl tertiary butyl ether (ETBE) which blends more easily with gasoline. Biogas, or biomethane,

Table 33.1 First-generation feedstock with their products, pros, and cons (Stevens and Verhe 2004; Singh et al. 2010; Mohanty and Swain 2019)

Feedstock	Pros	Cons	Product
Corns	Simple to convert starch to ethanol	Energy yield is low, use of fertilizer and pesticides	Ethanol
Sugarcane	Yield is more than corns, no land-use changes	Region-specific cultivation	Ethanol
Vegetable oil	Widely available feedstock	Important food feedstock, direct use harms the engine	Biodiesel
Soybean	Maintenance is easier, and it has 20% oil content	Direct threat to food chain, unprofitable feedstock	Biodiesel, ethanol

is a fuel that can be used in gasoline vehicles with slight adaptations. It can be produced through anaerobic digestion of liquid manure and other digestible feedstock. At present, biodiesel, bioethanol, and biogas are produced from commodities that are also used for food. The demands for edibles oils are increasing trend, so it is difficult to use agricultural food crop for biofuel production (Kulkarni et al. 2006).

There are some potential crops for biodiesel production, which can be taken up as an industrial crop on fruitless lands. Such versatile uses of oilseed crops can be introduced so that the biomass produced by them can be utilized for the production of various bioproducts. In that respect, the pattern of whole crop biorefinery has been discussed aiming at the incorporated utilization of Jatropha in India, attempting to supply sustainable biodiesel production and parallel use of its solid residues for the manufacture of other valuable chemicals and employment of its lignocellulosic biomass for second-generation biofuel production.

33.3.1.1 The Conversion Process for First-Generation Biofuels

Transesterification The vegetable oil-based unsaturated fat methyl esters (FAME), prevalently known as biodiesel, is gaining importance as a domain benevolent diesel fuel substitute or extender (Lee and Lavoie 2013). Biodiesel is an elective diesel, produced using inexhaustible organic sources, for example, vegetable oils and creature fats by synthetically responding oil or fat with liquor, within the sight of a homogeneous and heterogeneous impetus (Kulkarni et al. 2006; Meher et al. 2006). The result of the response is a blend of methyl esters, which are known as biodiesel, and glycerol, which is a high worth co-item.

Homogeneous Catalysis Transesterification is a reversible response and continues basically by blending the reactants in which the impetus is a fluid corrosive or a fluid base.

Heterogeneous Catalysis It is preposterous to expect to play out an essential transesterification measure for high free unsaturated fat (FFA) content oil and diminishes the change of oil to methyl ester because of saponification response. The utilization of strong impetus is suggested for high free unsaturated fat containing oil. This is because the strong corrosive impetuses catalyze the transesterification of fatty substances and esterification of free unsaturated fat (FFA) present in the oil to methyl esters. Strong corrosive impetuses have the solid impending to supplant harmonized impetuses, killing division, consumption, and ecological issues.

Whole-Crop Biorefinery An entire yield of biorefinery measures and devours the entire harvest to get helpful items. Crude materials, for example, oilseed, can be utilized as feedstock in the component tasks of an entire harvest biorefinery as portrayed in Fig. 33.4. In that specific circumstance, the case of the Jatropha oilseed crop has been talked about. Jatropha seed part contains 35–40% oil and 1–1.5 huge amounts of oil can be delivered. The way toward changing over biomass into vitality is started by the partition of biomass into various segments that are then treated independently. The oil obtained becomes the beginning material for biodiesel

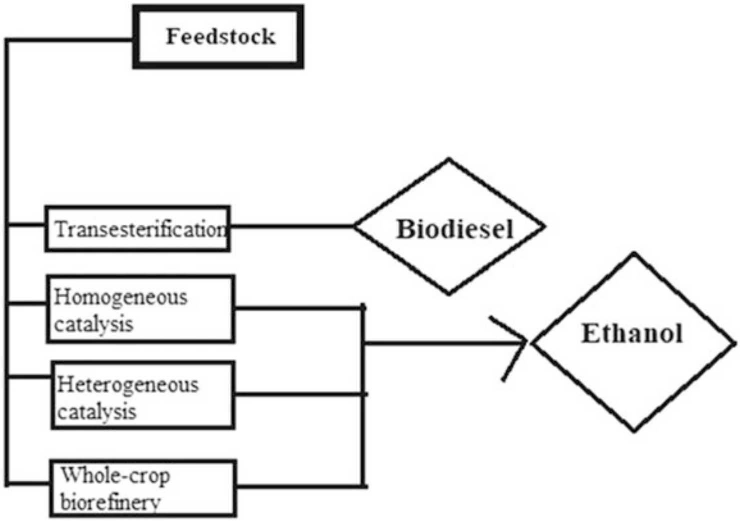


Fig. 33.4 Conversion of feedstock for first-generation biofuels

creation or can go through substance adjustment to deliver oleochemical items, while the de-oil cake division after cleansing can be utilized as the fundamental crude equipment for the union of significant synthetic compounds or gasification. The lignocellulosic biomass created during the treatment facility activities can go about as the beginning substance to the lignocellulosic biorefinery for the establishment of syngas, where syngas can be utilized as the essential stuff for the union of energies and methanol utilizing the Fischer–Tropsch process.

33.3.1.2 Advantages and Disadvantages of First-Generation Biofuel

Especially biofuels, maintainability is a key part of things to come with vitality improvement. The utilization of rapeseed biodiesel showed a better way and revealed the accomplishment of the European objectives regarding decrease of GHG discharge, estimated in CO_2 reciprocals which is observed as 56% to regular diesel. In any case, this outcome doesn't consider the negative natural effects brought about via land-use changes (immediate and roundabout), which would prompt GHG discharges, thus bringing about a lessening in the level of the assessed sparing. The land-use changes and strengthening of development following the expanded interest for biofuels possibly will source new GHG outflows and influence the biodiversity, dirt excellence, and ordinary assets.

Sponsoring biofuels along with bioenergy through the point of diminishing GHG emanations is a fewer powerful furthermore costlier method of accomplishing this objective than numerous other more financially savvy arrangements, for example, improving the vitality productivity and protection or empowering more compelling sustainable power source alternatives where practical. The structure of the current help (the whole of biofuel sponsorships and ranch installments) won't just keep on being noteworthy, yet is probably going to ascend after some time. Besides, citizen expenses on biofuel and sustainable power source approaches are high, particularly in comparison with their advantage, which can without much of a stretch be pessimistic. The transformation of broad agrarian frameworks and normal living spaces, such as prairies, into severe monocultures is one of the major threats to biodiversity; non-local feedstocks are also potentially intrusive and may affect biological systems; environment administrations, such as soil recovery, are also potentially intrusive and may affect biological systems.

33.3.2 Second-Generation Biofuels

Second-age biofuels are characterized as fills delivered from a wide cluster of various feedstocks, particularly however not restricted to non-consumable lignocellulosic biomass. Biomass utilized in favor of the creation of second-age biofuels is normally isolated into three primary classes: homogeneous, for example, white wood chips; semi-homogeneous, for example, horticultural and backwood deposits; and non-homogeneous, including low worth feedstock as metropolitan strong squanders (Lee and Lavoie 2013). The cost for this biomass is fundamentally not exactly the cost for vegetable oil, corn, and sugarcane, which is an impulsion. Then again, such biomass is commonly further unpredictable to change over and its creation is reliant on new advancements. Biofuel creation is connected to the item market; thus, the expense of changing over the first feedstock to the last item should be as low as conceivable to look after benefit (Neto et al. 1995). Then again, numerous biomasses (e.g., corn with the ethanol/animal feed duality) permit the chance to create an assortment of items elsewhere of a similar feedstock, consequently clinging to the idea of a "biorefinery." The transformation cycle for the creation of second-age biofuels is typically completed by two distinct methodologies, by and large alluded to as "thermo" and "bio" pathways (Table 33.2).

Table 33.2 Second-generation feedstock with their products, pros, and cons (Lee and Lavoie 2013; Neto et al. 1995)

Feedstock	Pros	Cons	Product
Wood	Better source than first generation	Deforestation	Ethanol
Lignin	Easy available	Yield is less	Ethanol
Cellulose	Easy available, energy yield is good	Difficult to extract from the source	Ethanol

33.3.2.1 Conversion of Second-Generation Biofuel

The Thermo Pathway

The "Thermo" approach covers explicit cycles where biomass is warmed with a negligible measure of oxidizing specialist, assuming any. All cycles in that class lead to the transformation of biomass into three parts: one strong known as biochar, one fluid as of now alluded to as pyrolytic oil or bio-oil, and one gas known as syngas, which is normally made out of carbon monoxide, hydrogen, short-chain alkanes, and carbon dioxide (Lee and Lavoie 2013). There is a point that when handled at low temperatures without oxygen, it goes through a vigorous cycle, and the significant transformation item is charcoal, made biologically. In more prominent temperatures (550 to 750 °C), additionally without oxygen, the cycle is known as pyrolysis (either quick or moderate contingent upon the warmth conversion scale with the biomass) and the signature item is bio-oil. Warm cycles are somewhat independent regarding vitality because the vitality needed to warm the biomaterial up to the mentioned temperatures can be provided by halfway or all-out oxidation of carbon from the biomass, responses that are normally exothermic.

Perhaps the most straightforward methodologies for the modern creation of synfuels out of syn-gas are to deliver methanol. Methanol can be created from carbon monoxide and hydrogen straightforwardly under the activity of a decreasing impetus. Methanol is its very own finished result, yet it can't be utilized as the added substance for fuel now. In this way, further, change is required. Depending on methanol as the beginning material, many final results have been delivered including alkanes utilizing the methanol-to-gas (MTG) cycle and ethanol through carbonylation measures and have the unmistakable favorable position of being easy to create under the activity of a corrosive impetus (Fig. 33.5).

The Bio Pathway

The "bio" pathway is to some degree equivalent with a pulping cycle because by and large a biological compound, cellulose, is first disengaged from the lignocellulosic

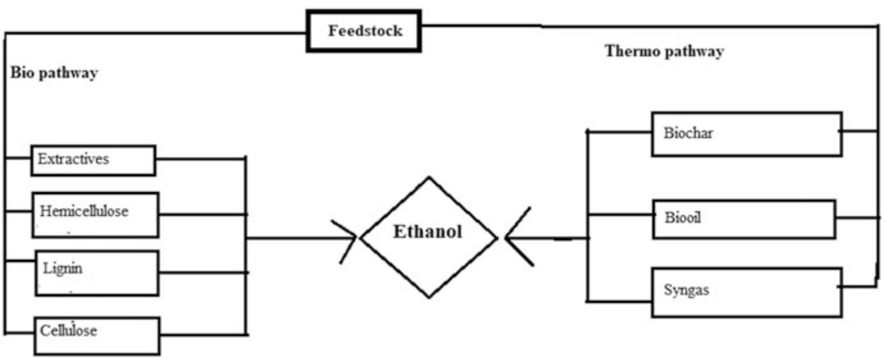


Fig. 33.5 Conversion of feedstock for second-generation to biofuels (Lee and Lavoie 2013)

biomass. Numerous cycles have been thought of including old-style addition measure steam blast and organo-solvent measures. Seclusion of cellulose is a mechanical test since it needs to create the most elevated virtue of cellulose to eliminate most inhibitors without devouring an excessive amount of vitality or such a large number of synthetic concoctions. Once cleansed, two methodologies are commonly utilized for saccharification of cellulose: either enzymatic or by concoction hydrolysis utilizing acids. Then again, concoction measures depend on rather modest synthetics (e.g., sulfuric corrosive), despite the fact that they must be recovered effortlessly to keep the cycle monetarily feasible. When segregated, the large molecules (i.e., starch) expect hydrolysis to be aged by yeasts.

Average North American timberland biomass weight (e.g., aspen) is made out of roughly 45% glucans which prompts a possible creation of 313 L of ethanol for each ton of crude biomass. Hemicelluloses are exceptionally vast starch-based polymers made out of both C5 and C6 sugars and the lignocellulosic biomass (dry weight) in them. Generally, the proportion among xylans and glucans in hemicelluloses differs a bit from the absolute sugar content. The principle bit of leeway of hemicelluloses is that, because of their exceptionally huge structure, they can be hydrated effectively utilizing water at high temperatures or a weakened watery blend of acids. The key issue is that C5 sugars don't mature with traditional yeast strains and require hereditarily adjusted creatures to deliver ethanol. Another methodology for the valorization of C5 sugars could be through concoction pathways.

33.3.2.2 Advantage and Disadvantage

There are not yet any business plants creating ethanol from lignocellulosic materials other than little plants that cycle squander sugar streams at sulfite mash factories. There are some enormous showing plants and various organizations are chipping away at plans to assemble business plants inside the following hardly any years. These organizations incorporate Iogen, Abengoa, Dedini, and numerous others (Lee and Lavoie 2013). The vast majority of these organizations don't deliver any definite data on their cycles or their financial aspects, and the focal points and inconveniences are accordingly to some degree theoretical right now. Similarly as with most second-era biofuels, the capacity to measure lignocellulosic feedstocks is an essential favorable position of this second-era pathway. These feedstocks are bountiful, geologically differing for the most part, and lower in cost than starch or sugar feedstocks, and huge amounts are created today that are as of now squandered. The yield of ethanol from the cycle can fluctuate from around 250 to 350 L/dry ton of feedstock. This is lower than the ethanol yield from harvests, for example, corn (460 L/dry ton) and wheat (425 L/dry ton) (Lee and Lavoie 2013). More feedstock must be shipped and handled in the plant for a given ethanol creation level contrasted with original innovations. The key element of the cycles is the use of lignin, which is a type of feedstock, to provide the vitality required for the preparation of work areas, hence reducing the need to purchase petroleum products for the activities. This has two favorable circumstances; it improves the life cycle ozone-depleting substance outflows for the cycle and brings down the working expenses by dodging petroleum derivative buys. It is in some cases expressed that the vitality equalization of lignocellulosic measures is better than it is for original biofuels. Carefully this isn't right. The complete vitality (counting the segment of the feedstock changed over to vitality) to add up is not as great for the present status of the workmanship of second era plant, yet the fossil vitality balance is better than original cycles that use non-renewable energy sources. Some original plants are changing over to biomass for their vitality needs, and their GHG emanation execution moves toward that of the second-era measures and their vitality adjustments are better.

33.3.3 Third-Generation Biofuels

The most acknowledged definition for third-age biofuel is that would be created from algal biomass, which is clearly identifiable development as contrasted with traditional lignocellulosic biomass. The creation of biofuels from green growth normally depends on the lipid substance of the microorganisms (Gerbrandt et al. 2016). Generally, species, for example, *Chlorella,* are focused on light of their high lipid content (around 60 to 70%) and their high profitability (7.4 g/L/d for *Chlorella protothecoides*) (Lee and Lavoie 2013). There are numerous difficulties related to algal biomass, some topographical and some specialized. Commonly, green growth will create 1 to 8 g/L/d of biomass in ideal development conditions (Neto et al. 1995; Netrusov et al. 2019). This infers enormous volumes of water are required for modern scale, introducing a significant issue for nations like Canada where the temperature is under 0 °C during a critical aspect of the year (Lee and Lavoie 2013). The more hydrogen and oxygen content is additionally an issue when lipids must be extracted from the algae, which require dehydration, through either centrifugation or filtering before separating lipids (Netrusov et al. 2019). Lipids acquired from green growth can be handled through transesterification by the recently depicted biodiesel measure to create lamp oil grade reasonable for use as avionics energizes.

33.4 Food Versus Fuel

Food vs. fuel is such a debatable topic. Food is a basic concern for humans as the need increases with the exponential growth of the population, on the other hand, its use in fuel production by biofuel method. This is the major concern of scientists while producing biofuel. Hunger is decently persuasive. If the people are thinking that the liquid to run the transportation by utilizing the plant will take food away from poor people, this is a making a great argument against the food is for automobile or the person to kill hunger. The improvement of the biological innovations of converting plant matter into fuels, as well as to research on agricultural and energy policies intended to increase utilization of biofuels. The first-generation biofuel is made from food materials which are food for animals or humans. Although the waste material is used, these unused materials of food and crops are animal feeding sources. The use of technology is becoming a problematic issue for peoples and researchers.

Fig. 33.6 Corn: food versus fuel

However, this does not vindicate the producers of various strategies for ever-increasing worldwide use of biofuel. Indeed, a more enough explanation of the ethical tensions between global food sanctuary and the growth of biofuel production requires the acknowledgment of errands that are, at present, only mutedly appreciated within the procedural and business communities (Fig. 33.6).

The rise of science from quite a while ago anticipated general rise in the cost of significant food items was the bigger setting for these articles and publications recognizing a connection between ethanol and oil-based biodiesel and a worldwide craving. Overall increase in the cost of basic food are typically related with fairly ephemeral impacts on global food gracefully, like as a large dry season or sickness in a single area, just like any pattern that covers a multitude of in any instance of isolated events. The overall genuine value patterns for rural items were descending for the greater part of the twentieth century, yet financial specialists, for example, Brown, had been anticipating this would be turned around sooner or later inferable from the blend of declining nature of ecological assets, all-out populace development (Brown 1995; Caetano et al. 2017).

US government endowments for producing ethanol from corn have been assaulted as the fundamental driver of the food versus fuel problem (Economic Analysis 2008). To protect them, the National Corn Growers Association (NCGA) has distributed their perspectives on this issue (National Corn Growers Association 2015). They consider the "food versus fuel" contention to be a misrepresentation that is "loaded with misinformed rationale, overstatement, and alarm strategies."

Cases made by the NCGA include (National Corn Growers Association 2015):

1. Corn cultivators have been and will keep on creating enough corn with the goal that gracefully and request meet and there is no deficiency. Ranchers settle on their planting choices dependent on signals from the commercial center. On the off chance that interest for corn is high and extended income per section of land is solid compared with different yields, ranchers will plant more corn. In 2007 US ranchers planted huge acres of land with corn, 19% a larger number of sections of land than they did in the previous year.
2. The USA has multiplied corn yields in the course of the most recent 40 years and hopes to twofold them again in the following years. With twice as much corn from every section of land, corn can be put to new uses without taking food from the hungry or causing deforestation (Krajewski 2013).
3. US shoppers purchase things like corn drops where the expense of the corn per box is around 5 pennies. So if the cost of a bushel of corn goes up, there might be no recognizable effect on US retail food prices [dubious – discuss]. The US retail

food cost list has gone up just a couple of percent for every year and is required to keep on having exceptionally little increments.

4. The majority of the corn delivered in the USA is field corn, not sweet corn, and not absorbable by people in its crude structure. Most corn is utilized for animals' feed and not human food, even the segment that is sent out.
5. Just the starch part of corn bits is changed over to ethanol. The rest (protein, fat, nutrients, and minerals) is gone through to the feed co-products or human food fixings.
6. One of the most noteworthy and quick advantages of higher grain costs is a sensational decrease in government ranch uphold installments. As indicated by the US Branch of Agriculture, corn ranchers got billions in government uphold. Due to higher corn costs, installments are required to drop to a few billion every subsequent year, a few percentages of decrease (Makarewicz-Marcinkiewicz 2013).
7. While the EROEI and financial matters of corn-based ethanol are somewhat feeble, it prepares for cellulosic ethanol which ought to have much better EROEI and financial aspects.
8. While essential sustenance is unmistakably significant, principal cultural requirements of vitality, versatility, and vitality security are as well. If ranchers' harvests can help their nation in these territories additionally, it appears ok to do as such.

The closing point is that an agrarian moral of biofuels involves a positive commitment for those in the science and business networks: They should recognize the contention between metropolitan, what's more, provincial interests and concede to settling it, if just through admonishment and cognizance rising. They will be left to deliver lower esteem crops or will be bankrupted during the beginning of progress to a bio-economy. It is hence that numerous social researchers composing on biofuels anticipate the grievous effect on poor or little scope makers in the creating scene. As sure researchers, these experts are for the most part severely disliked to consolidate moral decisions into their investigations. In moving from sociology to morals, it is consequently eminent that the experimental investigation of improvement brings about a similar determination as a studying light of moral hypothesis. The researchers, speculators, and policymakers who coordinate biofuel progress may be held collectively liable for monetary and individual losses, suicides, disengagement, and orderly craving and demise resulting from this change, at least to the extent that this network of biofuels engineers fails to respond to the ethical complexities that their innovations incite. Researchers, businesses, and perhaps at the same time lawmakers obtain moral duties to discover ways for alleviating the pressure between the interests of metropolitan and provincial poor. The observational investigation shows how troublesome this will be, yet it gives nothing that scatters the commitment to attempt. It is not that we should consider trailblazers ethically answerable for hurts that happen notwithstanding their best endeavors, yet the individuals who continue merrily ahead as though another person will address these issues merit judgment.

33.5 Biofuel Effect in the Environment

Fossil fuel has a lot of concern for the environment; thus biofuel takes the upper hand in the environmental factor to minimize the degradation of the environment. Biofuel advancement depends on the mixed-up presumption that during their burning just as much CO_2 is delivered as the plants consumed before. This shortsighted thinking doesn't consider the whole biofuel creation cycle (Duran et al. 2013). Also, the adjustments during the land use in addition to the vitality consumption designed for the developments as well as handling of biomass excluded beginning these appraisals. In creating nations, humid woodlands went through deforestation, and in their rest crops assigned for biofuels were developed. From the exploration led by Danielsen and his partners, CO_2 retention by the tropical backwoods is essentially more noteworthy than the developed plants in their position utilized for the creation of biofuels. Changing over rainforests and peatlands for the creation of biofuels prompting extra CO_2 discharge is a measure of around 55 mg of CO_2 for each hectare every year for a time of around 120 years (Piementel 2012). Along these lines, the utilization of biofuels acquired from crops frequently doesn't prompt a decrease in CO_2 emanations. The tropical timberlands are the territory for the greater part of the earthbound varieties. The most jeopardized are backwoods in southeast Asia. Enormous single yield estates, which we, as a rule, manage on account of biofuel crops, require a lot of herbicides and pesticides, which at that point infiltrate into the groundwater defiling it. Instances of the negative effect of pesticides are soya bean crops in Brazil. The pesticides and herbicides, utilized for a huge scope, compromise the Pantanal wetland region (WWF 2003; Junk and Cunha 2005) which is one of the most significant regions for many fledgling species, warm-blooded animals, and reptiles (Duran et al. 2013). The 20,000-hectare sugar stick ranch expected for ethanol creation may establish one of the models. It is situated in the Tana River Delta in Kenya. With an arranged take-up of 1680 m^3 of water/min, speaking to about 30% of the waterway stream rate, it represents a genuine danger to the nearby environment, territory for 345 types of water-flying creatures and bog fowls. Also, to create a biofuel, e.g., bioethanol, from corn, vitality is fundamental for development, creation of composts, plant assortment, and preparation into fuel during aging and refining. Utilizing the LCA method, it was uncovered that the measure of CO_2 delivered per unit of vitality got from bioethanol, produced from corn is higher as much as 60% contrasted with the measure of CO_2 delivered during the ignition of energizes utilizing raw petroleum (Neto et al. 2019). Indeed, even an account of bioethanol production from sugar stick in Brazil, where the biofuel creation is in the most evolved stage plus the rest of the biomass is completely utilized, for example, may be utilized in the creation of warmth, (Cizler 2013; Naqvi and Yan 2015), it has not to be conceivable to diminish CO_2 outflows per unit of yield vitality underneath biofuels that of the emanations from fluid got from raw petroleum.

33.6 Conclusion

This paper has talked about the first, second, and third generations of biofuel, the idea of biorefineries, various sorts of biorefineries, and related specialized difficulties. Nonetheless, developing worries over first-era biofuels in quite a while over their effect on food costs and the earth have prompted an undeniably terrible force down in the last year. The disastrous impact with the intention of biofuel is beginning to create an obstruction, especially in helpless nations utilizing natural plans. Since the substitution for petroleum derivative happens regardless of these worries, the best approach to evade the negative impacts of creating biofuels from food supplies is to make lignocellulosic consequent powers accessible inside the briefest conceivable time (e.g., second-era biofuels). Anyway, the prompt utilization of first-age biofuels includes setting up calculated changes to use biofuels. This pledge to biofuels currently will make the progress to the second-era biofuels all the supplementary financially helpful. Be that as it may, at present the innovation to create these substitution fills is as yet being created. Biorefineries dependent on lignocellulosic will have the option to get to a lot more extensive assortment of feedstock, including wood biomass. In this way, there is a demand to incorporate cycle activity, reactor, plus impetus configuration to progress the adequacy of various cycles utilized for bioproduct and biofuel creation in a regular biorefinery framework. The principle target of the biorefinery is to deliver different items utilizing a blend of advances. Besides the dedication of the science, especially the natural science, it is required that the idea of bio-based items with biorefinery frameworks must reduce constrain of the blend of the biotechnological tools and newer compound development.

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34 Coir Retting: Process Upgradation and Pollution Abatement Through Environmental Biotechnology

T. R. Satyakeerthy and I. S. Bright Singh

Abstract

Coir industry is one among the small-scale industries that earns copious foreign exchange and still continues to employ traditional practices and methods at various stages. Coconut husk is the raw material for the coir industry which is grown and cultivated extensively in India. Retting of coconut husk for the extraction of coir fibre is an age-old process for which the coconut husk is soaked in saline water for a period of 6–12 months. During the initial stage of retting, polyphenols from the coconut husk are released into the aquatic system causing pollution impairing the productivity of the ecosystem. The coir fibre thus obtained after retting should be of good quality for the sustainability of the industry and depends on various aspects of the natural environment like season, location tidal influence, etc. The unhygienic conditions of the people working in the retting yards, long duration for the completion of retting, wait for the returns from the money invested and socio-economic transitions in the society are a cause of concern for this traditional industry. Therefore, it is imperative to develop an alternative eco-friendly and economically viable process for the production of quality retted coir fibre. Thus, the study led to the concept of a coir retting bioreactor consisting of the polyphenol extraction process and the microbial retting of coconut husk in the reactor. The extraction process consists of crushing of the coconut husk, stripping the polyphenols, concentration and lyophilization. A microbial consortium from the retting grounds having saline water consisting of fungi and bacteria like *Aspergillus, Ascomycetes, Fusarium,*

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Enterobacteriaceae, Bacillus, Aeromonas, Pseudomonas, Alteromonas, Micrococcus, Acinetobacter and Planococcus was used for the microbial retting of the coconut husk and could reduce the retting period to 45 days from the usual 6–12 months extracting good quality coir fibre. The bioreactor as a controlled system reduces the aquatic pollution and could be developed as a small-scale enterprise for producing good quality fibre and employment to the weaker sections of the society.

Keywords

Bioreactor · Coconut husk · Consortium · Polyphenols · Retting

34.1 Introduction

The coir industry is one of the small-scale industries in India which earns a lot of foreign income to the country employing traditional age-old practices and methods at various stages of production of coir. The methods employed are time tested and have become part of our environment and the rich cultural heritage. Retting of coconut husk for the extraction of coir fibre, being the backbone of the industry, is one among such practices which has the traditional flavour and colour, though it is not unknown as to when the practices came into existence.

34.1.1 Traditional Practice of Coir Retting

Traditional coir retting is practised by soaking the coconut husk in saline/brackish waters or steeping in pits dug out within the reach of the tidal action. Retting is also carried out in canals which have the influence of water from a river and has free connection to the sea. In such areas, pits of four to five metres deep are dug out and the coconut husks are dumped and covered with mud. In estuaries, where there is tidal influence, husks are bundled together called ‘mallis’ and dumped into the water. Rocky shores are also used for retting where the cavities on rocks or space between adjacent rocks on the seashore are used. Another method practised is by arranging the husk in coir nets into bundles and floating them freely into the backwaters until they get soaked, become heavy and gradually sink into the bottom. Often the bundles are weighed down by piling on their top with mud collected from the bottom of the retting yards.

Unlike the other plant fibres which get released within a few days of retting, coir fibre takes a long time to get separated from pith, the binding material. Thus, the retting time varies from 4 to 12 months depending on the area and the variety of the yarn required to be produced.

Properly retted husks are removed from the retting grounds, washed free of adhering slime, mud or sand and the exocarp of the coconut husk is easily peeled off with the hand. Thereafter, the husks are placed on granite stones and beaten with wooden mallets so as to loosen the husk and separate the fibre from the coir pith. Further cleaning with water and lightly beating the fibre obtained are resorted to get rid of the adhering pith and the fibres are then spread out to dry (Fowler and Marsden 1925).

34.1.2 Environmental Impairment Due to Retting

Coir retting though a traditional and age-old process is not an environment-friendly practice due to the fact that it takes place in backwaters which support rich and diverse life forms and provide crucial nurseries for shrimp and fishes as well as serve as habitat for oysters, clams and mussels. Retting adversely affects the productivity of the backwaters (Najee and Philipose 2013).

During the initial phase of retting, enormous amounts of polyphenols are liberated into the water causing acute toxicity to the aquatic environment. In the retting process, lignolytic, pectinolytic, cellulolytic and hemicellulolytic organisms together degrade the fibre binding material of the husk liberating large quantities of organic matter into the environment which includes pectin, pentosans, polyphenols, etc. (Abraham et al. 2013). This leads to heavy biological oxygen demand with enormous production of hydrogen sulphide. Obviously, community diversity of plankton decreases (Remani et al. 1989), and ecosystem with micro aerobic and anaerobic properties emerge in retting zones which subsequently exterminates the flora and fauna (Abdul Azis and Balakrishnan Nair 1978). Survey of fishery wealth showed that the fishery in the retting zones are adversely affected in terms of community diversity as well as zoomass productivity. Only tolerant species like *Arius* sp., *Etroplus* sp., etc. were found in the retting grounds. Fish biomass in the retting zones were three times less than that in the non-retting zones. Similarly, the abundance of molluscs and crustaceans were also much lower in the retting zones when compared with the non-retting zones (Abdul Azis and Balakrishnan Nair 1978).

34.1.3 Inconsistency in the Quality of Fibre

For any industry to achieve sustainability, the raw material should be consistent and of good quality. This requirement is not always met within the coir industry, where the quality of coir fibre, which is the raw material, is left to the vagaries of nature as the quality of fibre varies from season to season and place to place. Retting yards with very good tidal influence generate comparatively good quality fibre because aeration leads to faster degradation of the products of fermentation which under normal conditions tend to accumulate adversely affecting the quality of fibre (Bhatt and Nambudiri 1971). A better control over the quality if this raw material has to be acquired for the smooth functioning of the coir industry.

34.1.4 Other Socio-Economic Considerations

Being a traditional industry, the people involved are also from the marginal section of the society and are especially true with those working in the retting yards. With augmentation of technology, opening up of the economy and other job opportunities, the new generation are reluctant to work in such environments (Akhila and Emilia Abraham 2008). This sociological transition will adversely affect the industry at large in the coming days. The long durations required for the completion of the retting makes one to wait for getting the return for the money invested. Further, it is also seen that the retrieval of the entire lot of coconut husk dumped in the retting grounds is also difficult leading to substantial loss. Painfully enough it is seen that people working in the unhygienic retting yards are also prone to skin diseases, headaches and nausea.

Considering all these factors, an alternate process for the production of retted fibre has been conceptualized which can perpetuate the pristine glory of the golden quality retted coir fibre. Thus, the concept of coir retting in a bioreactor has been envisaged so that it is environment-friendly and economically viable.

34.2 Coir Retting in a Bioreactor

Coir retting in a bioreactor consists of the polyphenol extraction process and the microbial retting of coconut husk in the reactor. The extraction process involves crushing of coconut husk, stripping the polyphenols, concentration and lyophilization. Once the extraction process is complete, the husk is prepared for microbial retting. The whole operation is aimed at bringing down the polyphenol content to facilitate the microbial process of retting immediately, the polyphenol extracted forms the by-product of the process.

34.2.1 Crushing

Crushing the coconut husk can be done either manually or mechanically wherein the process leads to loosening of the husk for faster liberation of polyphenols and an immediate invasion of the microbial flora for retting (Fernando and Amarasinghe 2017). Besides, crushing straightens the husk and facilitates spacing in the bioreactor, a requisite for economic viability.

34.2.2 Polyphenol Stripping

The stripping of polyphenols is done in a tank made of fibre glass (40 $\times$ 40 cm) having a perforated platform positioned 10 cm above the base to support the husk and can hold ten freshly crushed coconut husks weighing around 8–10 kg. At the bottom of the tank an outlet pipe of 2 cm diameter connects with a 0.25 HP

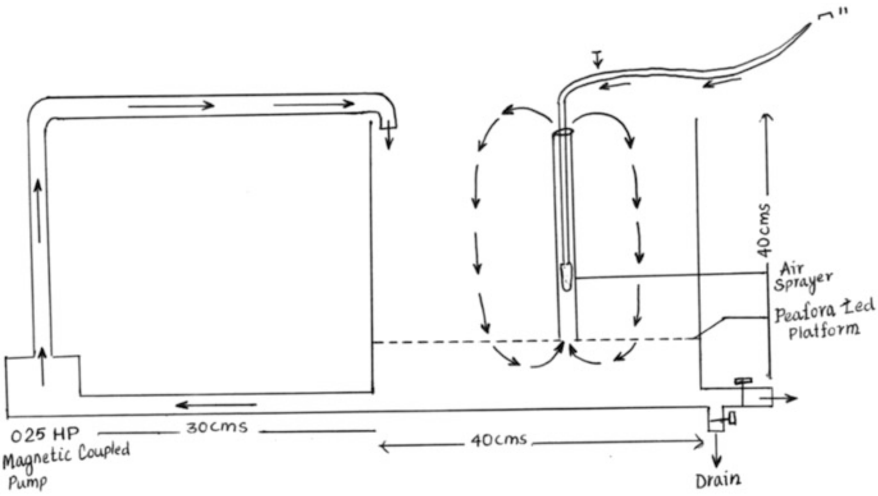

Fig. 34.1 Coir retting bioreactor

monoblock magnetic coupled electric pump which facilitates drawing water forcefully from the bottom and discharges at the top of the tank, thereby providing a strong circulation of water at a rate of 40 L per minute. Through another outlet pipe at the opposite side of the tank, the ret. liquor can be drained off for concentration of the polyphenols. The pipes connecting the tank and the valves provided for the outlets are made of PVC and polypropylene, respectively, to avoid corrosion (Fig. 34.1).

The stripping of polyphenols was carried out using 5 ppt diluted sea water to facilitate maximum liberation of polyphenols (Satyakeerthy 1999). Freshly split coconut husk is crushed either manually or mechanically and arranged in the tank with the split side upwards and is filled with 5 ppt sea water. After soaking for 6 h, the pump is operated for another 18 h with intermittent stops. After 24 h of operation, the ret. liquor is drained off as this contains the maximum quantity of polyphenols (Satyakeerthy 1999). Further maintenance of the ret liquor in the reactor leads to degradation of polyphenols, and a second stage of stripping with freshly added 5 ppt sea water does not yield any more polyphenols.

34.2.3 Concentration and Lyophilization of Polyphenols

In order to remove the polyphenols from the drained off ret liquor and for its concentration, several methods such as solvent extraction, adsorption onto activated charcoal and reverse osmosis were employed. Among all these methods, reverse osmosis was found to be the most efficient in terms of percentage separation and recovery of polyphenols (Satyakeerthy 1999). The experiment setup consists of a flat membrane module with a cellulose acetate membrane. An aliquot of ret. liquor is introduced into the module through its inlet port and subjected to a constant pressure of 100 psi. Initial concentration of polyphenols in the ret. liquor is determined, and the rejection of the polyphenols from the permeate is calculated using the equation,

$$R = 100 \times \left(1 - C_p/C_f\right)$$

where C_p is the permeate and C_f is the corresponding feed side concentration. By this method, 95% rejection of polyphenols could be obtained. The polyphenols concentrated by reverse osmosis were lyophilized using a benchtop lyophilizer (FTS System Inc., USA). The lyophilized mass is a whitish powder with a slight brown colouration which is Seitz filtered and stored at 4 °C for further use.

34.2.4 Development of Microbial Consortium for the Bioreactor

In nature, even though the changes made by the microbial population are very slow, the concerted action by a variety of microbes from the retting grounds in a justifiable proposition can be used to develop a consortium of microbes with the husk as the substratum and can be used as the inoculum in the bioreactor.

Partially retted husks along with 500 g sediment and 1 L from an ideal retting ground from Kollam wherefrom golden coir fibre is extracted were drawn and used as the source of microbial consortium. The husk and sediment samples were wrapped in a polythene cover and the water in a autoclaved saline bottle and are placed in a thermomould box and transported to the laboratory at 4 °C. In the laboratory two to three pieces of the husk obtained along with the water and sediment samples were further incubated in 20 ppt sea water in a 40 L container and aerated continuously. As nutrients, 5 g NH_4Cl/L, 1 g NH_4NO_3/L and 1 g KH_2PO_4/L were added and the pH adjusted to 6.5. Whenever the pH dropped to the acidic range, it was adjusted to 6.5 using 1 N NaOH. This preparation was subjected to differential enrichment and isolation for identifying the microbes in the retting ground. Based on a series of screening procedures, three fungal cultures, namely, *Aspergillus* sp., *Ascomycetes* and *Fusarium* sp. and eight bacterial cultures, namely, *Enterobacteriaceae, Bacillus, Aeromonas, Pseudomonas, Alteromonas, Micrococcus, Acinetobacter and Planococcus* were found predominant in the system and form the microbial consortium. After 2 weeks of incubation, four pieces of fresh husk stripped of polyphenols were introduced in the preparation and incubated for softening of the husk. Periodically, one fourth of the water was replaced with fresh 20 ppt sea water. The whole preparation is the main stock of the consortium.

34.2.5 Effect of Aeration and Maintenance of Stock

Three litres of sea water with the mineral base medium and pH adjusted to 6.5 were introduced into two 5-L jars each. Sufficient quantity of the above stock culture was inoculated into each jar. One of the jars was maintained with gentle aeration and the other in an anaerobic condition. Fresh coconut husks stripped off polyphenols were introduced into each jar and the pH was maintained at 6.5 throughout the experiment. After a period of time, it was found that the husk in the aerated jar was considerably softened, whereas the husk in the jar maintained in anaerobic condition had no remarkable softness. Moreover, the pH in the aerated jar fluctuated up to 4, whereas in the anaerobic jar there was no noticeable change.

20 ppt 500 mL of sea water supplemented with the mineral medium mentioned in the previous section is taken in two 1-L conical flasks each and autoclaved at 15 lbs for 15 min. From the main stock of consortium maintained, few pieces of husk are taken out and made into small pieces and introduced into the flasks. Along with this 50 mL of the consortium is also added into each flask and kept over a magnetic stirrer for proper mixing and aeration. The pH of the flasks is maintained at 6.5 throughout the experiment. The nutrient levels (phosphate and ammonia) were determined once in 2 weeks, and whenever there was a depletion in nutrients, it was replenished by adding fresh aliquots. Fresh small pieces of husk stripped off polyphenols were introduced into the above flasks and were observed periodically for softening.

The consortium thus developed in conical flasks and maintained over the magnetic stirrer exhibited profound activity as the piece of fresh husk into the system for disintegrated within a week along with drop in pH.

Continuous drop in pH during the maintenance of the consortium was a problem to be addressed. Adjusting pH every day is cumbersome and while commercializing the technology it would turn out to be expensive too. To address this issue, 20 g crushed pieces of clam shell were incorporated in the basal medium while autoclaving. Incubation of the husk along with the microbial consortium in this medium exhibited a steady state of pH and faster disintegration of the fresh pieces of the husk. Therefore, this was followed as the procedure for maintenance of the consortium.

34.2.6 Activity of Consortium

A mini reactor was set up and about 600ml autoclaved 20 ppt sea water mineral base medium was dispensed into it and pieces of coconut husk which are stripped off with polyphenols are introduced. It was then inoculated with 60 mL of microbial consortium maintained in the laboratory. It was aerated and the pH adjusted to 6.5 throughout the experiment. A similar setup without inoculation of the microbial consortium was maintained as the control.

It was found that the husks in the mini reactor that has been inoculated have remarkable softening than the uninoculated one. Also, the polyphenol content was very low with a declining trend of the pH value. All these indicate that the consortium developed is active and can be used effectively in the coir retting bioreactor.

34.2.7 Application of Microbial Consortium in Coir Retting Bioreactor

The coir retting bioreactor as described earlier is filled with ten freshly split and crushed coconut husks and arranged in the tank with the split side upwards and subjected to polyphenol stripping as mentioned earlier. After draining off the ret. liquor for concentration of polyphenols, the coconut husks are removed and reactor tank is filled with a 2 cm-thick crushed clam shell on the perforated platform and then packed with the coconut husks. The reactor is then filled with 20 ppt sea water, and commercial-grade ammonium phosphate to the final concentration of 1% is added and pH adjusted to 6.5 using 10 N NaOH. The bioreactor is inoculated with 1 L of the microbial consortium developed as above, and the airlift pump operated to keep the fluid under circulation and to oxygenate the system sufficiently. The performance of the bioreactor is monitored in terms of polyphenol content, pH and softness of the husk which are monitored intermittently (Table 34.1). Evaporation loss if any is supplemented accordingly.

Though there was an increase in the polyphenol content in the initial phase, the concentration decreased in the later phases and the colour of the water was very clear. After about 20 days, the husks started to become soft which progressed and attained satisfactory softening within 45 days, and at this stage the pericarp could be easily peeled off and the fibre recovered from the husk by the conventional method of beating it 10–15 times, removing the pith and washing.

In the coir retting bioreactor, one of the difficulties observed was differential retting whereby few husks were found to be less soft and less retted when compared to the majority of the husks. This sort of differential retting happens in the natural environment also; however, it has to be addressed specifically in the future.

34.2.8 Effluent Quality and Quality of the Pith

The quality of the effluent generated during the retting is assessed in terms of the polyphenol content summarized in Table 34.2. Accordingly, it is seen that the polyphenol content builds up in the initial phase of retting, stabilizes later and comes down drastically and registers zero towards the end. The effluent is very clear without any odour, almost neutral pH, and can be reused in the reactor after removing the retted husk completely.

Table 34.1 Performance of the coir retting bioreactor

	Parameters		
Days	pH	Polyphenol conc. (mg/mL)	Softness
1	6.5	0.093	–
2	6.0	0.091	–
4	6.0	0.081	–
6	6.0	0.051	–
7	6.0	0.042	–
10	6.0	0.019	–
15	6.0	0.016	–
20	6.0	0.012	+
25	6.0	0.009	+
35	6.0	0.003	+
45	6.0	0.001	+

‘–’ indicates no softness and ‘+’ indicates softness

Table 34.2 Quality of effluent generated in the coir retting bioreactor

Days	Polyphenol conc. (mg/mL)	pH	Turbidity and odour
1	0.117	6.5	+
5	0.179	7.0	+
10	0.276	7.0	–
15	0.284	7.0	–
20	0.252	6.5	–
25	0.125	7.0	–
35	0.059	6.5	–
45	0.012	6.5	–

'+' indicates turbidity and odour '–' indicates no turbidity and odour

For converting the pith to manure, the most appropriate way of utilizing this solid waste is to have a very low content of polyphenols (<0.1%). Generally, the pith generated from the husk retted naturally satisfies this requirement, in contrast to the pith generated during mechanical defibering. The pith generated from the husk in the bioreactor contained polyphenol comparable to that of the naturally retted husk (0.05 to 0.1%).

34.2.9 Bioreactor as a Controlled System

The bioreactor thus developed is a zero-pollution technology which bioremediates one of the perennial pollution problems of the aquatic ecosystem. The bench-scale reactor designed and developed is for retting ten coconut husks. This can be scaled up to a pilot plant and commercialized as a small-scale unit for retting 500 husks at a time to produce quality coir fibre in a shorter period of time and can be a better livelihood for the weaker section of the society who are working in this segment of the industry.

34.3 Conclusion

The process of retting of coir for the extraction of coir fibre being a traditional industry has to be sustained for the socio-economic development of the country. The traditional process though being practised from age-old times takes long durations for the retting process to be completed, causing heavy pollution to the aquatic environment and affecting the health of the people working in the retting yards. The retting of coconut husk in the bioreactor would invariably reduce the pollution of the aquatic environment and when scaled up into a medium scale industry would help in the economic development of the society at large. Traditional industries are the backbone of our country, and such industries are to be promoted for the sustainable development of the culture and traditions of our diverse nation. The coir industry exports exceed more than 1600 crores per year employing around 6.4 lakh people mostly from the economically weaker sections of the society (Vijitha 2010). Thus, the development of this new eco-friendly technology would invariably boost the revenues once it is accepted. Though other technologies of extracting out coir fibre are coming up, the process developed and explained in this chapter adheres to the traditional age-old process wherein good quality coir fibre could be extracted.

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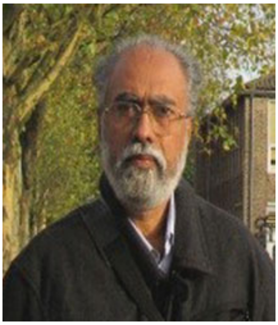

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Cadmium Toxicity in Rice: Tolerance Mechanisms and Their Management

35

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Abstract

Cadmium (Cd) is one of the heavy metal pollutants found in paddy fields, such as rice (*Oryza sativa* L.) paddy fields, which subsequently transfer to the global food chain in various ways. The Cd toxicity in food chain is also creating environmental issues worldwide. Understanding and managing Cd transport mechanisms can reduce Cd uptake and accumulation in rice. It may also improve rice growth and grain quality. Cadmium toxicity harms plant physiological parameters of seed germination, plant growth, mineral nutrients, photosynthetic rate, and grain yield. Plant response to Cd toxicity varies between cultivars, growth conditions, and duration of exposure. Rice plant defense mechanisms such as antioxidant stimulation, osmoregulation of osmolytes, ion homeostasis, and enhanced production of signaling molecules are activated in Cd stress. These are important tolerance mechanisms in rice. This chapter covers the toxic effects of Cd, transport mechanisms in rice, factors affecting Cd uptake (including physicochemical characteristics of soil and ecophysiological features of rice), and efficient measures for immobilization of Cd in soil to reduce rice uptake. These important agronomic, bioremediation, and molecular techniques contribute to food safety by reducing human exposure to Cd.

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Keywords

Rice · Cadmium toxicity · Heavy metal · CRISPR/Cas · Genome editing · *Oryza sativa*

35.1 Introduction

Cadmium (Cd) is a primary heavy metal pollutant in rice (*Oryza sativa* L.) fields, which is transferred to the food chain and creates major environmental concern around the globe. Agricultural soil contamination by heavy metals, like cadmium (Cd), is a widespread toxic environmental problem (Du et al. 2013). Organismal absorption of Cd through foods is well documented (Uraguchi et al. 2009). Rice (*Oryza sativa* L.) is considered as a major staple food for more than half of the world's population (Kosolsaksakul et al. 2014). Cadmium is reported to be readily absorbed by rice root cells and further translocated to shoots and finally into the grains (Wang et al. 2014; Song et al. 2015). In this way, it enters the food chain through rice consumption. Toxic total cadmium (Cd) concentration is generally considered >8 mg kg^{-1}in soil, >0.001 mg kg^{-1} bioavailable, or between 3 and 30 mg kg^{-1}in plant tissue (Chen et al. 2017). The excess amount of Cd in plants interferes with plant physiological processes such as respiration and photosynthesis (Feng et al. 2010; Volland et al. 2014), nutrient uptake, transport, mineral assimilation (Wang et al. 2007), and water uptake (Polle et al. 2013). Additionally, Cd stress alters gene and protein expression, induces or inhibits enzymes, enhances reactive oxygen species (ROS) accumulation and lipid peroxidation, and ultimately leads to plant metabolism disruption (Qian et al. 2010; Irfan et al. 2013).Cd-stressed plants exhibit stunted growth, root browning, chlorosis, necrosis, and even death (Zhang et al. 2014). A minimum concentration of Cd in contaminated soil can also enter into the food chain and cause toxicity to humans (Aziz et al. 2015). Cadmium is classified as a human carcinogen. Rice grown in Cd-contaminated soils challenges current standards of quality food production (Jallad 2015). Minimization of Cd toxicity in rice is crucial for agro-environmental sustainability and food safety worldwide.

Reduction of toxic Cd concentrations within rice grains is a promising approach for decreasing human food-chain exposure. In the recent past, several management practices have been adopted to reduce Cd levels. These include use of plant growth regulators (PGRs) such as abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), glutathione (GSH), phytochelatins (PCs), nitric oxide (NO), brassinosteroids (BRs), and polyamines (PAs) (Cai et al. 2011; Wu et al. 2015a, b; Cao et al. 2015; Farooq et al. 2015). For the paddy field-grown plants, Cd toxicity is mainly dependent on the bioavalability of Cd in soil and presence of other elements which is known to compete with Cd during mineral uptake by plants (Fahad et al. 2015). Studies have reported that applying nitrogen (N), zinc (Zn), iron (Fe), selenium (Se), and phosphorus (P) can reduce the uptake of Cd and its toxicity in rice (Fahad et al. 2015; Zhou et al. 2015; Du et al. 2009). Some other factors, including addition of liming materials fly ash, calcium magnesium phosphate, calcium silicate, and limestone to increase soil pH, are also reported to decrease Cd soil bioavailability (Gu et al. 2013; Mahar et al. 2015). Some inorganic soil supplements perform similarly, such as silicon (Si), gypsum, and bentonite among others. A few organic supplements like manure, compost, and biochars have also been shown to reduce Cd uptake in rice fields (Rehman et al. 2015; Srivastava et al. 2015; Zhang et al. 2015). Selection of a low-Cd-accumulating rice cultivar, adjustment of planting patterns, rotation of crops, water management, microbe application, and use of varying soil patterns also reduced Cd toxicity in this study of rice fields (Yu et al. 2014; Aziz et al. 2015; Yao et al. 2015). This chapter discusses Cd toxicity in rice fields, plant absorption of Cd from soil, methods used to reduce Cd in rice, plant tolerance mechanisms, management measures available for alleviating Cd phytotoxicity in rice, and use of plant-specific genes to produce Cd-tolerant rice.

35.2 Cadmium Toxicity in Rice

Cadmium (Cd) is non-essential and toxic for rice growth and human if accumulated at high levels. Cd toxicity within agricultural land is primarily due to human activity. Human industry contributes about 13,000 of the 30,000 annual tons of Cd added to the environment (Gallego et al. 2012). Both geogenic and anthropogenic (i.e., environmental pollution and pollutants generated from human activity) sources attribute Cd contamination in soils and groundwater. Anthropogenic Cd sources include mining, atmospheric deposition of combustion emissions, and the use of Cd-contaminated fertilizers. Atmospheric deposition of Cd is considered a major source of Cd input in agricultural soils. In paddy fields, both application of phosphate fertilizers and irrigation are major anthropogenic Cd-polluting sources (Kosolsaksakul et al. 2014). Most plants, including rice, take up Cd in the form of Cd^{2+} from the soils. Rice plants differ in uptake rates of cadmium based on soil pH and soil organic matter content (Zeng et al. 2011). Cd is absorbed by plant roots and transported to the stele, passing through endodermis and Casparian strips. At the stele, Cd translocates to shoots via xylem to accumulate within grains (Song et al. 2013). Xylem Cd is regulated by *OsHMA2* and *OsHMA3* (Sasaki et al. 2014; Ueno et al. 2010). Ueno et al. (2009) reported that some quantitative trait loci (QTL) are responsible for relocation of Cd from root to shoot in rice plants (Ueno et al. 2009). For example, rice *OsHMA2* transporter plays an important role in the delivery of Cd to developing tissues (Miyadate et al. 2011; Takahashi et al. 2012). *OsHMA3*, a P-type heavy metal ATPase, plays a critical role in Cd compartmentalization within root cell vacuoles. When Cd influxes into rice plant cell cytosol, Cd is transported to vacuoles through transporter *OsHMA3* and limits Cd mobility in cell cytosol. *OsHMA3* gene mainly expresses in the roots. Cd is preferentially transported to the upper node after being transferred from xylem to phloem and eventually the panicle, instead of leaves (Uraguchi et al. 2011).

Transport of Cd from shoot to grains has been hypothesized to translocate through phloem (Uraguchi et al. 2011, 2014). Cd concentration in phloem has been reported to correlate with rice grain Cd concentrations (Kato et al. 2010).

However, actual mechanisms behind Cd uptake and translocation have not been explored in depth. In summary, Cd is absorbed by the plant roots and translocated to shoots via xylem and finally remobilized and translocated to grains.

During grain maturation, when Cd is transported from roots to grains, two major pathways are involved: (a) either Cd gets directly transported from xylem to the developing grains, or (b) Cd gets transported through the transpiratory parts of the plants such as rachis, culms, flag leaves, and outer panicle parts, followed by rapid remobilization through phloem to grain (Rodda et al. 2011; Uraguchi et al. 2009; Yoneyama et al. 2010). Nodes are also reported to be the central organ for Cd transfer from xylem to phloem and translocation from soil to grain during the grain-filling stage (Fujimaki et al. 2010). The rice *OsLCT1* gene is also reported to be a Cd transporter which is mainly expressed at nodes and responsible for transporting Cd to grains (Uraguchi et al. 2011, 2014). Study also showed that in rice plant, leaf blades, and nodes, *OsLCT1* transporter gene is highly expressed during the reproductive stage of development (Uraguchi et al. 2011).

It is well known that plant roots are the primary tissue that comes in close proximity and direct contact with the soil to absorb heavy metals solubilized in the soil and form the first layer of Cd protection in protoplasts (Fu et al. 2011; Hall 2002). The Cd ions travel into roots via rhizodermis cell walls, from soil solution toward the vascular cylinder (Redjala et al. 2011). There are two parallel paths involved for the transport of Cd via root cortex toward shoot: (a) active transport from cell to cell via symplast (selective transport across membrane) and (b) passive transport via diffusion and convection through the apoplast, where both cell walls and intercellular spaces get utilized (Zhao et al. 2010). The compartmentalization of Cd in the plant root cell wall for exudation is one approach for suppressing plant Cd uptake (Qiu et al. 2011). One plant detoxification mechanism is found in root cell walls that provide functional groups that bond Cd ions together and restrain their movement across the cytoplasm membrane (Qiu et al. 2011). Additionally, vacuoles act as a subdominant Cd binding site to further reduce Cd interference (Wang et al. 2008). Cd is a non-essential plant element, which actively enters plant cells by using essential element (Zn, Ca, and Fe) uptake mechanisms (Lu et al. 2009).

35.3 Methods Used to Reduce Cadmium in Rice

Effective measures to reduce Cd concentration in soil would definitely reduce the uptake and transport of Cd in rice plants as well, and it may also minimize the exposure of humans to heavy metal. The possible measures to minimize Cd uptake in rice have been summarized in the chart (Fig. 35.1): (a) Implementation of various agronomic practices and strategies, for example, fertilizer management which is based on addition of different fertilizers (Yan et al. 2015), soil amendments (Guo et al. 2006), water management (Hu et al. 2015), tillage management (Yu et al. 2014), etc.; (b) second, bioremediation practices can also be utilized which includes phytoremediation (He et al. 2015), microbial remediation (Dixit et al. 2015), etc.; and (c) third important aspect which can be utilized to reduce Cd is through

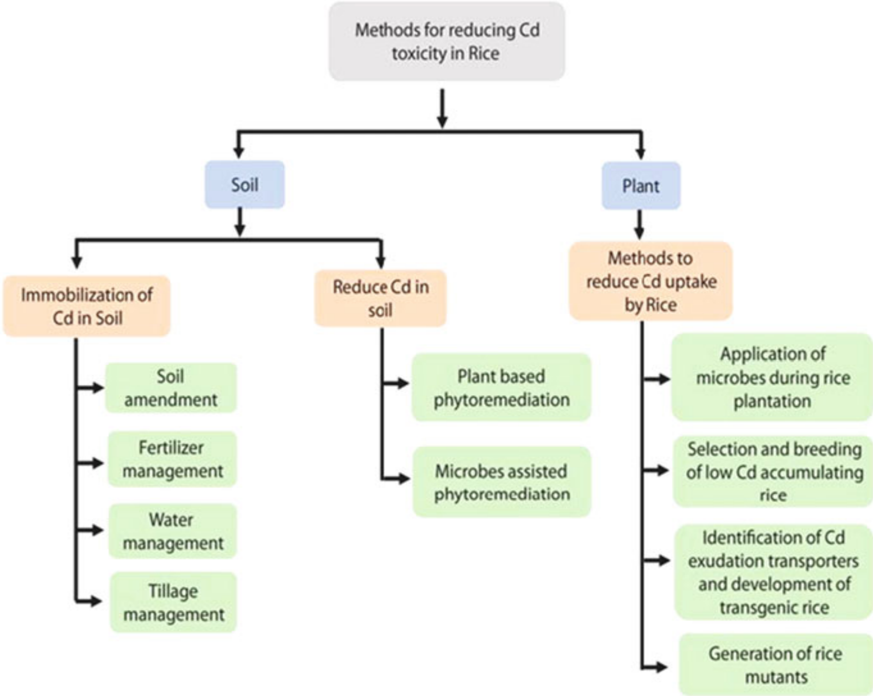

Fig. 35.1 Schematic map of various methods adapted for reducing Cd toxicity in rice

application of molecular biology technologies such as developing transgenic rice (Takahashi et al. 2014) and some rice mutants (Ishikawa et al. 2012) which can reduce the uptake and transport of Cd from soil.

35.3.1 Agronomic Practices

The in situ immobilization of Cd from the contaminated soil is the most viable approach to lower bioavailable Cd concentrations in rice-growing fields. Various agronomic practices, including use of various soil amendments (Guo et al. 2006), use of fertilizer (Yan et al. 2015), water quality (Hu et al. 2015), and tillage management (Yu et al. 2014), are potential strategies for improvement of the physicochemical characteristics of soil that also immobilize Cd. Besides these methods, there are several other available physical and chemical methods that include excavation, transport, and soil dressing (Bolan et al. 2013; Suthar et al. 2014) to improve soil properties. However, it has been observed that these methods are time-consuming, labor-intensive, and costly and can also cause secondary pollution. As a result, their use should be limited to highly contaminated areas.

Mechanisms of Cd immobilization by soil amendments are mainly adsorption, precipitation, cation exchange, and surface complexation (Guo et al. 2006; Shaheen and Rinklebe 2015). Recent efforts have been focused on novel, practical, and cost-effective improvements. Studies have been conducted to assess various emerging and low-cost improvements to immobilize Cd in contaminated soil. In one report, solubility of soil Cd decreased due to high calcium content from the functioning of a nearby limestone and sugar beet factory. This might be due to the presence of calcium carbonate that results in high soil alkalinity (Shaheen and Rinklebe 2015). Another report found in the form of application of biochar which reduced Cd contamination in flood plain soil, indicating biochar, has promise for immobilizing Cd in soil (Rinklebe et al. 2016). Biochar contains shell limestone (carbonate), resulting in high soil acidity. This induces Cd precipitation, reduces its solubility, and promotes sorption by increasing the net negative charge of soil constituents (Karami et al. 2011).

Addition of fertilizers alters the important soil characteristics such as pH, phosphate availability, and their surface charge. By reacting directly with Cd, the added fertilizer converts the mobile Cd into more stable forms (Yan et al. 2015). Efficiency of fertilizer with Cd immobilization in soil was evidenced by Yan et al. (2015), who studied a variety of phosphate fertilizers, including potassium phosphate monobasic (MPP), tribasic calcium phosphate (TCP), calcium superphosphateon (SSP), and diammonium phosphate (DAP). Each of these fertilizers provided the amount of phosphate required to decrease bioavailable Cd in soil. Several advantages of fertilizer treatments are increasing soil pH, enhancing sorption of Cd in soil, and promoting the formation of Cd-carbonate precipitates and their complexes. In a similar study carried out by Ahn et al. (2015), two phosphate-based agents [natural phosphate fertilizer (PF) and mono-potassium phosphate (MKP)] with red mud (RM) stabilizers were studied for stabilization efficiency using Cd, Pb, and Zn in mine tailings. Studies were conducted with both single and combined stabilizing agents. Results from plant-available and non-plant-available species showed MKP/RM stabilizer mix to be the most effective for plant growth and stabilization of Cd, Pb, and Zn in mine tailings. There are several fertilizers that contain NO_3 which neither significantly promotes rice growth nor plays any crucial role to elevate Cd accumulation in rice (Yang et al. 2016). Therefore, fertilizers can be selected to precisely balance rice growth and grain Cd concentration.

Water management is an alternative option for reducing Cd soil concentration. Water changes redox potential and pH of soil, subsequently affecting Cd solubility and availability. Manipulation can reduce Cd exposure to rice plants and accumulation in rice grains (Li et al. 2015a, b; Honma et al. 2016). Controlled experiments at pot level coupled with field experiments showed flooding before and after heading successfully reduced Cd concentration. This same study showed aerobic treatment to increase Cd concentration in rice (Hu et al. 2015). Flooding soil increased soil pH, enhanced mobility of Cd in soil, and lessened Cd in grains (Sun et al. 2007). To decrease the Cd accumulation in contaminated areas, Japan encouraged their farmers to keep paddy fields flooded prior to and after rice plantation. Cadmium ion (Cd^{2+}) is converted to cadmium sulfide (CdS) in flooded conditions (Iimura 1978), and CdS converts back to Cd^{2+} in drained field conditions (Ito and Iimura 1976). Appropriate water management is a practical, low-cost strategy to prevent Cd accumulation in rice.

Crop rotation systems and tillage management (e.g., reduced tillage, conventional tillage, etc.) improve physical, chemical, and biological properties of soil (Liu et al. 2016; Yu et al. 2014). Recently, Yu et al. (2014) reported rotating crops with a high Cd-accumulating oilseed rape reduced Cd contents of rice fields. Similarly, Gao et al. (2010) reported reduced tillage management decreased Cd concentration in grain and accumulation in wheat compared to conventional tillage. This may be due to increased organic matter in reduced tillage soil residual from previous crops, enhancing adsorption and complexation of Cd. The reduced tillage management may also decrease microbial activity and prevent Cd release from residue. Overall, agricultural management practices regarding Cd phytoavailability are vital for understanding and selecting a suitable strategy for optimizing crop yield and lowering Cd concentration in rice grains.

35.3.2 Bioremediation

Bioremediation is an eco-friendly and sustainable approach for eliminating the toxic heavy metals from the surrounding environment. The process utilizes green plants, secretory enzymes, and efficient microorganisms that have the capability to detoxify heavy metals to restore the contaminated areas (Gaur et al. 2014). Bioremediation process refers either to microbial remediation or phytoremediation or in combination of both. Phytoremediation refers to the application of heavy metal detoxifying green plants that have the capability to exudate the heavy metals inside their cells through molecular detoxification mechanism and make contamination free to the polluted sites or alleviate toxic environmental effects (Ali et al. 2013; Luo et al. 2016). Compared to other remediation techniques, phytoremediation installation and maintenance costs are relatively low. Among phytoremediation techniques, the most efficient approach is *phytoextraction* in which the removal of heavy metals and metalloids from polluted soil, sediment, or water can be done (Ali et al. 2013; Liu et al. 2011). Any plant which is capable of growing in a contaminated area with a capacity to absorb excess amount of Cd from soil can be selected for use in phytoextraction (He et al. 2015). Some Cd-tolerant plants, *Arabidopsis halleri* (Ueno et al. 2008), *Solanum nigrum* (Wei et al. 2014), and *Noccaea caerulescens* (Seregin et al. 2015), are known as Cd hyper-accumulators, as they generate relatively less shoot biomass but capture more pollutant Cd (Ali et al. 2013). Some other plant species can also hyper-accumulate Cd to remediate soil, such as moso bamboo. There are two types of bamboo: running and clumping. Some bamboo species that spread quickly through their underground rhizome systems are called “running” bamboos. The second one is the “clampers” type, which bamboo plants generally stay in one place. Moso bamboo (*Phyllostachys edulis*) is one of the running bamboo species. Moso bamboo with its greater biomass and deeper root system effectively remediates Cd-contaminated soil (Li et al. 2015a, b).

Bioremediation has been tested with hydroponic experiments, finding moso bamboo tissue can reach maximum concentration (leaf 25.6, stem 129.8, and root 377 mg kg^{-1}, respectively) at the highest levels of Cd (400 mM) application, although these levels significantly inhibited plant seedling growth (Li et al. 2015a, b). Moreover, some rice cultivars, such as *indica* type, absorb relatively high levels of Cd up to 21.0 mg kg^{-1} (dry weight) in shoots and 3.9 mg kg^{-1} (dry weight) in grains (Arao and Ae 2003). Studies indicate this cultivar can also be used to reduce soil Cd content (Murakami et al. 2007). Planting *indica* rice cultivars (Chokoukoku) for 2 consecutive years in a Cd-polluted area removed 883 g Cd ha^{-1} and eventually decreased grain Cd content of *Japonica* food rice grown subsequently by 47% without lowering grain yield (Murakami et al. 2009). Wide application of high Cd-accumulating rice cultivars for remediation of paddy fields contaminated with Cd is a reliable method, with proper Cd-accumulated plant disposal.

Soil microorganisms influence plant growth, mobility of nutrients, metals, and contaminants in soil. Therefore, microbes are a focus for remediating agricultural soil (Dixit et al. 2015). This requires understanding the complex interaction between metal-accumulating plants and their rhizosphere microbes for effective implementation (Muehe et al. 2015). A group of plant-growth-promoting rhizobacteria (PGPR) found in the plant rhizosphere is capable of increasing heavy metal tolerance and promoting normal plant development. Liu et al. (2015) isolated nine strains of Cd-tolerant PGPR from roots of Cd-accumulating plants. They found that Cd hyperaccumulator plant *Sedum plumbizincicola* inoculated with PGPR strains *Rhodococcus erythropolis NSX2* and *Cedecea davisae LCR1* demonstrated better growth and higher Cd accumulation in shoots: 0.59 and 0.57 mg for inoculating with *NSX2* and *LCR1*, respectively, compared with control of 0.50 mg (in a pot experiment). Similarly, use of rhizospheric microbe *arbuscular mycorrhizal fungi* (AMF) in phyto−/bioremediation of heavy -metal soil is beneficial and widely adaptable. Some AMF are key soil microbes, forming symbiotic relationships with most plant species to enhance water and nutrient absorption, plant development, and tolerance to the plants under heavy metal, drought, and salinity stress (Guo et al. 2013; Shahabiv et al. 2012). Use of AMF has been found to reduce more than one type of heavy metal toxicity in rice. A recent report found rice inoculated with *Funneliformis mosseae* significantly reduced copper (Cu; from Latin: cuprum) concentration in shoots and roots (Zhang et al. 2009). Rice plants inoculated with either single or combined AMF microbial strain decreased arsenic (As) uptake and enhanced grain yield simultaneously (Chan et al. 2013; Wu et al. 2015a, b). Another study conducted by Zhang et al. (2005) suggests inoculation with AMF enhances rice growth and lessens potential shoot toxicity by combining Cu, Zn, Pb, and Cd. Based upon current knowledge, screening beneficial microorganisms to combine with rice and reduce Cd uptake would be beneficial. Before applying microorganisms in any paddy field, field studies are needed to investigate the effect of exogenous microorganisms on indigenous microorganisms. Monitoring of rice growth is a necessity, since rice is more susceptible to a variety of microbial pathogens.

35.3.3 Molecular Technologies

Molecular biology offers widely adapted, alternative options for reducing heavy metal toxicity in soil. Selection and breeding of low-grain Cd-accumulating cultivars can be a good step in reducing Cd accumulation in rice grains directly and decreasing human Cd dietary exposure. Adapting transgenic technology or rice mutants may reduce Cd levels in high Cd-accumulating cultivars. Identification and characterization of heavy metal tolerance genes provides a means of understanding the molecular mechanisms of tolerance and presents a viable option for generating low Cd-accumulating transgenic rice (Sun et al. 2015). A variety of transporter genes are known which have the ability of Cd exudation within plant cells to vacuoles. Appropriate promoters regulate transporter genes in Cd-sensitive rice cultivars. Several genes have been identified which are responsible for translocation of Cd in rice and also varying expression results in differing Cd accumulation. The *OsNRAMP5* gene product is known to function as an important transporter of manganese, iron, and cadmium (Cd). In a recent study, Takahashi et al. (2014) found reduced Cd uptake by gene knockdown of *OsNRAMP5* gene in rice. Transgenic management of Cd transporters has proven to be a viable option to decrease Cd accumulation in rice or promote phytoremediation efficiency (Ueno et al. 2010; Uraguchi et al. 2011). In other Cd-stress-condition rice studies, overexpression of gene fragments derived from two rice *OXS3* (*OXIDATIVE STRESS3*) homologs reduced the toxic Cd accumulation throughout the plant without decreasing total grain yield or nutritional content of Cu, Zn, Fe, and Mn (Wang et al. 2016a, b). Truncated gene fragments derived from rice genes *OsO3L2* and *OsO3L3* were overexpressed (Wang et al. 2016a, b). Similarly, "limited" expression of rice genes *OsO3L2* and *OsO3L3* reduced accumulation of cadmium in transgenic rice (Wang et al. 2019). Use of truncated gene fragments or "limited expression" of full-length *OsO3L2* and *OsO3L3* genes, in a preliminary study in *Arabidopsis*, has showed constitutive overexpression of *OsO3L2* and *OsO3L3* to affect seedling viability (Wang et al. 2016a, b, 2019). A similar study in *Arabidopsis,* overexpressed maize *OXS2* (*OXIDATIVE STRESS2*) homolog genes (from sweet corn, *ZmOXS2b* and *ZmO2L1;* both genes transiently induced by Cd treatment), demonstrated enhanced cadmium tolerance by activating the putative SAM-dependent methyltransferase gene (He et al. 2016).

35.3.3.1 Genome Editing (GE) Technology

In recent years, genome editing technologies have successfully improved traits in various crops. These GE technologies include LAGLIDADG homing endonucleases (meganucleases), zinc-finger nucleases (ZFNs),transcription activator-like effector nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated Cas9 (CRISPR/Cas9). CRISPR technology is widely accepted than the other GE tools because of its simplicity, efficiency, and versatility. CRISPR/Cas9 has revolutionized genome editing for biotic/abiotic stress resistance, improved yield, improved nutritional content, and detoxification of heavy metals. A few years after the technology was invented, the inventors of CRISPR/Cas9 technology were awarded Nobel Prize in Chemistry in 2020 (https://www.nobelprize.org/prizes/chemistry/2020/press-release/). The inventors are Dr. Emmanuelle Charpentier from Max Planck Unit for the Science of Pathogens, Berlin, Germany, and Dr. Jennifer A. Doudna from University of California, Berkeley, USA. The technology has also been widely used to improve rice traits (Zhou et al. 2016; Xu et al. 2016; Li et al. 2016; Wang et al. 2016a, b; Sun et al. 2016), including Cd-in-plant study. For example, Tang et al. (2017) obtained *OsNramp5*-knockout *indica* rice mutants through CRISPR/Cas9 technology. The past studies depicted that the transgene-free homozygous rice mutants had low Cd content in grains without compromising their yield when it was cultivated under high Cd-contaminated soil. It has been found that a rice homolog of wheat low-affinity cation transporter 1 (*OsLCT1*) is highly involved in translocation of Cd and some other important elements from the leaf blades to the grain. In a study, rice mutant lines were generated through CRISPR/Cas9-mediated mutagenesis by knocking out the genes *OsLCT1* (Liu et al. 2019). Cd accumulation and plant growth were analyzed to evaluate the possibility of generating low-Cd-accumulated rice genotypes without compromising plant growth and yield. In a Cd-contaminated field study (contaminated with ~0.9 mg/kg Cd), the researchers observed that CRISPR mutant line grains contained approximately 40% (0.17 mg/kg) Cd content of the wild-type parental line, less than the China National Food Safety Standard (0.20 mg/kg) (Liu et al. 2019).

35.3.3.2 Non-transgenic Approaches

However, conventional genetically modified transgenic rice (or even current CRISPR-generated rice varieties) raises public concerns and is prohibited in many countries. In a study, three non-transgenic *japonica* Koshihikari rice mutants were developed by carbon ion-beam irradiation. Koshihikari is the most popular Japanese temperate *japonica* rice cultivar. Seeds of rice (*Oryza sativa* L. cv. Koshihikari) were irradiated with 320 MeV carbon ions ($^{12}C^{6+}$) at a dose of 40 Gy (Ishikawa et al. 2012). Mutants produced using this method are more likely to be accepted by the general public. These mutant rice cultivars grown in Cd-contaminated paddy fields yielded grains nearing undetectable Cd levels. Moreover, these mutant rice cultivars did not impair plant/grain/straw yield, grain morphology, or consumption quality thus clearly indicating applications for inbreeding programs. They found that the three mutant lines each had a different mutation (a transposon (*mPingA1*) insertion, a single-base pair deletion, and a large deletion) in the same gene *OsNRAMP5* (Ishikawa et al. 2012). Molecular mechanisms behind natural variation between *japonica* and *indica* need further evaluation.

Another alternative strategy is marker-assisted breeding, in which molecular markers are getting used to develop low-Cd rice cultivars (Collard and Mackill 2008). To generate a low-Cd rice cultivar, initially QTL has to be identified from a known low-Cd-accumulating cultivar, and then the low-Cd QTL is introduced into a high-Cd cultivar through breeding approach. This is considered a feasible method for generating new low-Cd rice cultivars (Uraguchi and Fujiwara 2012). Overall, we can say that both the transgenic and non-transgenic methods can provide reduced Cd levels in rice and reduced Cd exposure to human via the food chain.

35.4 Conclusions

Cadmium exposure causes adverse effects on a variety of organisms and negatively ecologically impacts communities. Understanding rice Cd translocation mechanisms and factors affecting Cd accumulation is vital to development of efficient environmental reduction strategies. Substantial advancement regarding basic understanding of Cd tolerance mechanism, uptake, and transport into different parts of plants has been made, and several Cd transporter families in rice have been identified in recent past studies. Additional exploration of rice Cd transport mechanisms in rice requires identification of currently unknown transporters or other molecules identified by advanced molecular biology techniques. The past research progress for the identification of rice Cd transporters can also be adopted and applied to other cereals. Both the physicochemical properties of soil and ecophysiological features of rice play a crucial role in the bioavailability of Cd in rice. Various approaches such as flooding the paddy soils before and after heading, availing feasible bioremediation methods as per the climatic conditions, screening of potential microorganisms which have the ability to detoxify Cd from soil, and transferring of QTLs from low-Cd-accumulating cultivars to high-Cd-accumulating cultivars are considered as feasible methods for reducing Cd toxicity in rice. Further progressive research is still needed to confirm extensive application of these devised approaches. Further study to evaluate residual influences of these amendments used to reduce Cd uptake in rice under variable environmental conditions is important. Considering the significant food safety risk posed by Cd worldwide, future research should be mainly focused on (1) identifying novel transporters related to Cd translocation or tolerance in rice, (2) screening of various microbial populations to identify specific microorganisms which have the capability of reducing Cd uptake by rice, (3) adapting conventional breeding methods to generate non-transgenic rice that are publicly accepted, and (4) generating low-Cd-accumulating rice cultivars with improved yields through advanced molecular biology approaches.

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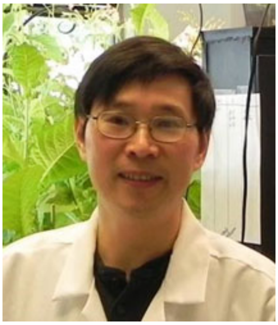

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36 Evaluation of Residual Toxicity of Synthetic Pyrethroids in the Environment

Shashi Meena, Vinod Kumari, and Rakesh Kumar Lata

Abstract

Pyrethroid insecticides are widely accepted because of their high efficacy at lower concentration and relatively safe for humans and other non-targeted animals. Pyrethroids spreading in the nature are mainly due to drift and sprays or surface runoff from agricultural lands and domestic applications. They are rapidly degraded in the environment due to various environmental biotic and abiotic factors. The effect of temperature and relative humidity on persistency and residual toxicity of fenvalerate and cypermethrin deposits on glass surface was evaluated in the laboratory. Temperature along with relative humidity (RH) considerably affected the persistency and residual toxicity of fenvalerate and cypermethrin deposits on glass surface stored at 20 ± 1 °C coupled with 50% RH and 30 ± 1 °C in combination with 70% RH, respectively. High temperature led to increase the concentration of pyrethroids against adults of *Callosobruchus chinensis* exposed to treated glass surfaces, recorded after 1 to 37 days. Rate of mortality declined at lower temperature when kept continuously.

Keywords

Abiotic factors · Cypermethrin · Fenvalerate · Residual toxicity · Synthetic pyrethroids

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36.1 Introduction

Pyrethroids have evolved over the few decades back and comprise a broad-spectrum insecticide family that has been applied for more than 60 years. In the beginning, pyrethrins have been identified as effective chemicals but not have been so far used for crop protection at broad level in the agriculture sector. Currently pyrethrins represent 80% of the entire market of plant-based insecticides. The pyrethrins are chemically modified into synthetic pyrethroids to improve their stability in sunlight (UV). The allethrin was first formed of synthetic pyrethroids synthesized in the year 1949, but the first commercial form came in 1978 as fenvalerate. Till, now more than 1000 pyrethroid molecules have been synthesized. Worldwide, sale of pyrethrins and pyrethroids was positioned second behind organophosphorus in the market of insecticides in the year 1995 with 23% of worldwide sales (Fenner et al. 2013).

The plant-based pyrethrins are a combination of six components which have insecticidal activity: pyrethrin (1 and 2), cinerin (1 and 2), and jasmolin (1 and 2). According to toxicity and molecular structure, pyrethrins were categorized in two main classes, type I (absence of α-cyano group) and type II (presence of α-cyano group). The different isomers may be more or less toxic, but the stereoisomers are known mainly toxic to insects and mammals. Pyrethroids of type II are more potent neuropoisonous than type I pyrethroids, where a-cyano substituent governs the toxicity of type II pyrethroids. First-generation synthetic pyrethroids were developed in the 1960s and significantly more active than natural derivates of pyrethrins which involved many pyrethrin derivatives such as allethrin, bioallethrin, bifenthrin, tetramethrin, d-phenothrin, resmethrin, bioresmethrin, and tetramethrin without cyano group. Synthetic pyrethroid compounds can be recognized with the use of the suffix *-thrin*. They were of limited use due to high photodegradability and lower efficacy against insect pest. Further in the 1970s, a second generation of synthetic pyrethroids was developed including cyhalothrin, permethrin, cypermethrin, cyfluthrin, and deltamethrin with a cyano group in their structure. Later on, other compounds such as fenvalerate, lambda-cyhalothrin, and beta-cyfluthrin were synthesized in the subsequent years. Synthetic pyrethroids were developed after 1970s, were found much effective against agriculture crop pests, are used for indoor homes and public health pests, and became a very popular insecticide in a wide variety of fields. Synthetic pyrethroids are an analogue of naturally occurring botanical insecticide pyrethrum, although having some deviations from the basic chrysanthemic acid ester structure, being composed of an ester linkage between chrysanthemic acid and an aromatic alcohol. Pyrethroids contain chirality of acid moiety, the alcohol moiety or both. A pyrethroid compound consists of two to eight isomers of different biological properties in commercial products. The production of pyrethroid compounds with varied toxicity of the same insecticide has varying isomeric ratios. Several studies have reported that the degradation mechanism of pyrethroids is also based on selection of particular isomers. Synthetic pyrethroids are newly synthesized molecules that tend to be more active and are also used at lower doses (Gupta 1999; Ross 2011).

The environmental hazards posed by conventional insecticides have necessitated the search for some alternative source for applying ecologically viable pest control strategies. Pyrethroids have assumed greater importance in recent years all over the world due to environmental deterioration and health hazards associated within discriminate use of synthetic organic chemicals. Application of synthetic pyrethroid has arisen in the past few years because they are heavily applied to replace the more toxic synthetic chemical organophosphates and other hazardous insecticides. Currently, synthetic pyrethroids cover more than 25% of total pesticide of the world's market. Pyrethroids are the domain insecticide for mosquito's vector control having most common active ingredients in commercially available insect sprays. They have antifeedant and repellent properties; in addition to these they maintain insecticidal activity over a greatly extended period which helps in controlling overlapping generations of pests. These insecticides have direct impact on cells of the nervous system of insects with high toxic potency, when they bind to target receptor proteins, alter sodium channel activity inducing flux of sodium ions, and disrupt the impulse transmission of the neurons, thus leading to paralysis and the eventual death of insects (Clark and Symington 2012; Ross 2011).

Pyrethroids are neurotoxic, interfering with the nerve impulse transmission and involving blockage of sodium ion channel gates which causes elongation in nerve impulse duration. This leads to muscular paralysis and eventually death of the organism. Pyrethroids primarily affect the sodium channel gates of nerve cells; they slow down the opening and closing of these gates, resulting in the excitation state of the neuron, followed by hyperexcitable condition due to high concentration of sodium ions inside the neuron. This leads the neuron in prolonged depolarized state, causing continuous firing of these sensory fibers. Pyrethroids also affect the voltage-dependent chloride channels found in the brain, neurons, muscles, and salivary glands and regulate cell excitability. In the insects, pyrethroids are strongly bound with the sodium channels at low temperatures than at high temperatures unlike as in mammals. Insects' ambient body temperature is approximately 25 °C in comparison to mammals that is 37 °C. Stereospecificity of pyrethroids also affects the binding of pyrethroids upon the sodium channel gates. The *cis* isomers of these insecticides are usually more toxic than the *trans* isomers. At higher concentration, pyrethroids may also act on chloride channels. In the other way, they represent secondary mode of mechanisms which with gamma aminobutyric acid (GABA) receptors, modifying nicotinic cholinergic transmission, induce rate of noradrenaline release and interference with calcium channels. These insecticides showed less chronic health effects to vertebrates although neurotoxicity may occur as an adverse acute effect on high-dose exposures. Synthetic pyrethroids are neither dermal irritants nor sensitizers and non-teratogenic in mammals. Inhalation toxicity and dermal toxicity of these compounds are extremely low. Subacute and chronic doses of these insecticides invariably induced some histopathological changes in the tissues of mammals, though tumorigenicity was not reported yet (Ross 2011).

To date, synthetic pyrethroids have been applied due to mainly their high toxicities to insect pests, allowing them to use in each field with a relatively low concentration. These are broad-spectrum insecticides having extremely high insecticidal activity at very low doses, rapid biodegradation, and increased safety for users. Furthermore, these pesticides were reported to be less toxic to mammals and thus got high preference among insecticides for agricultural industries. Pyrethroids are widely used in domestic, commercial, agricultural, and public health (against insect vectors, e.g., mosquitoes), medical, and veterinary sectors. Synthetic pyrethroids have been introduced in households, agriculture, vegetable, forestry, and public health because of their significant activity against a wide range of insect pests worldwide. In 2018, synthetic pyrethroids had been known to cover a global market value of $ 1.63 billion (Tang et al. 2018). These are used mainly as an emulsifiable concentrate, wettable powders, ultralow-volume concentrates, and various combined liquid formulations (Saillenfait et al. 2015; Rehman et al. 2014).

In comparison to synthetic chemical insecticides (organochlorines, organophosphates, and carbamates), synthetic pyrethroids (SPs) are less harmful to environment because of extreme sensitivity to light, pH, temperature, and moisture. The half-life of the pyrethroids has been significantly reduced in hours at extreme environmental conditions and plasma half-life is generally reported less than 8 h. In the environment, pyrethroids are rapidly degraded by one or more abiotic or biotic factors. Photodegradation, hydrolysis, and the microbial degradation are well-known mechanisms in the breakdown of synthetic pyrethroids which are invariably associated with pH, temperature, presence of oxygen, humidity, and adsorption to sediment particles. Generally, decomposition of pyrethroids is faster in alkaline environments. Natural pyrethrins are decomposed rapidly in sunlight and in the presence of humidity. Synthetic pyrethroids are considered to have half-life between 30 and 100 days in the aerobic conditions, but limited sunlight and air led to increase this duration to 1 year. Cypermethrin is relatively more stable with 8 weeks half-life and showed persistency for 3 months after indoor treatments. Hydrolysis is considered to be slower at indoor treatments, whereas rapid decomposition occurred in field treatment in the exposure of sunlight and high temperature (Clark and Symington 2012; Bereswill et al. 2013; Kumari et al. 2019).

Ecological decomposition is prerequisite for the use of the pyrethroids in agricultural purposes. However, these insecticides rapidly undergo abiotic transformations because of less stability in soil, air, water, and light. Metabolism of synthetic pyrethroids accelerates with hydrolysis of ester linkage, conjugate formation, and oxidation with less bioaccumulation in the organisms. Hydrolysis is a main process of pesticide degradation in alkaline environmental conditions and promotes elimination behavior in soil and water bodies. Pyrethroid compounds are sensitive to temperature, which induce some alterations such as isomerization or ester link cleavage. In the environment pyrethroid metabolism is followed by hydrolysis of the central ester bond and oxidation process at several sites to produce acid and alcohol derivatives. The rate of chemical degradation depends on the type of synthetic pyrethroids, climate, soil, and microbial population. Moreover, degradation pathway and derivates of synthetic pyrethroids have been observed in an oxidizing environment during heating. *Permethrin*, α-*cypermethrin*, λ-*cyhalothrin*, *deltamethrin*, *fenvalerate,* and τ-*fluvalinate* undergo rapid evaporation, oxidation, and char combustion upon thermal exposure. The degradation process accelerates more with increasing temperature with the formation of low molecular weight derivatives. The process of oxidation is followed by production of CO, CO_2, and H_2O at increasing temperature range. Further studies confirmed that synthetic pyrethroids are known to be less efficient at higher temperature; this depends on the implications of many climatologic conditions (Palmquist et al. 2012; Meyer et al. 2013).

The present study was carried out using two synthetic pyrethroids, cypermethrin and fenvalerate. Cypermethrin has been reported as a highly active synthetic pyrethroid against insect pests, synthesized in the year 1974 and marketed first in 1977. Chemically, cypermethrin is an α-cyano-3-phenoxybenzyl-(1RS)-cis, trans-3-(2,2dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate. It is produced by esterification of α-hydroxy-3-phenoxy-phenylacetonitrile with 3-(2,2-dichrovinyl)-2,2-dimethylcyclopropanecarboxlic acid. This molecule embodies three chiral centers, two in the cyclopropane ring and one on the alpha cyano carbon. Isomers of this molecule are formed of four cis- and four trans-forms, the cis-group being the more powerful insecticide. The technical cypermethrin contains more than 90% of the active material and brown-yellow viscous liquid to a semisolid. Cypermethrin is less soluble in water but highly soluble in the various organic solvents and has very low vapor pressure. Fenvalerate is (RS)-α-cyano-3-phenoxybenzyl-(RS)-2-(4-chlorophenyl)-3-methylbutyrate. It is an esterification product of 2-(4-chlorophenyl)-3-methylbutyrate acid with α-hydroxy-3-phenoxy-phenylacetonitrile. Fenvalerate has four stereoisomers with no cyclopropane ring in the molecule. Technical fenvalerate is yellow viscous liquid with mild chemical odor. It is almost insoluble in water, but soluble in most organic solvents (Gupta 1999; Callinan-Hoffmann et al. 2016).

Pyrethroids are found to be more soluble in fats than in the water and may be runoff from the treated surfaces during rain. Agricultural runoffs may cause higher concentration in the summer in surface waters than in the winter. Since pyrethroids tend to bind strongly to particles, their toxicity in sediments might be more important than that expressed by the dissolved water concentrations. Organic pyrethrins are degraded quickly by the sunlight (photodegradation) and in the presence of the moisture (hydrolysis). Although synthetic pyrethroids are more stable, this family of pesticides is generally considered to degrade rapidly in the environment as compared to other insecticides. The synthetic pyrethroids are degraded through different routes depending on the ecological biotic or abiotic conditions in soil or water. In the environment by one or more biotic and abiotic processes, degradation of pyrethroids is very fast and adsorption to sediments may be a very rare process through degraders. The environmental persistence of pyrethroids is usually measured very low, but this varies with the active component of interest and with ecological conditions. The patterns of uses and precipitation are two most significant factors affecting pyrethroid amount and deposition in surface water (Chapman and Cole 1982; Todd et al. 2003).

The toxicity of the insecticide must continue for a long duration with minimum change and degradation of its effectiveness on a surface. The amount of initially laid down insecticidal chemical after application on the surface is termed as “deposits,” while the amount of insecticide left over after a lapse of time may be referred as "residue." Thus, residue is the ultimate product of the initial deposit of insecticide leftover to be formed by the climate and other factors in due course of time. An insecticide with the property of long-lasting persistency and residual toxicity is required on the various surfaces to control insect pests, which appear more often at same place, so that they can be actively involved continuously in killing, that rested on them thus avoiding frequent retreatment. Effectiveness of the persistency and residual toxicity of insecticides is of considerable practical importance in making recommendation for their use to control pest. Moreover, ecological factors greatly affect the insecticide-treated surface and play an important role in reducing persistency and residual toxicity of an insecticide. Further, low humidity and high temperature lower the effective duration of the residual deposits of insecticides, and high level of relative humidity is needed for efficient use of insecticides (Boina et al. 2009). Various studies also reported that lower temperature reduces toxicity and increases persistence of insecticides (Pradhan 2009; Ismail et al. 2012). Several workers also evaluated the effect of temperature and relative humidity on the persistency and toxicity of synthetic pyrethroids (Boina et al. 2009; Harwood et al. 2009; Muturi et al. 2011; Glunt et al. 2014). High temperature increases volatization of insecticides and showed positive relationship with temperature. Decomposition of natural pyrethrins, bioallethrin, bioresmethrin, and tetramethrin by thermal fogging using the Swingfog Model SN6 and the Lightweight TIFA have been also documented (Schummer et al. 2010). Residual effectiveness of insecticides is of considerable practical importance in making recommendations for their use.

36.2 Residual Toxicity of Fenvalerate and Cypermethrin

Kumari et al. (2019) investigated the combined effect of temperature and RH on the persistency and residual toxicity of fenvalerate and cypermethrin films on the glass surface. Formulated concentrations of fenvalerate (40, 80, and 160 ppm) and cypermethrin (4, 8, and 16 ppm) were deposited as insecticidal films (1 mL of each concentration) on glass surface (10 cm diameter) by micropipette only on one side of glass surface. The surface was allowed for drying for 15 min to evaporate off solvent, leaving behind a thin film of respective concentration of fenvalerate and cypermethrin. Carbon tetrachloride only served as control. Each treatment was replicated three times. *Callosobruchus chinensis* (20 adults) were used as test animal to analyze two levels of temperature 20 ± 1 °C and 30 ± 1 °C in combination with relative humidity of 70 and 50%, respectively. Data of mortality was calculated and corrected by using Abbott's formula (Abbott 1925) and probit analysis was subjected to determine the lethal concentration (Finey 1971) (Tables 36.1 and 36.2).

Table 36.1 Residual toxicity of fenvalerate and cypermethrin on the glass surface to *Callosobruchus chinensis* (Linn.) adults under different temperature and relative humidity conditions

		Percent corrected mortality							
		Fenvalerate concentration (ppm)				Cypermethrin concentration (ppm)			
Ecological factors	Days after treatments	40	80	160	Control	40	80	160	Control
30 ± 1 °C in combination with 50% RH	1	87.76	100	100	1.67	81.93	96.39	100	16.67
	5	73.33	95	100	0	71.13	91.75	100	3.33
	9	50	86.67	98.33	0	56.67	83.33	96.67	0
	13	33.68	66.32	94.74	5	40	68.33	93.33	0
	17	16.33	43.88	84.69	1.67	25	50	85	0
	21	5	23.33	65	0	11.83	35.48	69.89	6.67
	25	1.02	8.16	38.78	1.67	5.26	17.89	52.67	5
	29	0	1.67	15	0	1.67	6.67	36.67	0
	33					0	3.33	15	0
20 ± 1 °C in combination with 70% RH	1	83.87	97.85	100	6.67	77.55	94.9	100	1.67
	5	76.47	96.47	100	15	73.33	93.33	100	0
	9	66.67	93.33	100	0	63.33	86.67	98.33	0
	13	48.33	86.67	98.33	0	51.81	75.9	96.39	16.67
	17	33.33	70	95	0	38.95	65.26	91.58	5
	21	16.67	48.33	86.67	0	23.33	50	83.33	0
	25	6.45	26.88	69.89	6.67	11.67	33.33	70	0
	29	2.11	10.53	50.53	5	5	16.67	53.33	0
	33	0	5	26.67	0	2.11	7.37	36.84	5
	37					0	4.35	18.48	8.33

Table 36.2 Toxicity of fenvalerate and cypermethrin deposits on glass surface to *Callosobruchus chinensis* (Linn.) exposed to different temperature and relative humidity conditions

Ecological factors	Days after treatments	LC_{50} (ppm)	
		Cypermethrin	Fenvalerate
30 ± 1 °C in combination with 50% RH	1	1.9498	–[a]
	5	2.6002	26.9153
	9	3.5892	38.9045
	13	5.188	56.8853
	17	7.4989	83.1764
	21	10.8393	127.3503
	25	15.6675	199.5262
	29	23.7137	316.2278
	33	37.4111	–[b]
20 ± 1 °C in combination with 70% RH	1	2.1878	20.6538
	5	2.4547	25.1189
	9	3.1989	30.1995
	13	4.1687	39.355
	17	5.4325	54.325
	21	7.6736	78.5236
	25	10.9648	116.1449
	29	16.4059	167.8804
	33	23.2243	239.8833
	37	33.1131	–[b]

LC_{50} (ppm) concentration in ppm to give 50% mortality
[a]Deposit gave 100 or 0% mortality at two concentrations; hence LC_{50} could not be calculated
[b]No observations

36.2.1 Fenvalerate

Corrected mortality on deposits of 40, 80, and 160 ppm of fenvalerate was recorded as 87.76, 100, and 100%, respectively, on day 1 and decreased to 0.00, 1.67, and 15%, respectively, on day 29 at 30 ± 1 °C in combination with 50% RH. On the contrary, at 20 ± 1 °C in combination with 70% RH, corrected mortality was observed to be 83.87, 97.85, and 100% on day 1 and 0.00, 5, and 26.67% on day 33 on exposure of 40, 80, and 160 ppm, respectively.

The residual toxicity of 40 ppm fenvalerate deposits on glass petri dish was significantly affected by high temperature (30 ± 1 °C) and low RH (50%) and remained effective till 25 days, whereas at low temperature (20 ± 1 °C) and high RH (70%), the same concentration lasted for 29 days. Similar trend was also observed at high concentration treatments. In case of higher concentration of fenvalerate when exposed to high temperature with low humidity, the toxicity was observed only up to 29 days. However, deposits of higher concentrations remained toxic even after 33 days at low temperature with high humidity. The LC_{50} of 20.6538 ppm for deposits of day 1 and 239.8833 ppm for deposit of day 33 on exposure to low temperature coupled with high RH was recorded, as compared to 26.9153 ppm for deposits of day 5 and 316.2278 ppm for deposit of day 29 on exposures to high temperature with low humidity.

36.2.2 Cypermethrin

Corrected mortality of 81.93, 96.39, and 100 was observed after day 1on deposits of 4, 8, and 16 ppm of cypermethrin which reduced to 0, 3.33, and 15, respectively, on day 33 of deposits stored at 30 ± 1 °C and low RH (50%). However at temperature 20 ± 1 °C coupled with 70% RH, deposits of same cypermethrin concentrations caused percent corrected mortality of 77.55, 94.90, and 100 on day 1 which decreased to 0, 4.35, and 18.48, respectively, on day 37.

Increased residual mortalities on treated petri dishes were observed for all three treatment concentrations (40, 80, and 160 ppm) at low temperature and high humidity indicating high persistency of toxicity with this combination. The results showed that at 20 ± 1 °C coupled with 70% RH, the toxicity of three concentrations of cypermethrin lasted till 33, 37, and 37 days, respectively, while it was only 29, 33, and 33 days at (30 ± 1 °C) and 50% RH. LC_{50} of 1.9498 ppm on the first day of deposit increased to 37.4111 ppm on the 33rd day of deposit stored at high temperature in combination with low RH. On the contrary, LC_{50} of 2.1878 ppm and 33.1131 ppm, respectively, was recorded for first day and 37th day of deposits when stored at low temperature and high RH combinations.

36.3 Degradation and Persistence

The residual toxicity was greatly influenced by high temperature and had a more deleterious effect on the residual toxicity of fenvalerate and cypermethrin than low temperature. In contrast with organophosphates, pyrethroids tested against *Musca domestica* showed a negative association with temperature. The toxicity of cypermethrin decreased by 1.46- and 1.52-fold at 27 and 34 °C, respectively, when compared with the toxicity at 20 °C, with an overall 22.21 temperature coefficient (Ali Khan and Akram 2014). Similarly, the toxicity of deltamethrin decreased with the increase in temperature range, with an overall negative temperature coefficient of 22.42. The results are in accordance with the already reported impact of temperature on pyrethroid toxicity with different insect species (Kookana et al. 2010; Muturi et al. 2011; Glunt et al. 2013).

Present study shows that required rate of synthetic pyrethroid concentration increased with passing of days after 1 to 33 at high temperature (30 ± 1 °C) coupled with 50% RH, and residual toxicity was comparatively low at minimum of 20 ± 1 °C in combination with high humidity (70%). Earlier, it was reported that elevated relative humidity, temperature, and photo exposure increased rapid pesticide degradation and volatization (Kookana et al. 2010). Results are in accordance with the findings of Singh and Kavadia (1998) who reported that high temperature (45 °C) coupled with low humidity (60 ± 5%) had more deleterious effect on the residual toxicity of insecticides than low temperature (25 °C) and high humidity (80 ± 5%). Palmquist et al. (2012) demonstrated effects of increasing temperatures on low persistency, and residual toxicity of insecticide deposits on glass surface is due to increased volatization. A positive relation between temperature and rate of volatization of insecticides has been reported by several workers (Harwood et al. 2009; Glunt et al. 2013). Other possible reason for reduction in persistency and residual toxicity at high temperature and low RH could be high volatility of the solvent which also plays an important role in retaining the activity of insecticide deposits, resulting thereby in a decrease in effectiveness. Further it is quite likely that high temperature has created hot conditions which may be adverse not only to deposits for retaining toxicity but also to physiological state of insects and thus cause a loss of insecticide residue, and low humidity accelerated the rate of loss and helped in creating such conditions. These findings were supported by Glunt et al. (2014) who reported that cooler conditions after spraying do increase the toxicity of pyrethrins, lauryl thiocyanates, and DDT.

Mobility of insecticide particles also depends on ecological factors. Possibly the decreased uptake of insecticides by insects at high temperature and low RH accounts for greater loss and degradation of residual toxicity as compared to low temperature and high RH (Trunnelle et al. 2014).Temperature can affect the degradation of toxicant which accumulated on leaf surfaces, soil, and water surfaces and finally reduce the accumulation on the food chain (Radford et al. 2014). Many documented research revealed that temperature potentially affects insecticide degradation and involves in rapid breakage of ester linkage. Isomerization usually did not produce during heating of the pyrethroids in the presence of potassium chlorate in solution. Salt-catalyzed thermal degradation of *cis*-permethrin and β-cypermethrin occurred when potassium chlorate was present, in which cyclopropane isomerization, ester bond cleavage, and oxidation of the resulting products were observed. Permethrinic acid, 3-phenoxybenzyl chloride, alcohol, aldehyde, and acid were identified along with 3-phenoxybenzyl cyanide from β-cypermethrin. A similar decomposition pattern occurred in pyrethroid fumigant formulations after combustion. However, some investigations have been revealed that decomposition of thin film of trans-allethrin, trans-tetramethrin, trans-dimethrin, and pyrethrin I on the glass surface occurred with a sunlamp at 40 °C in the presence of oxygen. The rate of decomposition of these pyrethroids varied from 0.2, 4, and 8 to 16 h, respectively. More than ten acids, including trans-chrysanthemic acid, in saponification of the products are yielded. Subsequent oxidation during decomposition gave rise to alcohol, aldehyde, and carboxylic acid derivatives of the trans-methyl group of the isobutenyl moiety. Oxidation mechanism of the isobutenyl double bond gave rise to keto derivative and esters of trans-caronic acid. Epoxidation of the isobutenyl double bond along with photodegradation produces cyclic ozonide-type peroxide intermediate, with further forms many ester derivatives. The (+)-trans-chrysanthemumic acid, phenylacetic acid, benzyl alcohol, and benzoic acid are formed, whereas the alcohol component, 5-benzyl-3-furylmethanol, is absent. In environment the isobutenyl double bond and the terminal methyl group of the chrysanthemumic acid moiety existed for a very less time period. A thin film of (+)-trans-resmethrin decomposed rapidly within a few hours, permethrin showed half-life more than 20 days moreover, and phenothrin intermediate half-life is of 6 days. Trans-resmethrin decomposed within 1 day when exposed to outdoor sunlight (Delorenzo et al. 2009; Boina et al. 2009; Liu et al. 2009; UN 2011). Temperature critically affects the adsorption equilibrium and rate of pesticide elimination (Kaneko 2011; Thatheyus et al. 2013; Otieno et al. 2013). Furthermore, temperature range of 14 to 39 °C critically affected the intoxicated adult *Anoplotrupes stercorosus* survivability with insecticides belonging to phosphoorganic insecticides (diazinon), carbamate (pirimicarb), quinazolines (fenazaquin), oxadiazine (indoxacarb), benzoylurea insecticides (teflubenzuron), neonicotinoids (acetamiprid), and pyrethroids (beta-cyfluthrin). These results had confirmed significant positive temperature correlation (Piechowicz and Grodzick 2013). Toxicity of fenvalerate was affected by changing temperature gradients to silkworms (Li et al. 2013). A negative correlation between temperature and toxicity of synthetic pyrethroid was confirmed and suggested that higher temperature conditions will promote the elimination rate of pesticides (Harwood et al. 2009). Moreover, temperature and exposure time period influences the rate of persistence of esfenvalerate (ES-FV). He reported the amount of recovery of ES-FV was 14.63 and 9.28 ppm, after 1 day and 3 days of 45 °C exposure, respectively. Rapid chemical modifications of chiral pesticides provide instability under various temperature conditions and may undergo racemization between isoforms (Soliman 2012). In open conditions, temperature and pH significantly influenced the dissolution and rate of elimination of fenvalerate isomers. The results showed that the half-life and elimination rate ranges of trans-FV were 4.75–11.95 days and 65–93%, respectively, while those of trans-FV were 4.60–11.82 days and 67–93% and those of cis-FV were 4.94–12.04 days and 64–92%, respectively. The rate of elimination of trans-FV was reported better than that of cis-FV (Zhang et al. 2018).

Synthetic pyrethroid residues are degraded or broken down by both biotic and abiotic mechanisms. The efficacy of natural pyrethrins and synthetic pyrethroids is known to be more at lower temperatures than higher to targeted organisms as well as non-target organisms. The structure of the soil, the weather, and the profusion and diversity of microbes influence the degradation rate of the pyrethroids. In general, pyrethroids have 25 to 100 days of average half-life in aerobic conditions and are believed to have alike physicochemical properties affecting their movement and persistence in the environment. Multiple studies have shown that insecticide degradation occurs mainly due to mechanism of hydrolysis. In particular, insecticides such as organophosphates and synthetic pyrethroid, both containing ester linkages, are susceptible to hydrolysis. In a similar way previous work was supported that dialkyl phosphates (DAPs) and other hydrolysis pesticide degradation residues were observed in the food. Pyrethrins, pyrethroids, and some OP insecticides also are susceptible to photolysis in both soil and water (Radford et al. 2018).

The presence of ester linkages in OPs and synthetic pyrethroid insecticides makes them susceptible to the process of hydrolysis (Todd et al. 2008). The degradation of organophosphates (malathion, chlorpyrifos, and diazinon) and synthetic pyrethroids (cypermethrin, permethrin, deltamethrin, and cyfluthrin) was studied in water, white grape juice, red wine, and orange juice. The pesticide undergoes different transformation processes as a result of its distribution patterns and behavior of transport. Atmospheric transformation of pesticide is determined by its chemical composition, structural affinity, and the environmental conditions where it is represented. Redox gradients in the soils, sediments, or in water often determine transport potential which biotic and/or abiotic transformations can occur (Fenner et al. 2013).

Synthetic pyrethroids generally degrade under both aerobic and anaerobic conditions. In general, fast degradation occurred under aerobic conditions than anaerobic conditions. Degradation of 11 pyrethroids was measured for bifenthrin, cypermethrin, ζ-cypermethrin, cyfluthrin, β-cyfluthrin, deltamethrin, esfenvalerate, fenpropathrin, γ-cyhalothrin, λ-cyhalothrin, and permethrin over 100 days in three sediment/aquatic systems under both aerobic and anaerobic conditions at 25 °C in the dark. Under aerobic conditions half-life of insecticides ranged from 2.9 days to 200 days, with an average value of 18 days. For half-lives under anaerobic conditions, the range was observed to be 20 days to more than 200 days, with an average value of 70 days (Brian et al. 2013).

However, insecticide adsorption to the glass wall of the storage container is observed to be significant mechanism of loss or volatilization of OP and pyrethroid insecticides. The study of the adsorption of insecticides on glass surface conferred that bulk of fluid gets lost under varying environmental conditions. Overall, multiple evidences suggested that temperature grading affects the loss of pesticides on the jar wall, but the concentration of insecticide found their loss largely due to a sum of other mechanisms. Loss of analyte at high range of temperatures is more rapid and obvious to analyze. However, statistically significant results were observed when studies were conducted at the temperature of a refrigerator at 4 °C and also did show ideal volatility at 2.5 °C. In the environment degradation or transformation comprises various mechanisms including oxidation, hydrolysis, and photolysis as these are key processes in abiotic degradation that transports and governs the environmental fate of pyrethroids. During these mechanisms, cleavage of ester is a main process resulting in the production of cyclopropane acid, 3-phenoxybenzyl alcohol, 3-phenoxybenzaldehyde (3-PBA), or 3-phenoxybenzoic acid. In the abiotic degradation of pyrethroids, 3-phenoxybenzyl alcohol also often is an intermediate in the photodegradation, followed by oxidation to produce corresponding carboxylic acid and accumulate in the soil. Abiotic degradation of pyrethroids often slows, depending on soil types and initial pyrethroid derivatives. The half-life values of bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenpropathrin, fenvalerate, and permethrin in non-sterilized soils have been estimated to be 12.4–1410, 7.8–54.6, 17.1–52.1, 8.3–105.3,37.1, 17.7–41.3, and 5–55 days, respectively. The degradation processes significantly affected soil temperature, pH, and moisture. The process of degradation of fenvalerate was greatly affected by the temperature and the moisture content, which resulted in the decrease of the insecticide half-life. The rate of the degradation of deltamethrin correlated with texture of soil and organic matter content. Generally, a less content of clay and organic matter in the soil resulted in a higher degradation of deltamethrin, cypermethrin, and fenvalerate (Cyco'n and Seget 2016).

Overall, transformation and degradation of pyrethroids may eventually depend on environmental conditions both aerobic and anaerobic and their adsorption on environmental compartments such as soil or water. The time period required to disintegration of half amount of the original product of chemical or insecticide is known as half-life or measurement in days for half-life ($t_{1/2}$). As cyfluthrin is broken down with half-life of 11.5 days with a soil component well supplied with oxygen and microbes, in poorly aerated soil however bifenthrin degrades at a very slow rate and might have remained for 3 years (half life = 425 days). In other study, altering environmental conditions may lead to variability for degrading product compounds of cypermethrin (half-life = 1.9–619) and indicate longer persistency in the environment. Pyrethroids are a class of insecticides, strongly bound to soils and sediments and degraded at varying rates, used to control a wide range of pests for both agricultural and urban uses. The amount of pyrethroid application rate increased in urban area after increasing limited use of organophosphate compounds. An active component in insecticides persists for longer duration in the environment, with more chances of toxicity to both environment and human. With regard to persistency, synthetic pyrethroids have a low environmental persistency, however differ with the amount of active ingredient of specific concern in the environmental conditions. Most of the studies monitoring environmental fate of insecticides have been used to provide detailed information of residue concentrations, degradation products in various sediments, and integrated information for assessment of ecological risk of pesticides carried out in both laboratory and outdoor areas. Earlier multiple studies have reported the degradation of the deltamethrin in aquatic system to be rapid and half-life was observed to be less than 1 day. A series of study were conducted and designed to determine the ecological fate and residual persistence of two synthetic pyrethroid insecticides, tralomethrin and deltamethrin, under spray drift and field runoff treatment regimes (Palmquist et al. 2012).

The efficiency and residual action of pyrethroids is greatly affected by abiotic conditions. For instance, at lower temperatures, natural pyrethrins and synthetic pyrethroids are considered to be more efficient insecticides with higher toxicity which is also harmful to non-target organisms at cooler temperature. Physical state suitable for availability to insects of residual film of insecticides can influence temperature and relative humidity. Impact of these two abiotic factors to insecticides and solvent decreased the intake or uptake of insects which may be due to the physiological conditions of insects themselves or the less suitable physical state of residual film or both. In the environment pyrethroids are exhibited to degrade out rapidly by abiotic mechanisms, though adsorption of these degrading derivatives to sediments was also demonstrated. The varying ratios of active ingredient of insecticides degrade at variable rates influenced by the composition of the soil, the climate, and the abundance and diversity of biotic factors. In direct sunlight, cypermethrin may persist for 3 months in indoors or domestic treatments while considered relatively stable in direct photoperiod are with a half-life as long as 8 weeks or travel to untreated rooms via air. In indoors the rate of hydrolysis and photolysis is considered to be slower, with a rapid degradation occurring within a few days. In areas with limited exposure of sunlight, temperature, and air circulation, most of the applied pyrethroids (*d*-phenothrin) remain for longer period as until 1 year. In normal environmental conditions, cypermethrin may persist more than 50 days, but lower temperature and sunlight may require 100 days. A class of pyrethroids, permethrin showed stability under UV light. But adsorption to soil particles may lead to a half-life of 43 days, and in some formulations it is used to kill termites (termiticides) and can persist up to 5 years. In general, synthetic pyrethroids are considered to have similar physicochemical properties that affect their transport and fate in the environment, and the average half-life in soils with the presence of air (aerobic) varies between 30 and 100 days (Fenner et al. 2013; Ismail et al. 2012).

Hence, incorporation and impact of environmental factors affect pyrethroid's sustainability and degradation. The study will be helpful to generate the information in selection of appropriate insecticide and weather conditions to have best use of these synthetic pyrethroids.

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Sustainable Sanitation as a Tool to Reduce Land Degradation

37

H. Kate Schofield

Abstract

Although a fundamentally unappealing topic, human excreta management has long been, and remains, one of humankind's most significant dilemmas. Human excreta is one of the few resources that is increasing with the growth of the population. The highly organic, nutrient-rich nature of human excreta makes it a valuable resource; however, without adequate processing, it can pose a risk to environmental quality and human health through the presence of pathogens, pollutants (e.g. pharmaceuticals) and high concentrations of mobile nutrients. Sustainable innovation of sanitation technology is required to address this management issue.

Another issue of growing global concern is the loss of productive agricultural land and with it the loss of food security, which has further implications for human health and environmental quality. The diversity of causes and the growing extent of land degradation means that there is no 'silver bullet' solution and a range of location- and environment-specific solutions are required to address the issue.

This chapter will explore the use of sustainable human excreta management practices for the provision of a solution to two global needs: sustainable human excreta management and improvement of degraded lands. There are a range of sanitation solutions designed to suit a diversity of environments and needs, but in order for a sanitation system to be considered sustainable, it must address social, economic and environmental needs for the community it serves. Meeting the three requirements for sustainability remains a common issue globally, but particularly in low- and middle-income countries where access to sustainable

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sanitation is one of the leading barriers to improved health in the general population and where reliance on the productivity of agricultural and urban garden lands is high.

Keywords

Soil · Land degradation · Sanitation · Soil degradation · Sustainable development

37.1 Introduction

Soil health is inextricably linked with food security and public health. As such, soil sustainability is key to addressing the pressures of a growing global population. Unfortunately, the value of soil, as with many natural resources, has long been overlooked. Recent decades have seen a shift in attitudes towards greater understanding and appreciation of our environment, and issues such as pollution and climate change have amassed widespread concern. However, issues relating to soil quality have not yet gained the same level of attention and awareness of other issues within the environmental sphere.

The idea of using products derived from human excreta to improve soil quality is not new, but it has seen many iterations and developments throughout human history. Human excreta, in the form of urine and faecal matter, are a rich source of essential plant nutrients and its relative abundance makes it appealing as a fertiliser. Historically, this was widely recognised with human excreta, termed ‘Nightsoils’, collected from cities, towns and villages to be spread, either raw or following composting, onto the surrounding agricultural lands as a means of increasing crop yield.

The use of raw human excreta is may be regarded as risky practice due to the potential presence of pathogens, which pose a risk of faecal-oral transmission of disease. Indeed, in order for the application of human excreta to land to be considered viable, it must undergo appropriate processing. The Industrial Revolution saw a significant increase in urban populations, and the discovery of a link between raw sewage and disease saw the implementation of large-scale sewage systems. However, in regions where municipal sewage works are not available or do not function adequately, the practice of informal human excreta treatment and application to land is still conducted (Ferguson 2014).

Across much of the agricultural sector, chemically synthesised inorganic fertilisers have been widely adopted in the place of the more labour-intensive systems of nightsoil conservation and application. However, these bring their own disadvantages as they are often produced through energy intensive processes and/or are derived from finite materials that are subject to increasingly limited availability. In addition, such inorganic fertilisers, if not properly applied and managed, are susceptible to leaching from the soils, risking detrimental environmental impacts (e.g. eutrophication). Growing concern surrounding the future availability of synthetic inorganic fertilisers has re-emphasised the need for better nutrient management, including comprehensive recycling of nutrients contained in human excreta for the remediation of degraded agricultural lands.

By focusing on the potential for human excreta to become a value-added product, rather than a burdensome hazard, there is the opportunity to generate political and legislative support and funding for greater provision of sustainable and safe sanitation facilities. Access to safe and sustainable sanitation has been linked to many direct and indirect benefits. Lower disease transmission and increased hygiene have been linked to increased community health, which indirectly leads to better school attendance and subsequently provides better opportunities for the individual along with benefits to the economy. However, a complex of financial, political and social barriers restrict progress towards the provision of safe and sustainable sanitation.

This chapter explores the potential applications for human-waste derived products, nature of human-waste derived products and the necessary considerations required for its utilisation as a valuable resource for the improvement of degraded lands.

37.1.1 Sustainable Development Goals

The United Nations Sustainable Development Goals (SDGs) are a collection of 17 interlinked goals designed as a blueprint to achieve a better and more sustainable future for all (UN 2020). The SDGs were outlined in 2015 and are intended to be achieved by 2030. The use of human excreta products for the reduction of land degradation links to multiple SDGs, the three most relevant ways being:

1. Increasing access to safe sanitation through optimisation of composting latrines as improved sanitation systems (SDG.6).
2. Reducing degraded land area by generation of a human waste-derived soil amendment (SDG.15).
3. Increasing food security through the sustainable improvement to agricultural land productivity (SDG.2).

37.2 Land Degradation

To appreciate how the application of human excreta to land can reduce land degradation, it is important to understand the causes and implications of land degradation.

Land degradation is an example of a widely appreciated, but poorly understood, globally significant problem that influences environmental issues such as food security, reduced productivity, diminished quality of freshwater resources, loss of biodiversity and global climate change.

Land degradation mainly occurs as a result of various natural and human activities and takes a number of forms including soil erosion, organic matter depletion, fertility decline, pollution or contamination, desertification, salinisation, deforestation and acidification.

37.2.1 Causes and Scale

Land degradation is the result of various interlinked physical, chemical and biological processes and is primarily human-induced (Ahmad et al. 2018; Bindraban et al. 2012). The main causes of soil degradation are erosion, by water or wind; compaction; salinisation; nutrient depletion—due to a decline in organic matter content, leaching or extraction by plant roots without adequate replacement; contamination; and soil sealing, e.g. by urbanisation or road construction (Bindraban et al. 2012).

Globally, it is estimated that more than two billion hectares of land, an area the approximate size of South America, would benefit from some degree of land restoration (WRI 2014). Worsening land degradation caused by human activities is already undermining the well-being of 40% of humanity, driving species extinctions (loss of biodiversity) and intensifying climate change, all of which serves to undermine the peace and stability of land-dependent communities (Barbut and Alexander 2016).

The convergence of pressures from sustained population growth and dwindling land quality as will be seen over the coming decades is unprecedented. Estimates suggest that by 2050 the global population will reach 9.1 billion. This will require agricultural productivity to increase by between 70 and 100% in order to provide adequately healthy diets for all as targeted by the United Nations Sustainable Development Goals (FAO 2011). Therefore, the design and implementation of policies that stimulate positive environmental performance whilst also increasing agricultural productivity will likely become a key policy issue for concerned governments (Lankoski and Thiem 2020). This presents a significant task as much of the required agricultural production will need to be performed using existing agricultural lands. This issue is further compounded by the growing demand for non-food items such as biofuels and biomaterials (Bindraban et al. 2012).

37.2.2 Implications

Land degradation puts global ecosystems under strain and reduces their capacity to provide vital services. Meanwhile, with forecasts of continued global population growth, demand for these services is expected to increase.

Land degradation manifests as the disruption of the healthy balance between five key ecosystem functions: food production, fibre provision, microclimate regulation, water retention and carbon storage (Ahmad et al. 2018; El-Zein 2018). The far-reaching impacts of this include loss of soil fertility, loss of biodiversity, soil erosion, contribution to climate change and economic losses.

A report by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES 2018) estimated that the well-being of at least 3.2 billion people is being adversely affected by land degradation brought about as a result of human activities, pushing the planet towards a sixth extinction. To avoid, reduce and reverse this problem, restoration of degraded land is an urgent priority to ensure the well-being of all life on Earth.

Some soil degradation processes are directly observable: erosion, landslides, sealing or, to an extent, decline in organic matter; however, soil contamination, compaction or decline in soil biodiversity and reduced food security cannot be directly assessed (FAO, ITPS 2015).

Less than 25% of the Earth's land surface remains free from substantial human impacts. It is estimated that, by 2050, this will drop to less than 10%, with most of the remaining area being unsuitable for human use or settlement such as in deserts, mountainous areas, tundra and polar areas (IPBES 2018). Globally, we cannot afford to lose land from agricultural production because of degradation, and, therefore, action is needed to prevent further land degradation and remediate areas of already degraded land.

37.2.2.1 Food Production

The global food production system has an extensive influence on the planet's natural cycles. The quantity of food that can be grown is limited by the amount and availability of essential nutrients within the soil. Where natural nutrients have been insufficient, inorganic fertilisers (nitrogen, phosphorus and potassium, NPK) are recognised to have played a key role in the growth of food production (Erisman et al. 2008; Kidd et al. 2017). However, the extensive use of these fertilisers raises questions relating to their sustainability and environmental impact. Prolonged regular usage of inorganic fertilisers has been linked to increases in soil acidity (Pillai et al. 2015) and alters soil microbiological communities (Kidd et al. 2017).

Inorganic fertilisers are generally derived from finite materials: the nitrogen (N) component is derived from either natural gas, which is subject to price change and tied to the availability of methane (Pillai et al. 2015), or from nitrogen in the air—which is converted to ammonia fertiliser through the energy-intensive Haber-Bosch process. Both potassium (K) and phosphorus (P) are a product of mining. Remaining phosphorus stocks are projected to become exhausted within the next century (Vaccari 2009) and are concentrated predominantly in a few countries linking the mineral's availability inextricably with international politics. Based on the past patterns of world fertiliser consumption, projects suggest that demand for fertiliser is unlikely to diminish, with 263 million tonnes of fertiliser expected to be used annually by 2050, an increase of 60 million tonnes on the present day (Alexandratos and Bruinsma 2012). As mineral resources become scarcer, supply and demand mechanisms are likely to see a rise in the cost of synthetic fertiliser making food production less affordable. Access to renewable sources of nutrients could enhance the global resource systems sustaining human development (Trimmer et al. 2017).

Global estimates of reuse from human sanitation are highly variable but suggest 0–15% of nitrogen and 0–55% of phosphorus are currently recycled to cropland (FAO 2020; Cordell et al. 2009; Bouwman et al. 2009). There is suggested to be potential for sanitation to contribute to improved resource accessibility and sustainability at local and global scales, e.g. the total phosphorus available globally in human excreta in 2009 represented approximately 22% of worldwide phosphorus demand (Mihelcic et al. 2011).

37.2.2.2 Biodiversity

The impact of land degradation on the decline in biodiversity cannot be directly assessed. However, evidence supports that human activity is having an undeniably large impact on biodiversity. Between 1970 and 2012, the index of the average population size of wild land-based species of vertebrates dropped by 38% and freshwater species by 81% (McRae et al. 2017). The strongest drivers of biodiversity loss to date have been agriculture followed by forestry, infrastructure, urban encroachment and climate change (Alexandratos and Bruinsma 2012). In the 2010–2050 period, climate change, crop agriculture and infrastructure development are expected to be the drivers of biodiversity loss with the greatest projected increase, with biodiversity loss projected to reach 38–40% by 2050 (IPBES 2018).

37.2.2.3 Climate Change

Soils are the largest terrestrial carbon store, storing approximately four billion tonnes of carbon—ten times as much as the world's forests (Ontl and Schulte 2012; Lal 2004). Carbon is stored as organic, soil organic carbon (SOC) and inorganic, mineral-derived forms. The process of soil degradation results in the release of carbon formerly stored in the soils, as carbon dioxide (CO_2), which contributes to increased atmospheric greenhouse gas (GHG) concentrations, which are closely linked to climate change (Ontl and Schulte 2012). Approximately two-thirds of the total increase in atmospheric CO_2 is a result of the burning of fossil fuels, with the remainder coming from SOC loss due to land use change and degradation (Lal 2004), such as the clearing of forests, the cultivation of land for food production and soil erosion.

The release of carbon from soils feeds into a cycle called the soil-carbon-positive feedback mechanism (Melillo et al. 2017). The basic premise here is that increases in global temperatures (resulting from climate change) cause increased microbial activity in soils, which accelerates the breakdown of soil organic matter and releases more CO_2 to the atmosphere, which then contributes to further global warming and so on.

Whilst carbon loss from soils contributes to atmospheric CO_2 concentrations, soils also offer the opportunity to store (sequester) carbon, whereby CO_2 is removed from the atmosphere and stored within the soil carbon pool (Smith 2016). This may be seen as a way to potentially 'break' the soil-carbon-positive feedback loop. Some potential strategies for this include biochar application, reforestation and terrestrial enhanced weathering (Royal Society 2018).

37.2.2.4 Economics

As observed by the IPBES (2018) report, high-consumption lifestyles in more developed economies, combined with rising consumption in developing and emerging economies, are the dominant factors driving land degradation globally. The full impact of consumption choices on land degradation worldwide is not often visible due to the distances that can separate many consumers and producers. In addition, many of those who benefit from overexploitation of natural resources are among the least affected by the direct negative impacts of land degradation and therefore have the least incentive to take action (IPBES 2018).

37.3 Sustainable Sanitation

Human excreta is one of the few resources that will increase in proportion with human population growth, and with human population growth projected to continue long into the future, the management of human excreta poses a significant and growing challenge.

In 2017, it was estimated that 2.4 billion people were living without access to improved sanitation, of which 673 million (9% of the global population) are still practising open defecation (WHO/UNICEF 2019), with the rest relying on other basic systems such as pit latrines, cesspits and septic tanks.

The improper or lack of management of human excreta can have serious implications for human health and environmental quality. Sanitation is broadly defined as the separation of human excreta from human and environmental contact and is fundamental to human health and well-being, as well as to social and economic development.

The unsafe management of excreta and wastewater exposes populations to disease and leads to the degradation of ecosystems and the services they provide (Andersson et al. 2016). Sanitation is considered a primary barrier to infection by preventing the exposure of humans to pathogens found in faeces and urine. Poor sanitation is associated with a number of infectious and nutritional outcomes, which places a heavy burden of disease globally. Further to its impact on human health and well-being, poor sanitation management also has an impact on the environment through the contamination of water bodies, soils and food sources.

In order for us to progress towards achieving the Sustainable Development Goals, there will be an increased need for the provision of improved sanitation facilities and their associated management, which has a significant associated cost. Many of those who are presently without access to equitable sanitation live in remote and/or decentralised locations. These often lack municipal infrastructure, such as road access, electricity and water supply, without which human excreta management is expensive, intensive and unpleasant (Hill and Baldwin 2012).

Sustainable sanitation practices recognise that human excreta is not a waste product, but rather, a valuable resource. By shifting away from today's paradigm which focuses on what must be removed from wastewater to a new paradigm focusing on what can be recovered, sanitation systems may begin to be respected as resource recovery systems. In order for sanitation to be considered sustainable, sanitation must minimise the depletion of the resource base, protect and promote human health, minimise environmental degradation and be technically and institutionally appropriate, socially acceptable and economically viable in the long term (Andersson et al. 2016).

37.3.1 Excreta Treatment Methods

The safe disposal of human excreta is essential for population health and welfare, as well as for the prevention of pollution to the surrounding environment. Globally, the range of sanitation technologies in use is extensive. some of the extensive list of human excreta treatment methodologies include disinfection, heat treatment, radiation, composting, aerobic digestion, anaerobic digestion, desiccation (drying) and incineration (Tilley et al. 2014).

Initially, the World Health Organization and UNICEF outlined a two-level system for the classification of sanitation systems: improved or unimproved, where improved sanitation facilities are designed to hygienically separate excreta from human contact. WHO/UNICEF (2019) have further refined this to a ladder classification system with five levels:

– *Safely managed*—Use of improved facilities, which are not shared with other households and where excreta are safely disposed of in situ or transported and treated off-site.
– *Basic*—Use of improved facilities, which are not shared with other households.
– *Limited*—Use of improved facilities shared between two or more households.
– *Unimproved*—Use of pit latrines without a slab or platform, handing latrines or bucket latrines.
– *Open defecation*—Disposal of human faeces in fields, forests, bushes, bodies of water, beaches and other open spaces with solid waste.

According to the ladder system, safely managed, basic and limited systems are considered improved and include flush/pour flush to a piped sewer system, septic tanks or pit latrines, ventilated improved pit latrines, composting toilets or pit latrines with slabs. One of the aims of the Sustainable Development Goals (SDG 6.2) is to achieve access to adequate and equitable sanitation and hygiene for all and end open defecation by 2030 (UN 2018).

37.3.1.1 On-Site Treatment

On-site, also referred to as decentralised wastewater treatment, is typically used in two scenarios: (1) in locations where housing density is sufficiently low that centralised wastewater treatment is not economically feasible and (2) in areas where technology and resource limitations do not permit centralised wastewater treatment systems.

There exists extensive variation in on-site sanitation technologies, which include pit latrines, unsewered public ablution blocks, composting latrines, septic tanks, aqua privies and dry toilets. However, the common purpose of on-site wastewater treatment systems is to reduce the concentrations of contaminants within the waste to acceptable levels before it reaches the environment or comes into contact with the human population (Yates 2011). In addition, some on-site systems promote the recycling of human excreta, which can be returned to the soil as fertilizer after pathogen reduction, transforming the human waste material into a value-added resource.

37.3.1.2 Off-Site Treatment

Off-site sanitation systems collect and remove excreta and wastewater from where they are generated. Off-site sanitation systems rely on sewer systems and centralised wastewater treatment. Such systems require high economic investment.

Off-site treatment is most appropriate for dense urban and peri-urban settlements, where there is not enough space for on-site containment technologies or emptying; however, such systems are less appropriate for rural areas with low population densities.

Off-site treatment of sanitation products is conducted at wastewater treatment plants and results in the conversion of wastewaters to sewage sludge (see Sect. 37.3.2.4) and effluent (Sect. 37.3.2.3).

Wastewater treatment is the process used to remove contaminants from wastewater and convert it to an effluent that is safe to be returned to the water cycle. Treatment processes and technologies vary; however the broad principles of wastewater treatment generally follow a common sequence (Fig. 37.1), the products of which are sewage sludge and effluent.

37.3.1.3 Direct Environmental Discharge

Direct environmental discharge refers to wastewater discharged directly into a stream or other receiving body. Open defecation is the practise of defecating in

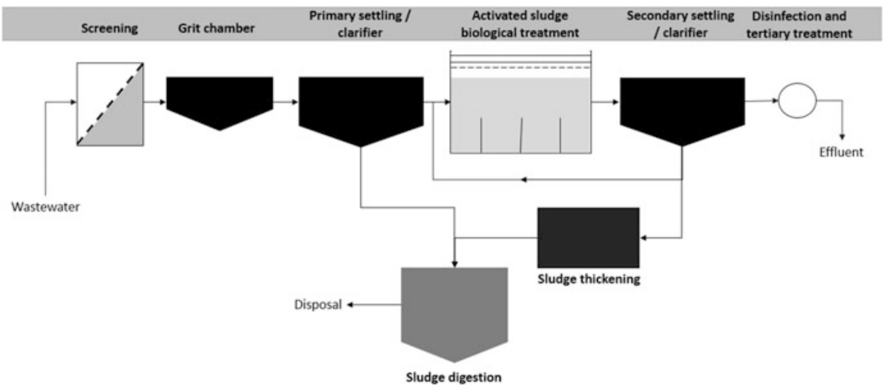

Fig. 37.1 The basic principles of the wastewater treatment process

open fields, waterways and open trenches without any proper treatment or disposal processes and is classified as unimproved sanitation (Jones et al. 2012). Direct environmental discharge does not provide any form of treatment and is thus a form of point source pollution to the environment and results in an increased risk of human exposure.

37.3.2 Sanitation Products

The sanitation technologies by which human excretions are treated are highly varied; thus, the nature of the sanitation outputs is similarly varied. This has implications for its suitability for application to land, with not all products being appropriate.

The term sanitation product is used to refer to materials directly generated by humans, those required to ensure the functioning of sanitation technologies or those generated by the storage or treatment process (Tilley et al. 2014). Herein, the focus is on those sanitation products most prevalent or suitable for application to land. An extensive compendium of sanitation products and technologies has been complied by Tilley et al. (2014).

37.3.2.1 Excreta

Excreta consists of a mix of human urine and faeces. Whilst the precise proportions vary significantly with diet, health, the average adult will produce one stool per day, with a wet weight range of 35–796 g (median 128 g) and 1.42 L urine per day (Rose et al. 2015; Ciba-Geigy 1977).

Faeces

Human faeces are semisolid and composed of water, protein, undigested fats, polysaccharides, bacterial biomass, ash and undigested food residues (Rose et al. 2015). Faeces have are typically composed of between 63 and 86% water (Penn et al. 2018), with variation attributed to a difference in diet (Reddy et al. 1998; Eastwood 1973).

The remaining proportion of faeces (37–14%) is composed of solid material of which between 84 and 93% is organic material (Rose et al. 2015). The major elements in faeces as a percentage of wet weight are oxygen (74%), hydrogen (10%), carbon (5%) and nitrogen (0.7%) (Rose et al. 2015).

Urine

Urine is the liquid produced by the body to rid itself of urea and other waste products. The composition of human urine consists of 90% water by weight, with the remainder being inorganic salts and organic compounds (Rose et al. 2015). The dried solids are comprised of carbon (13%), nitrogen (14–18%), phosphorus (3.7%) and potassium (3.7%) (Rose et al. 2015).

Both urine and faeces contain a range of micronutrients such as magnesium (Mg) and selenium (Se), the amount of which varies depending on dietary intake, whilst the digestibility of the diet determines the partitioning of nutrients between urine (digested) and faeces (undigested) (Jönsson et al. 2004). Generally, urine contains the majority of nitrogen and about half of potassium and phosphorus contained in human excreta, whilst faeces are rich in potassium and phosphorus and contain the majority of the carbon (Harder et al. 2019).

37.3.2.2 Wastewater

Wastewater is defined as any water that has been contaminated by human use; thus, it comprises used water from any combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water and any sewer inflow or sewer infiltration (Tilley et al. 2014). Thus, wastewaters vary significantly depending on the source, but, in general, they comprise a complex mix of solids (faeces, sand grit, rubbish and other debris), dissolved particulate matter, microorganisms and chemical compounds (nutrients, heavy metals and micro-pollutants) (Warwick et al. 2013).

The reuse of wastewaters for irrigation has been suggested as a strategy for improved water management practices by a number of bodies: Environmental Protection Agency (EPA), World Health Organization (WHO) and European Union (EU).

37.3.2.3 Effluent

Effluent is any liquid that leaves a technology; in terms of sanitation, effluent is typically released once processing and treatment are complete. Due to the variation in WWTP processes, the quality of effluent also varied; thus, effluent may be completely sanitised or may require further treatment prior to disposal. For instance, once wastewater has undergone solid separation and sanitisation within a wastewater treatment plant (WWTP), it may be discharged to the environment as effluent or undergo tertiary treatment to further reduce levels of contaminants and microorganisms. Whereas effluent from a septic tank undergoes further treatment as it is passed through the drainage field prior to environmental discharge.

37.3.2.4 Sewage Sludge

Sewage sludge is rich in nutrients such as nitrogen and phosphorus and contains valuable organic matter, which is useful for the improvement of degraded soils. However, it also has a tendency to concentrate the potentially toxic elements (PTEs, see Sect. 37.3.3.3) and poorly biodegradable organic compounds (xenobiotic organic compounds, see Sect. 37.3.3.3) as well as pathogenic organisms (see Sect. 37.3.3.3) that are present in wastewaters (EC n.d.). Effective treatment methods are needed to remove pollutants, some of which include dewatering, thickening, drying, anaerobic digestion and composting (Xu et al. 2014).

Sewage sludge disposal is often subject to governmental regulation and thus varies between countries; however, the main options for sewage sludge disposal are landfill, incineration and agricultural use, with some very small quantities disposed of at sea.

37.3.2.5 Faecal Sludge

Faecal sludge differs from sewage sludge in that it has not been transported through a sewer system. It is the product of on-site sanitation technologies such as septic tanks and pit latrines. It may be broadly defined as either a raw or partially digested, slurry or semisolid and results from the collection, storage or treatment of combinations of excreta and black water (the mix of urine, faeces and flush water along with any cleansing materials from toilets), with or without grey water (water generated from washing food, clothes, dishware, as well as bathing) (Strande 2014).

As with sewage sludge, faecal sludge has a high nutrient concentration; however, the variability of physical, chemical and biological characteristics of faecal sludge is largely varied due to differences in climate, user habits and numbers, system design and pumping frequency; thus the suitability of different end-uses varied.

Ideally, faecal sludge would be collected from the on-site sanitation system and transported to a treatment site, where it will undertake further treatment processes. These processes aim to further stabilise the sludge – reducing its biological oxygen demand (BOD) and chemical oxygen demand (COD), pathogen concentrations and the sludge volume (Wang et al. 2007). However, in some instances, there is a lack of infrastructure to prevent the legal disposal of faecal sludge, resulting in it being dumped illegally into the environment where it poses a threat to human health and environmental quality (Niwagaba et al. 2014).

37.3.3 Excreta-Associated Hazards

The nature and severity of human excreta-associated hazards are highly dependent on the mode of sanitation through which the excreta is processed. Wastewater treatment plants result in the introduction of heavy metals and organic pollutants from non-excretory origins into the sludge product, whilst raw faecal material is likely to contain higher pathogen concentrations.

There exist numerous potential exposure pathways, which should be considered in relation to the application of human excreta-derived products to land. The most likely routes to human ingestion are via consumption of contaminated plants or animals or consumption of contaminated drinking water.

37.3.3.1 Pathogens

Human excreta may contain a range of disease-causing organisms: viruses, bacteria, eggs or larvae of parasites. The microorganisms contained in human faeces may enter the body through either direct, handling or inhalation of airborne microbes, or indirect contact – contaminated food, water, eating and cooking utensils and by contact with contaminated objects. Diarrhoea, cholera and typhoid are spread in this way and are major causes of sickness and death. Indeed, diseases associated with inadequate sanitation have been estimated to account for 10% of the total disease burden worldwide (Prüss-üstün et al. 2008).

Persistence of pathogens varies between different bacterial, viral and parasitic organisms; however, it is essential that any sanitation products considered for application to soil undergo processes to reduce the pathogenic load to safe levels. Pathogen reduction is reported to occur by either dehydration or biodegradation (Jenkins 2005).

A further concern is the role of vectors in the transmission of excreta-derived pathogens. Vectors are any living organisms (insects, birds, rodents and domestic animals) that transport pathogens found in the excretory product to humans, therefore, providing a further pathway for disease transmission. Vectors are attracted to excreta products as a food source. Vector attraction reduction can be accomplished in two ways: (1) by treating the excreta product so that it is no longer attractive to vectors or (2) through the use of a physical barrier between the excreta material and the vectors.

37.3.3.2 Antimicrobial Resistance

Antimicrobial agents are a keystone of modern medicine and have been largely responsible for the reduction in morbidity and mortality associated with infectious disease and routine medical practice (Sanderson et al. 2019). However, the ability of antimicrobial agents to combat deadly infections is at risk of compromise through the development of tolerance or resistance to the active compound (Davies and Davies 2010).

Naturally occurring antibiotics are generally only present in very small quantities (Gottlieb 1976), with these being isolated from their respective environments to be used medicinally (Mullis et al. 2019). Thus, the generation of environmental reservoirs of antibiotic resistance is indisputably linked to human activity with commercial production providing the bulk of antibiotics found within the biosphere (Davies and Davies 2010).

Many microbial and chemical contaminants in wastewater cannot be degraded by the treatment process or inactivated through disinfection of the effluent (Sanderson et al. 2019; EC 2016). As a result, genes that allow bacteria to survive against antibiotics (AMR genes) have been detected at all stages of the wastewater treatment process. For those contaminants that can be degraded, the resulting metabolites may still have antimicrobial or selective activity. WWTP effluent and solid waste products not only have a high prevalence of AMRs but also release selective agents into receiving environments (Jury et al. 2011).

The risk posed by antimicrobial resistance (AMR) is recognised by international, national and regional agencies, with many resolutions and recommendations proposed, however, mechanisms and processes that contribute to AMR are highly complex and the threat posed by antibiotic resistance continues to develop. Further research is required to ensure that the application of human excreta-derived products to degraded land will not exacerbate and contribute to the threat posed by AMR.

37.3.3.3 Pollutants

The application of human waste-derived products, particularly sewage sludge, to land has raised questions relating to the risks posed by heavy metals and xenobiotic organic compounds (XOCs) present (Eriksson et al. 2008).

Plant growth is dependent on the water solubility of nutrients; thus water is the transporting vector. In this regard, contaminants with low water soluble (hydrophobic) are less likely to be taken up by plants and so pose a lower risk of harm (Langenkamp and Part 2001).

Nutrients

As reported in Sect. 37.2.2.1, human excreta is rich in three key agricultural nutrients: N, P and K along with other micronutrients, which are vital for plant and human or animal nutrition. When the application of human excreta to land is properly managed, these can be beneficial for agricultural productivity; however, without proper management these can have detrimental environmental impacts (e.g. eurtrophication).

Potentially Toxic Elements

Potentially toxic elements (PTEs) are chemical elements including both metals and non-metals that have the potential to cause harmful effects to organisms in the environment if present in high concentrations (Antoniadis et al. 2019). Potentially toxic elements are naturally occurring with most originating from the weathering of parent materials. PTEs include cadmium (Cd), chromium (Cr III and Cr VI), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn). Due to their usefulness for a variety human activities and applications, they have become ubiquitous in human influenced environments (Pan et al. 2018). In general, PTEs as environmental contaminants are derived from municipal, electronic and industrial wastes, which, are transported via the sewage network to wastewater, although domestic sources and faecal inputs do also contribute to the presence of some PTEs, albeit, in relatively small quantities (EC 2001b). It has been reported that plants have the potential to uptake and accumulate PTEs from soils, posing a risk of human exposure via food chain (Ortiz and Alcañiz 2006; Eriksson et al. 2008).

Xenobiotic Organic Compounds

Xenobiotic organic compounds (XOCs) do not naturally occur in living organisms and may have high persistence in the environment (are not easily biodegraded by natural processes). Over 6000 XOCs have been detected in raw wastewater (EC 2001b). The predominant organic pollutants of concern include polycyclic aromatic hydrocarbons (PAHs), (PCBs), di(2-ethylhexyl) phthalate (DEHP), linear alkyl benzene sulphonates (LAS), nonylphenol polyethoxylates (NPE), dioxins (PCDD), furans (PCDF) and per- and polyfluoroalkyl substances (PFAS). Whilst some of these compounds are highly persistent, others are readily biodegraded by wastewater treatment processes (Langenkamp and Part 2001; EC 2001b).

Fries (1996) reported that of organic contaminants in sludges, only lipophilic halogenated hydrocarbons accumulate in animal tissues and products. Compounds like phthalate esters, PAHs, phenolic acids, nitrosamines, volatile aromatics and aromatic surfactants are metabolised and do not accumulate. Thus, the majority of organic contaminants in human excreta-derived products are not expected to pose major health problems in the human population when sludge is reused for agricultural purposes although monitoring is advisable (EC 2001b).

Microplastics

The threat posed by plastic pollution to ecosystems around the world is the subject of increasingly extensive documentation. Microplastics have been found in sludge from wastewater treatment plants around the world (Rolsky et al. 2020). The use of sewage sludge as an agricultural amendment has led to microplastics occurring in the amended soils. At present, little is known about the impact of microplastics within the soil environment; however, their degradation can trigger the release of manufactured additives (e.g. phthalates) and adsorbed contaminants (e.g. persistent organic pollutants), which concentrate on the surface of the microplastics (Ziccardi et al. 2016). If ingested, the distribution and toxicity of chemical contaminants may increase and concentrate up the food chain, threatening humans and animals similarly (Barboza and Gimenez 2015).

37.4 Application of Sanitation Products to Address Land Degradation

Human excreta has a long history of being used as a fertiliser and organic soil amendment; however, urbanisation, the introduction of water closets and sewer networks, the growing and often widespread use of synthetic fertilisers and a shift in public perceptions have contributed to a significant departure from this practice (Harder et al. 2019; Ferguson 2014). Although it varies by location, faecal waste products are not currently well utilised, and it is not uncommon for them to be disposed of at landfill, discharged to the environment or given away or sold at a very low price (Diener et al. 2014).

Sustainable sanitation, or resource-orientated sanitation, has been advocated as an approach to promote circularity in the flow of (waste) resources from the built to the natural environment. By enabling the separation of human excreta at source (households) and re-channelling them back to agricultural areas for use of crop fertilisers, a closed-loop system is promoted.

The use of human excreta to improve degraded lands is a form of ecological restoration and requires an interdisciplinary approach that incorporates soil science, hydrology, agronomy, horticulture, forestry and conservation biology, together with socioeconomic and political frameworks (Aradottir and Hagen 2013).

Human excreta is rich in essential plant nutrients and has a high organic matter content. The predominantly present nutrients are N, P and K; however, there is also a presence of micronutrients (Fe, B, Cl, Cu, and Zn) which are also required for plant growth and human and animal nutrition. These micronutrients are rarely present in the inorganic synthetic fertilisers.

Andersson (2015) summarised the benefits of recovering, treating and safely reusing human excreta-derived nutrients for agriculture. These are varied with context and are often location dependent; however, they include:

- Low cost replacement or supplementation of commercial fertilisers.
- Reduced reliance on bought or imported commercial fertilisers.
- Direct improvements in agricultural productivity at minimal cost for smallholders who use no fertilisers and have on-site sanitation systems.
- Reduced health risks for farmers in communities practising open defecation in fields or applying excreta and other wastewater directly to crops.
- New business opportunities in the production and sale of fertilisers from recovered resources.

37.4.1 Land Application Strategies

In all cases, the appropriateness of human excretory products for application to degraded land is dependent on three key factors: (1) the nature of the product, (2) the condition of the soil and (3) the conditions under which application is performed. Application strategies will require careful consideration of the environmental parameters (such as soil conditions, potential impact on the crop, weather conditions at the time of application and proximity to watercourses) and the nature of the human excreta product (as described in Sects. 37.3.2 and 37.3.3).

37.4.1.1 Sanitation Product Treatment

The ability to separate, collect and concentrate useful products (nutrients) whilst removing or excluding the non-desirable components (pathogens, micropollutants, potentially toxic elements) is highly advantageous where human excreta is being considered for application to land (Simha et al. 2017).

The universal requirement for effective treatment of human excrement is the reduction of pathogens, pollutants and vector attraction to ‘safe’ levels. The means by which this is achieved are highly variable (Sect. 37.3.1), yielding similarly varied products. This variation poses a significant regulatory challenge. Tilley et al. (2014) provide a thorough review of sanitation technologies and their products and suitable modes of and incorporation into soils.

Whilst some products present little risk of detrimental human or environmental affects and are thus relatively safe for application to land, others contain high levels of hazardous compounds and/or pathogens, which can pose a significant risk to human health and environmental quality. An already established approach to this is the use of classification system based on the product characteristics (US EPA 1995; EC 2001a; UK Government 2015). These set acceptable limits for pollutant and pathogen concentrations within the product and also annual land loading limits for each contaminant, enabling the selection of appropriate reuse applications or disposal pathways.

Whilst the adoption of these classification systems for decentralised waste systems could be an effective way of assessing the safety of sanitation products, the costs associated with parameter testing (heavy metal or pathogen concentrations) may be prohibitively expensive. It is therefore crucial for decentralised sanitation waste treatment processes to provide consistently adequate treatment so that the risk of any potentially hazardous constituents is sufficiently removed.

37.4.1.2 Conditions of the Soil

Whilst the classification of the excreta product is important for reducing the direct risk to human health, ensuring the suitability of the receiving land is also important for ensuring a low risk of indirect environmental and human harm.

The application of any fertilisers or soil conditioners to land is only ever appropriate where there is a need for nutrients. When there is no such need, their application is at a higher risk of becoming a source of pollution. For instance, the application of a nitrogen-rich sewage sludge to a soil already rich in nitrogen increases the risk of nitrogen leaching from the soil and into groundwater and surface waters. Nutrient enrichment of watercourses can have detrimental impact at and ecosystem scale via eutrophication and reduced water quality – directly affecting environmental quality and indirectly affecting human health.

37.4.1.3 Application Conditions

The environmental conditions under which any soil amendments are applied and the mode of application have a significant impact on how effective the application is in terms of boosting soil quality and productivity. They can also have serious implications with regard to whether applications are retained within the soil or lost to groundwater or volatilisation.There is a range of application mechanisms, which include ridge and furrow irrigation, spreading using a splash plate or band spreader, subsurface injection, spray irrigation and burial. It is important that such applications are not performed during or prior to rainfall or on to frozen ground as this poses greater risk of runoff and leaching into groundwater and adjacent watercourses. In addition, it is also important to give consideration to application rate which can depend on the soil type, slope, depth of application, drainage efficiency and hydraulic loading (US-EPA 1999).

37.4.2 Regulation and Legislation

37.4.2.1 Improvement of Degraded Lands

The implementation of socially acceptable and environmentally desirable solutions to land degradation challenges is often limited by (1) fundamental gaps between evidence bases and different disciplines and (2) an implementation gap between science-based communications, policy makers and practitioners (Blake et al. 2018).

37.4.2.2 Sanitation Access and Management

In Europe, legislation is in place to ensure that infrastructure facilities and services are accessible to all. Whilst these systems may not function at optimal efficiency, they ensure that there is no debate on the principle of access for all (Jones et al. 2012). This is not always the case in low-income countries. Improving accessibility to water and sanitation at a community level can be technically straightforward and low cost; however, institutional and social barriers pose a challenge (Jones et al. 2012).

37.4.2.3 Application of Sanitation Products to Land

Any use of human excreta-derived products for application to land must be conducted in a manner that is safe for the environment and public health. Careful attention must be paid to the characteristics outlined in Sect. 37.3.3 of this chapter to ensure that human excreta-derived products do not have a detrimental environmental impact.

Legislation and control measures that govern the use of human excreta-derived products vary significantly with location. In areas studied such as the USA, Australia and the European Union, agricultural and land application of sewage sludge is commonplace, with each having their own set of regulations posing different usage restrictions and acceptable parameters (Christodoulou and Stamatelatou 2016). The most common parameter inspected, however, are heavy metal concentrations in sludge and soil, whilst in some cases, organic compounds and pathogen content are monitored as additional quality control parameters.

Christodoulou and Stamatelatou (2016) suggest that the general characteristics of legislative frameworks, ideally, should be:

- The continuous and long-term risk assessment of hazardous substances and novel contaminants (emerging pollutants, e.g. pharmaceuticals, micropollutants, etc.)
- The promotion of efficient and sustainable sanitation waste management schemes.
- The simplicity and consistency of regulations.
- The promotion of available markets for viable sanitation recovered products.
- Consumer safety.
- Public involvement in policy making and acceptance of the final product.

37.4.3 Public Opinion

Public opinion is not only based on economic, environmental and political factors, as social and cultural influences play an important role in the level of trust placed in a technology or process. The use of human excretory products on agricultural lands receives varying levels of social acceptance, something that regulators should take into account. The presence of pollutants in human excreta-derived products has resulted in its application to land for agriculture being considered socially unacceptable in some countries (Eriksson et al. 2008). Additionally, the odours from some forms of sanitation products are often considered offensive to work with or have nearby.

The standardisation of chemical, physical and biological characterisation of human waste-derived products and the provision of clear guidelines for management practices are an effective way of improving regulatory consistency, compliance and public confidence in proper sanitation product management (Christodoulou and Stamatelatou 2016).

37.5 Conclusions

Restoring degraded lands into productive and healthy systems provides a suite of benefits for both humankind and the environment, including offset and prevention of soil-carbon emissions.

The application of human excreta-derived products to degraded land has the potential to enhance the biological, chemical and physical properties of soils and represents the opportunity to turn agriculture into a closed-loop system whereby the unused nutrients consumed in food products are returned to the soil in a plant available form. If conducted in combination with other land remediation strategies and sustainable land management practices, it has the potential to contribute towards a sustainable and long-term increased food security.

As observed in Sect. 37.2 of this chapter, there exists an extensive range of sanitation technologies. The suitability of the product generated by these for application to land is similarly varied. In general, organic matter and nutrient concentrations are the two main factors that make the application of human excreta to land to reduce land degradation appealing. However, as highlighted in Sect. 37.3.2.2, the presence of potentially harmful constituents in the form of pathogens and PTEs and their ability to accumulate in soils represents a potential barrier to acceptance and a threat to environmental quality and human health and cannot be ignored. This is particularly true of products derived from centralised waste management systems, such as WWTPs, where the human excretory material is mixed with waste from other sources.

Whilst sanitation and waste processing technology continues to develop, yielding processes with higher efficiencies and lower costs, there remains a need for systems that efficiently separate, collect and concentrate the most useful components of human excreta whilst removing the non-desirable and harmful components.The benefits of centralised waste management systems are undeniable in terms of hygiene and efficiency. However, the contamination of the potentially valuable human excreta resource with pollutants from other waste streams renders the sewage sludge product more hazardous for application to land. In order to combat this, a more cautious approach to sewage sludge application to land is required, with careful management of loadings to ensure that accumulation of PTEs is avoided. In contrast, systems where the excretory material is kept separate from other sources of wastewater produce products with lower levels of PTEs, which, provided pathogenic loadings are suitably reduced, have greater suitability for application to land; however, these systems are often more labour intensive and require management at a household or community level.

As highlighted in Sect. 37.1.1, in order to achieve the SDG target of access to safe and sustainable sanitation for all by 2030, there will be a need for significantly increased provision of improved sanitation systems. Where possible, consideration should be given to treating the human excreta as a resource, through the provision of systems, which enable optimal processing for application to land as a value-added product.

Whilst the barriers to access for sanitation are often a matter complex of political, economic and practical arbitrations, the potential for the utilisation of sanitation products in land remediation may place a greater monetary value and incentive on the provision of suitable safe sanitation. However, for the potential for universal access to sustainable sanitation to be fully recognised, cooperation is required between regulators and practitioners.

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Duckweeds: The Tiny Creatures for Resolving the Major Environmental Issues

38

Kuldeep Luhana

Abstract

The superfast and unstoppable race for urbanization and industrialization has tried to improve quality of human life, but simultaneously it has maltreated the nature so seriously by striking the various components of environment. The elixir of life, water, has now become polluted due to anthropogenic activities contaminating its reservoirs. Water bodies are polluted by heavy metals, agrochemicals, nanomaterials, petroleum products, pharmaceuticals, medical wastes, toxins, dyes, and salts of nitrogen and phosphorus. Many of these are capable of bioaccumulation becoming more dangerous to the environment and so must be eliminated from the biosphere. Bioremediation and in particular phytoremediation have exhibited its potential to treat such pollutants by overcoming the limitations of conventional methods of wastewater treatment. Several plant species have made their marks in this direction. However, duckweeds, the smallest aquatic angiosperm of Lemnaceae family, have proved themselves as one of the best choices for phytoremediation, assessment of ecotoxicity, and suitable toxicity biomarker. These plants are being most effective because of their quick growth, net primary productivity, high bioaccumulation capacity, biotransformation, and biodegradation efficiency, simultaneous dealing with multiple pollutants in contaminated water bodies. Biotechnological tools and techniques have added more values to duckweed-based phytoremediation protocols. Along with this, duckweeds are capable of recovering the nutrients and produce quality proteins when incorporated to wastewater treatment. This ability of duckweeds can be useful to produce feedstock for animal farms, poultries, and fisheries. This effectiveness of duckweeds is making them one of

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the promising agents of clean and green technology for environmental sustainability and better livelihood of human being.

Keywords

Duckweed · Lemnaceae · Phytoremediation · Wastewater treatment · Water pollution

38.1 Introduction

Several environmental issues have emerged due to a variety of anthropogenic activities. This has altered normal functioning of environmental factors. Our intensive activities in the direction of urbanization and industrialization have made us overuse chemicals (agrochemicals, organic solvents, etc) and heavy metals, radioactive materials, etc. (Showqi et al. 2017; Nafea 2019). Along with this accumulation of all these materials increases the pollutant load on this planet and causes serious threat (Patel and Kanungo 2010; Beniah Obinna and Ebere 2019). This has also affected aquatic ecosystems so dangerously. When these toxic substances enter water bodies like rivers, lakes, streams and oceans, they get dissolved or remain suspended in them (Showqi et al. 2017). Discharge of heavy metals and other chemical pollutants potentially changes biological, chemical, and physical properties of water making it turbid, unpleasant, foul smelling, and unsuitable for routine uses like bathing, washing, drinking, and other household purposes. Ultimately this type of water is being detrimental to human health as well as entire aquatic ecosystems.

The control of our anthropogenic activities can minimize this challenge. Along with this we must have treatment facilities to solve this problem, but we hardly have such treatment plants to treat this huge quantity of wastewater and we simply discharge untreated water in water bodies. There are some conventional treatment methods like chemical precipitation, ion exchange, adsorption, a variety of filtrations like reverse osmosis, ultrafiltration, coagulation, and flocculation for remediating the environmental pollution (Jagtap and Jagtap 2018). All these methods obviously have many advantages like operation, flexibility, capacity, and efficiency but simultaneously have several limitations like secondary waste generation, seed money investment, maintenance cost, and high energy requirements (Ugya 2015). In this regard, nature-based solutions become answer to this serious question. Nature-based solutions are utilizing natural resources to manage and treat socioeconomic and environmental challenges like water pollution, eutrophication, etc. (Ceschin et al. 2020). Phytoremediation of contaminated water bodies and wetlands is one such option using natural principles and methods. Being cost-effective, environment-friendly, aesthetically pleasing, potential, and accurate over conventional methods, phytoremediation is becoming an attractive alternative (Ugya 2015; Lakshmi et al. 2017). New research carried out in this direction has identified a number of plant species to be used for clearing the toxic pollutants from the environment. Research has also shown the effectiveness of phytoremediation against tremendous number of pollutants like pesticides, insecticides, petroleum products, polychlorinated compounds, aromatic compounds, surfactants, explosives, heavy metals, etc. (Jagtap and Jagtap 2018). Aquatic plants have greater efficiency and capacity of treating the vast variety of chemicals compounds and heavy metals, reducing BOD (biochemical oxygen demand) and reducing the threat to aquatic life. There is a range of aquatic microphytes and macrophytes like *Eichhornia crassipes*, *Pistia stratiotes*, *Mentha aquatica*, and *Vallisneria spiralis* and some species of common duckweeds like *Lemna minor*, *Spirodelai ntermedia*, and *Spirodela polyrhiza* which have been introduced to be obeying the principles of nature-based solutions for phytoremediation (Beniah Obinna and Ebere 2019).

38.2 Duckweeds

38.2.1 Duckweed Biology

Duckweeds are floating or submerged aquatic monocot plants belonging to Lemnaceae family. They are the smallest and simplest flowering plants in the entire plant world. Duckweeds consist of four genera and 37 species and are found worldwide in static and slow flowing freshwaters (Laird and Barks 2018; Klaus et al. 2013). Duckweeds have very tiny plant body called frond bearing zero to several roots. The fronds (also called thallus) have surface area in the range of 1 mm^2 to 1 cm^2. Though they are flowering plants, majority of the duckweeds reproduce through asexual means. Proliferation of duckweeds creates dense mat on surface of water bodies. The fronds have doubling time of 1–2 days. The protein content of duckweeds varies with respect to species and ranges from 15 to 45% dry weight (Ziegler et al. 2016; Stomp 2005).

38.2.2 Promising Platform for Environmental Applications

Wastewaters, in particular municipal and domestic wastewaters, have high concentration of nutrients, and other organic matters support the growth of duckweeds in water bodies. The intrinsic growth rate of duckweeds is fast and as it reproduces mainly through asexual mode doubling time also gets reduced. Clonal propagation of duckweed can be achieved in a very short time duration under controlled or natural environmental conditions (Forni and Tommasi 2015). On the other hand, other plants may require many months or years to establish in novel conditions. The same property of the duckweed makes it more suitable when it comes to reproducibility of results, biomass uniformity, and optimization of process and validation of method. Duckweeds also require very simple nutrient compositions, but simultaneously it can utilize a wide range of organic substances and also accumulates high concentrations of heavy metals making it a suitable versatile agent of phytoremediation (Stomp 2005). Moreover, some duckweed species are capable of dealing with very high concentrations of toxic compounds and their sequestering in cell organelles, achieving morphological and other physiological plasticity, biotransformation of recalcitrant compounds, hormesis response to heavy metals, and synthesizing organic compounds by switching over metabolic pathways (Ziegler et al. 2016). Also, they can have resource trade-off and hormesis-based mechanisms, allozymic variations to achieve phytoremediation of organics. Being aquatic floating plants, they are in association with some microorganisms where they come up with great possibility to deal with pollutants of water bodies and especially nutrient recycling (Mkandawire and Dudel 2007).

Duckweeds are not limited to phytoremediation only to solve environmental issues. Instead they are useful to produce feedstock for fisheries, poultries being protein rich (Stomp 2005), and also useful for production of biofuels, ethanol, and green manure as they have low lignin content and high starch concentration (Cui and Cheng 2015; Chen et al. 2018). Many a times genetic engineering protocols are used to create transgenic variety of a plant to use it as platform for many commercial and environmental applications. In the same way duckweeds have been transformed for similar applications and proved itself as an easily manipulative plant platform and more suitable for such applications. All these advantages make duckweed a matter of concern to solve major environmental issues.

38.3 Duckweeds for Phytoremediation

38.3.1 Heavy Metals

Many studies have reported about adverse effects of heavy metals on environmental health. Heavy metal accumulation is seriously threatening the normal functioning of aquatic ecosystems, making water unusable (Ali et al. 2020). These heavy metals disrupt the food chains and webs by disturbing the intra- and interspecific interaction, thereby reducing the functionality and productivity of the ecosystem (Singh et al. 2011). The effects of heavy metals on health are also well studied particularly on wildlife as well as on humans. These heavy metals can drastically change the normal physiology and morphology of aquatic plants (Beniah Obinna and Ebere 2019; Ali et al. 2020). On the same time these heavy metals like Pb, Cd, Hg, and As can affect the aquatic animals like fishes at cellular and molecular levels causing damage to their kidney, liver, reproductive system, and their normal reproductive cycles. Several studies have shown impact of bioavailable heavy metals on health of human. These heavy metals can have a vast range of negative impact like osteoporosis; liver and kidney damage; deformities of cardiovascular, respiratory, urinary, reproductive, and nervous systems; and malfunctioning of systems causing abortion, nausea, anemia, allergy, weight loss, dermatitis, joint and muscle pains, low intelligence, and stroke (Diatta and Grzebisz 2011; Verla et al. 2019).

Many studies have supported the use of different species of duckweeds for phytoremediation of heavy metals. Duckweed *Lemna minor* can eliminate 82% nickel and 76% of lead from wastewaters of electroplating industry, and its accumulating higher concentration of nickel exhibited promise to deal with other heavy metals. *L. gibba* has the ability to decrease concentrations of Cu, Pb, Zn, and Cd by 100%, 100%, 93.6%, and 66.7%, respectively, when allowed to treat for 8 days (Nafea 2019). In one study *Spirodela polyrhiza* has cleared the heavy metal load simultaneously for more than one metal and removed 95% lead, 79% copper, 66% zinc, 53% chromium, 45% mercury, 26% cobalt, 20% manganese, and 7% nickel (Chaudhary and Sharma 2014). In Kashmir Himalayas a study was carried out to check the bioaccumulation efficiency of *L. minor,* and interestingly the wild variety has accumulated all the six metals (Cu, Zn, Ni, Cr, Cd, Pb) with more than 90% efficiency. It is important to note that along with this it has eliminated N, P, K, Ca, and Mg with the same potential (Showqi et al. 2017). *L. minor* was used as bioindicator of heavy metal pollution in Skadar lake, Montenegro. The study reveals the efficacy of the plant to treat a total of ten metals, and at the end of the study it was found that other than Zn all other nine metals have the following order of accumulation: sediment >*L. minor* (root) > *L. minor* (frond) with decreasing concentration of the metals found in plant tissues in the order Mn > Zn > Sr > Cu > Ni > Pb > Co > V > Cr > Cd (Kastratović et al. 2015).

The *L. minor* plant can reduce Cu concentration by 55% from the wastewater as reported in a study focused against 8 mg/L Cu in the sample. According to studies to check the effect of pH on Pb and Cd removal efficiency of *L. gibba,* neutral and slightly acidic conditions are favorable for bioremoval. Bioconcentration factor is lowered as alkaline pH indicating that bioremediation is inversely proportional to pH. The same study has also revealed that concentration of the metals in wastewater is also one of the factors concerned with removal of heavy metals, and higher concentrations favor the bioremediation of Pb and Cd (Verma and Suthar 2015). A study focused on bioremediation efficiency of *L. minor* against mixtures for Cu, CR, and Pb has explained that the plant has great efficiency in dealing with mixtures, but it has also showed that higher concentrations of Cu are being detrimental to the growth of the plant. High Cu concentration is damaging the antioxidant system of the plant and reducing biosorption rate for Cu making it unsuitable for the bioremediation of heavily Cu contaminated sites (Üçüncü et al. 2013).

In a study dealing with a variety of free-floating plants against chromium wastewaters, duckweeds stood at second position just after water hyacinth in removal of both the ionic states Cr (III) and Cr (VI). In vitro studies on *S. polyrhiza*, *L. aequinoctialis*, *L. turionifera,* and *L. punctata* against various concentrations of manganese exhibited that other than *L. punctata* all others are being sensitive against higher doses of Mn. The *L. punctata* is being most tolerant against Mn, but overall biomass is decreasing for all the plant species (Zhou et al. 2019). Another important reality is that the crude oil production sites are also contaminated by heavy metals due to their presence as impurities. During oil spillage these metals are exposed to environment. In a study focused to treat heavy metals in crude oil-polluted waters, using duckweeds has shown promising results. The plant *L. paucicostata* bioremediated Cd, Cr, Pb, and V up to 60 days and increasing the exposure time showed increased potential of plant (Ekperusi et al. 2020). Similar results were obtained using *L. minor* in a study focused at Kaduna refinery, Nigeria (Ugya 2015). The response toward heavy metals and its ability to recover from the stress of higher metal concentrations suggest that duckweeds can be used as bioindicator/biosensor to detect the presence of metals in contaminated waters or freshwater bodies. This type of response was studied against Pb (Hegazy et al. 2017). Bioaccumulated metals can be recycled and the combusted plant can be used as manure. A study has revealed this possibility using *S. polyrhiza*. This may add more importance to use of duckweeds with reference to environmental and ecological applications (Abubacker and Sathya 2017).

38.3.2 Crude Oil and Petroleum Products

A study targeted toward studying the crude oil bioremediation efficacy of *L. minor* showed that increasing the crude oil concentration has decreased the biomass doubling rate than the controlled conditions. The study suggested that *L. minor* can degrade crude oil up to very low concentrations (Kösesakal et al. 2015). On the other hand, in another study *L. paucicostata* exhibited great efficiency against petroleum hydrocarbons cleaning them about 97.91% in 120 days. The interesting fact of the study was only less than 1% of petroleum hydrocarbons were accumulated in plant body while around 97.74% were biodegraded (Ekperusi et al. 2020). These results indicate promising model for removing oil spillage and a great threat of petroleum hydrocarbons posing on aquatic ecosystems. According to above studies, extensive research is required on various species of duckweeds to check their effectiveness against crude oils and petroleum hydrocarbons.

38.3.3 Agrochemicals, Dyes and Other Chemicals

Agrochemicals play an important role in increasing agricultural productivity, but on the contrary they become a serious threat to the environment increasing huge load of fertilizers, pesticides, herbicides, and weedicides every year. Large quantity of all these agrochemicals are ultimately drained into the water bodies such as streams, lakes, and rivers posing serious threat to the environment and human health. Many of these compounds are xenobiotics and recalcitrant. Duckweeds can utilize a variety of commercially available agrochemicals like 2,4-D, DDT, propanil, dimethomorph, isoproturon, glyphosate, halogenated agrochemicals, etc. (Dosnon-Olette et al. 2011). *S. oligorrhiza* has proven its efficiency to biotransform DDT and organophosphorus pesticides. *L. minor* has shown its efficacy to remove rice herbicide, propanil (Mitsou et al. 2006), and simultaneously *L. gibba* has proven itself against sulfonylurea (Rosenkrantz et al. 2013). In a study it reported that *L. minor* can utilize isoproturon and glyphosate, both the herbicides simultaneously from the same medium at 25% and 19%, respectively (Böttcher and Schroll 2007). *L. minor* can sequester around 90% of the 2,4-D indicating its great efficiency to deal with this compound. *L. minor* and *S. polyrhiza* have exhibited their potentials for biodegradation of commonly used fungicides, dimethomorph, and copper sulfate (Dosnon-Olette et al. 2011). Majority of the studies indicate that duckweeds have a competitive advantage over other macrophytes irrespective of type of agrochemical. According to the reported studies *L. minor* is more efficient over *S. polyrhiza* when employed against agrochemicals as an agent of phytoremediation.

Azo dyes are important class of the commercially utilized dyes in textile and other industries. *L. minor* has proven itself against industrial dyes like methylene blue with 80.56% efficiency and also cleared contamination of toluidine blue dye and other blue dyes present in textile industry effluent (Imron et al. 2019). In the floating wetland ecosystems, duckweeds complete bioremediation of dyes through phyto-uptake, phyto-stimulation, rhizodegradation, phyto-extraction, phyto-degradation, and phyto-volatilization as the rhizosphere of the plants are in direct contact with dye molecules (Imron et al. 2019).

Many chemical compounds are considered as emerging pollutants creating novel challenges to the environment. Pharmaceutical and personal care products are considered as one of the upcoming threats for environmental health as the unused compounds disposed and finally discharged into water bodies are increasing at considerable rate (Forni and Tommasi 2015). Duckweeds are promising agents to clean up these compounds. Common duckweeds are generally tolerant to flumequine and other drugs. They are able to eliminate cefadroxil, metronidazole, trimethoprim, and sulfamethoxazole with 100%, 96%, 59%, and 73% removal in 24 days (Iatrou et al. 2017). *L. punctata* is also removing ibuprofen, fluoxetine, and triclosan with same ease and pattern (Reinhold et al. 2010). Still some studies report that some of the duckweed species have less tolerance and clearance ability to many of the drugs. But the results and range of chemical compounds already studied to check phytoremediation potential of duckweeds make them most promising candidates to solve this novel challenge.

38.3.4 Treatment of Domestic Wastewater

Domestic wastewaters have different qualities and quantities of compounds discharged with. Mostly domestic wastewaters contain nutrients coming out of household activities, kitchens, etc. Nowadays water pollution is increasing at alarming speed and with increase in population size, ignorance, or citizens' lack of proper channels to treat it, domestic wastewater is becoming a major issue of concern to the environment. Particularly aquatic environments, i.e., water bodies like streams, lakes, ponds, and rivers, are becoming more and more polluted as domestic wastewater is changing their quality of water. Discharge of domestic wastewater into water bodies is continuously adding nutrient-rich compounds (Patel and Kanungo 2010). It is constantly increasing nitrogen and phosphorus. Elevated concentrations of both these elements are detrimental to aquatic animals in many ways. As the ammoniacal nitrogen can be toxic to small aquatic animals, the derivatives produced by both of these elements are oxygen demanding, depleting dissolved oxygen of the water bodies and thus posing a serious threat to aquatic organisms. Also, being growth limiting nutrients, they enhance the growth rate of algae causing eutrophication of pond. All these changes lead to make water unsuitable for common uses as it has turned to be unhygienic (Patel and Kanungo 2010).

Phytoremediation based on aquatic plants is one of the solutions for treatment of wetlands and water bodies contaminated with domestic wastewaters. Among different aquatic plants such as water hyacinth, water lily, and duckweeds, duckweed *S. polyrhiza* has proved its importance in this direction (Loveson 2013). In one of the studies, *L. minor* has increased DO (dissolved oxygen, 5.5 mg/L to 126.1 mg/L), pH, and percentage oxygen saturation value and simultaneously decreased CO_2, COD (chemical oxygen demand), hardness, nitrogen, phosphorus, and chloride (137.57 mg/L to 5.27 mg/L) concentration. It has significantly reduced different forms of nitrogen like nitrite, nitrate, ammoniacal, and different forms of phosphorus like organic and inorganic (Patel and Kanungo 2010). This shows its utility and versatility to eliminate such compounds from water. One of the interesting facts of this study was NPP (net primary productivity), and fresh weight of *L. minor* was increased after completion of study indicated it can utilize domestic wastewater compounds for its normal metabolism and not just sequestering them. Another study focused on comparative analysis of *L. minor* and *L. minuta* to treat domestic wastewaters. *L. minuta* has proved itself more reliable and robust against high concentrations of phosphorus and nitrogen and created thick mat on the water surface. *L. minor* has proved itself hyperaccumulator of phosphates but not for nitrates (Ceschin et al. 2020). A study carried out using *L. minor* to treat wastewaters in tank and pond revealed that both in natural and artificial habitats *L. minor* has proved its efficiency as an agent of phytoremediation. According to final results concentration limits of some parameters couldn't achieve values set by WHO, but majority of the parameters remained under WHO standards (Iqbal et al. 2019). Really, these are promising results to treat a huge quantity of domestic wastewaters produced and discharged daily in freshwater bodies.

38.4 Other Potentials of Duckweeds

38.4.1 Protein-Rich Biomass Production

Duckweeds are one of the important phytoremediation agents causing removal of a variety of wastes from water bodies. This is really proving to be one of the upcoming best practices to solve the issue of sanitation. Along with this the fascinating property of duckweeds is its high growth rate with better accumulation of nutrients with biotransformation of toxic pollutants. This might solve the problem of hunger. It can also help to provide high-protein- and high-starch-containing feed for livestock and agriculture. Duckweeds when allowed to grow on domestic wastewaters in two different ponds with varied conditions and nutrient concentrations, together they produced around 13 tons of fresh weight in a year. The dry weight was accounted around 68.8 ton/ha year from the pond having higher nutrient concentration. According to one more study, dry biomass of *L. minor* and *L. gibba* reach up to 33 tons in just 8 months. The capacity of *L. valdiviana* was measured at 50 ton/ha year dry weight while growing on domestic wastewaters. The total protein content of the dry weight of duckweeds reached up to 35–45% (Mohedano et al. 2012). Duckweed protein content was estimated to be 10.1 ton/h year in one study. The commercial value of this much protein is around 4800 USD/ha year with comparison to market value soybean protein of 465 USD/ton. Interesting to note that both of these plants have similar protein quality and contents. This is really acting as tiny protein factory (Roman and Brennan 2019). However, composition of duckweed biomass largely depends on the nutrients of the aquatic systems its growing on. Many times, it has been observed that duckweeds accumulate heavy metals, but studies focused on to check the concentration of such heavy metals in duckweed biomass have suggested that their contents are very low enabling duckweeds as promising source of trace elements as well as organic supplements for farm animals. It can be used for livestock production as it contains essential amino acids, vitamins, and starch required by poultry – ducks, broiler chickens, laying hens, pigs, cattles, sheep, goats, fishes, etc. (Sońta et al. 2019). All these are having large economical value with respect to their products. Duckweeds help to minimize the cost of their feed with better nutrient quality provisions.

38.4.2 Biomanufacturing Platforms

Duckweeds have proved themselves as platforms for production of recombinant proteins belonging to different classes, e.g., biopharmaceuticals, therapeutic proteins, enzymes, etc. Duckweeds have efficient protein synthetic pathways (Stomp 2005). They have rapid growth, vegetative reproduction, protein modification systems similar to animals, and ease of genetic modification. But when it comes to environmental concerns, we have faced many issues related to the release of the genetically modified varieties due to lack of proper containment, use as agricultural crop, etc. These issues can be solved when it comes to duckweeds, they can be contained in a dedicated pond, and as they are not agricultural crop, there shouldn't be issues regarding human safety. The ease of downstream processing while using duckweeds as platforms also poses less impact on environment (Chhabra et al. 2011).

38.4.3 Miscellaneous

High starch accumulation capacity of duckweeds has made them one of the most promising sources for production of clean energy options like bioethanol, biobutanol, and biogas (Chen et al. 2018). During these days cornstarch is used for production of such fuels, but as we now know that duckweeds have high efficiency to store starch and have efficient switching between metabolic pathways, it has marked its way in this dimension too. Reports suggest that duckweeds, particularly *L. punctata,* are used in bioreactors as carbon source with *Clostridium acetobutylicum* CLCC 8012; it has produced butanol, acetone, and ethanol. The butanol production was around 14.11% higher than that with corn syrup (Gupta and Prakash 2014; Cui and Cheng 2015). Duckweeds harvested from freshwater ponds can be used as organic fertilizers for rice and other cereals. It has great effects as compost and fertilizer, and obviously it is cheap and non-hazardous compared to classical fertilizers used in routine practice. Due to high starch accumulation efficiency, duckweeds are now on target of fermentation industry. A duckweed *Wolffia arrhiza* has been used for alcohol fermentation using yeast.

When duckweeds produce dense mat on water surface, it reduces water evaporation by around 20% in comparison with open wastewater treatment plants. The duckweed mats have been proved to have algicidal activity and so greatly reduce the issue of eutrophication (Mkandawire and Dudel 2007). Some research outputs have also suggested very interesting applications of duckweeds dealing with environment and vector-borne diseases simultaneously. An evidence-based study reports that *L. minor* extract has insecticidal activities against mosquito larvae *Culex pipiens* and *Aedes aegypti,* the vectors of dengue, malaria, and chikungunya. *L. minor* extracts have also malformed the larvae of *Piophila casei* and *Spodoptera littoralis*. It has been reported that when the water bodies are covered with duckweeds, the populations of mosquitos *Anopheles albimanus* and *C. pipiens* were greatly reduced (Gupta and Prakash 2014). This can be a nature-based solution to combat vector-borne diseases without use of synthetic insecticidal.

38.5 Conclusion and Future Perspective

Environmental issues are increasing at an alarming rate and require nature-based solutions to have wide acceptance among the stakeholders from policy makers to common man. Many conventional methods have tried their best to solve these challenges, but at the same time many of them have ended up with developing another challenge. One of the novel approaches to deal with many environmental issues is use of duckweeds. Duckweeds, one-handedly, have exhibited their superlative role in many aspects. They have proved their potential in environmental sustainability through a variety of means. Different species of duckweeds have proved themselves promising for remediation of a vast range of pollutants like heavy metals, organic compounds, agrochemicals, pharmaceuticals, petrochemicals, etc. It is required to carry out extensive studies on metabolic pathways of duckweeds to understand the mechanisms of phytoremediation at biochemical and molecular levels. As heavy metal bioaccumulation is posing toxic effects in plant bodies, it is important to fully understand the mechanisms of heavy metal removal by duckweeds. It is also a matter of concern to have detailed study on protein biosynthesis pathways in different conditions and different types of wastewaters to check the efficiency and safety aspects. More research is required to check the quality of the proteins produced by duckweeds in a variety of wastewaters. Many plants have been studied, but only few have been proved successful platforms for production of recombinant proteins; duckweeds are among them, but it is also required to check glycosylation patterns and other posttranslation modifications. It is also important to develop more containment-based practices to use duckweeds as platforms for recombinant protein production. Efficient techniques to develop duckweed-based biofuel production systems are needed to be studied. Though duckweeds have exhibited insecticidal activities, it is required to study the insecticidal chemical compounds, their mechanisms, and possibility to develop them as commercial insecticidal agents to fight against vector-borne diseases as well as it should be checked against insects affecting crops. Now it is the duty of the scientific community to study, validate, and explore these fascinating tiny creatures with respect to different aspects of their ecological and environmental applications and industrial utilities for sustainable development.

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39 Influence of the Electrical Stimulation Using IrO_2-Ta_2O_5|Ti and RuO_2-Ta_2O_5|Ti Anodes in the Edaphological Properties for the Germination and Growth of *Zea mays L*

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Abstract

This chapter explains the use of manufactured Ti-based electrodes IrO_2-Ta_2O_5|Ti (70:30) and RuO_2-Ta_2O_5|Ti (30:70), generating a coat of the desired metal oxides by electrophoresis to test the electrofarming technology on seed germination and growth of maize plants in *Vertisol pelic*. Experiments were conducted to compare the effectivity of electrode materials on seed germination and maize plants' growth rate by applying an electric field using a 2D array with five anodes around a Ti cathode. RuO_2-Ta_2O_5|Ti (30:70) or IrO_2-Ta_2O_5|Ti (70:30) anodes were prepared by electrodeposition at a constant current density of 14 mA cm^{-2} 20 min at continuous stirring. Soil characteristics before and after the electrofarming tests were carried out to evaluate the electric field application, which show that RuO_2-Ta_2O_5|Ti (30:70) used in electrofarming technology favored seed germination rate and maize plant growth compared to IrO_2-Ta_2O_5| Ti (70:30) electrodes generating a homogeneous growth in the treated maize plants, as well as an increment of the number of leaves. They might be caused by

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an amelioration of the soil properties when subjected to electrofarming treatment. Finally, the use of RuO_2-Ta_2O_5|Ti (30:70) electrodes in electrofarming promotes seed germination rate and growth rate of maize plants compared to IrO_2-Ta_2O_5|Ti (70:30) electrodes generating a homogeneous plant growth in the treated maize plants, as well as an increase in the appearance of leaves in turn with the control soil. In this way, the edaphological properties have been positively affected when undergoing electrofarming treatment.

Keywords

Electrofarming · Maize · Metal oxide electrodes

39.1 Introduction

The electrofarming technology consists of applying an electric field on the cultivation soil, increasing ion and nutrient availability and, therefore, plant growth. It has been reported that this treatment can accelerate plant growth rates and improve crop quality; it has also been proven to help protect plants against diseases, insects, and frost (Acosta-Santoyo et al. 2018).

Plant growth and the biological processes for seed germination can be accelerated or inhibited using high-intensity electric fields (0.1 to 0.2 V cm^{-1}). The mechanism by which this phenomenon occurs is not completely understood. Electrostatic treatment increases the vigor of seeds. It influences the biochemical processes of free radicals or stimulates proteins and enzymes (Morar et al. 2008).

The process of electrofarming consists of soil preparation and conducting initial analysis, soil wetting, insertion of electrodes, pretreatment application for 4 h, seed planting, and germination process of 7 to 10 days (Acosta-Santoyo et al. 2018). Several naked and modified electrodes have been used to test this technology, which allows the generation of hydroxyl radicals to promote different transport phenomena during electrofarming (Acosta-Santoyo et al. 2018). There are several reports on the use of electrofarming (Pohl and Todd 1981; Volkov 2012) to ornamental plants (Volkov 2012), lettuce (Yi et al. 2012), pepper (Yi et al. 2012), and maize (Méndez-Berlanga 1985).

In a previous study from our laboratory, electrofarming showed promising results in different plant carbon fixation metabolisms: C_3, C_4, and CAM. All cases showed a favorable response to the applied electric field (between 0.1 and 0.2 V cm^{-1}) using Ti|Ti or IrO_2-Ta_2O_5|Ti both in 1D and 2D electrode arrangements (Acosta-Santoyo et al. 2018), as well as an imposition of alternating, direct, and direct current with potential imposition. The electrodes include titanium as a cathodic material, graphite sheets, stainless steel, and aluminum rods for some laboratory-level studies. Modified IrO_2-Ta_2O_5|Ti were prepared according to the specifications previously reported by our research team to be used as anodic materials in the laboratory, pilot, and field-level studies (Herrada et al. 2016).

In this way, the best conditions to carry out the electrofarming treatment determined the imposition of direct current with an electric field of 0.2 V cm^{-1} using a 2D arrangement with anodes of IrO_2-Ta_2O_5|Ti and Ti cathodes. In this case, IrO_2-Ta_2O_5| Ti electrodes are well known as suitable radical generators of $^{\bullet}OH$ (Yousefpour and Shokuhy 2012; Herrada et al. 2016), and in contact with the soil, it is possible the increase of oxygen to favor the germination of the exposed seeds. Ru-modified electrodes have been reported to be suitable oxygen generators (Cheng et al. 2009; Trasatti 1984) and allow photo-oxidation and water reduction. Besides, there is a linear relationship between the link energies of O_2 and $^{\bullet}OH$ on the oxides' surfaces. Calculations are performed at different levels of oxidized and reduced surfaces, resulting in link energies of O_2 in a range of more than 5 eV. The similarity of powers was in Ru and Ir (Rossmeisl et al. 2007; NOM-021-RECNAT- 2000).

Therefore, this research shows the use of these electrophoresis-modified surfaces of IrO_2-Ta_2O_5|Ti and RuO_2-Ta_2O_5|Ti with a relation Ir:Ta and Ru:Ta of 30:70 and 70:30 for seed germination and growth of maize plants in *Vertisol pelic* to test electrofarming technology.

39.2 Materials and Methods

39.2.1 Electrode Preparation

Titanium plates (20 × 30 x 1 mm) were pretreated in two steps. Firstly, they were sandblasted and then etched with a 40% oxalic acid solution for 20 min, and then they were rinsed with deionized water and dried. Different precursor solutions were prepared to dissolve $IrCl_3$ (Strem Chemicals, 99.9%) or $RuCl_3$ (Strem Chemicals, 99.9%) in HCl and $TaCl_5$ (Strem Chemicals, 99.9%) in isopropanol (J. T. Baker, 98%), stirring vigorously. The Ir:Ta and Ru:Ta ratios used in the precursor solutions were 70:30 and 30:70, respectively. Electrodes were obtained by electrodeposition at a constant current density of 14 mA cm^{-2} for 20 min at continuous stirring. After electrophoretic deposition, metal oxides were promoted by thermal decomposition in two steps, the first one at 523 K for 10 min and the second at 723 K for 1 h (Herrada et al. 2016).

39.2.2 Sampling of Vertisol Pelic

Vertisol pelic samples were taken from a nearby farm and collected at a depth of 15 cm. The samples were sieved through a 2 mm mesh to remove small rocks, roots, or other non-soil components. After sifting the soil, it was left dry at room temperature and stored under shade for later use.

39.2.3 Electrofarming Cell Preparation

Plastic cells used for germination tests and plant growth evaluation had dimensions 21.5 x 10 x 5 cm. Tubular electrode type used for electrofarming was modified using a relation of concentration Ir: Ta 70:30 or Ru: Ta 30:70 to have IrO_2-Ta_2O_5|Ti and RuO_2-Ta_2O_5|Ti (0.6 cm long and 0.7 cm diameter) around a cathode of Ti connected in series, as shown in Fig. 39.1. In this way, a set of two different treatments were placed (with RuO_2-Ta_2O_5|Ti and IrO_2-Ta_2O_5|Ti, respectively) to test electrode material efficiency in electrofarming and a new group without treatment as a control group; these three groups performed using previously sieved uncontaminated *Vertisol pelic*.

39.2.4 Germination Rate Analysis

Maize seeds (*Zea mays* L) were used to test the germination rate obtained from a local trading house. These seeds were disinfected with 10% commercial chlorine for 3 min and sown at a depth of about half a centimeter. In this way, 35 seeds were placed per cell (seven seeds per quadrant), forming a pentagon around the cathode (as shown in Fig. 39.1). Thus the cells with a thickness of 5 cm of soil were previously hydrated until saturation with tap water. Subsequently, an electric field of 0.2 V cm^{-1} (Pohl and Todd 1981) was applied during a 4-h exposure period in every cell to stimulate the seeds' germination. This procedure was only on the first day to promote this germination.

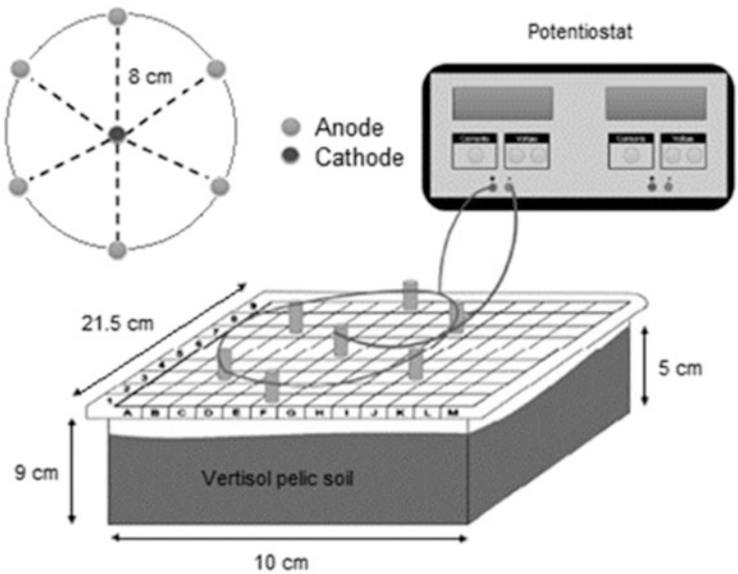

Fig. 39.1 Assembly of electrodes in the electrochemical cell for the electrofarming of maize

After a week, the cells are placed in a growth chamber with artificial light (to continue with the growth stage). Plants were watered daily to keep the soil moist and saturated with water to facilitate electric potential. The germination percentage analysis was performed under laboratory conditions. The germinated seeds began to count when the first seed broke the testa, considering when the mesocotyl sprouted. From this time, daily counts were performed until no more seeds germinated and the percentage calculated. All the experiments to germinate the seed of maize were by triplicate.

39.2.5 Plant Growth of Maize Plants Using RuO_2-Ta_2O_5| Ti and IrO_2-Ta_2O_5| Ti Anodes

This stage is performed in a growth chamber with artificial light (Fig. 39.1), applying 0.2 V cm^{-1} with an exposure period of 3 h every 12 h in each cell and a photoperiod of 8 h of light used for 16 h of darkness, at room temperature. Irrigation continued to be daily. For plant growth tracking, the measurements were initialized after the first pure leaf came out.

The experiments were performed for 2 weeks, and after this time, the plantlets were harvested, and the following values recorded: stem length, root length, and the number of leaves in each plant, leaves and stem width, as well as dry stem weight, to determine the effect of the electric field on maize plants when applying the electrofarming treatment on biomass production. All the experiments on plant growth of maize were by triplicate.

39.2.6 Edaphological Characterization

The characterization of soil samples treated with electrofarming was carried out to evaluate the properties of the soil before and after the treatment and to establish the mobilization of more abundant nutrients such as Ca, Mg, Na, and K in the exchange sites as a result of the treatment (also known as exchangeable cations). The following variables were determined: pH measurement (in H_2O and KCl), cation exchange capacity (CEC), electrical conductivity (EC), bulk density, and soil organic matter (SOM). Analytical determinations of properties were made at least by triplicate. Soil pH and EC were measured with a combined Beckman-branded glass and calomel electrode for soils. All analyses are made as specified in NOM-021-RECNAT- 2000.

The determination of exchangeable cations carried out in the ground was selected for cultivation, taking a homogeneous sample. The soil sample, once the electrofarming was carried out, was divided into three groups, according to the section in the cell, being close to anode (CA), middle cell (MC), and close to the cathode (CC), as shown in Fig. 39.1. The exchangeable cations determined were calcium (Ca^{+2}), magnesium (Mg^{+2}), sodium (Na^{+}), and potassium (K^{+}), using the titration method with EDTA and flame emission spectrophotometer.

Also, the contents of soluble ions are measured as chloride (Cl^{-}), carbonate (CO_3^{-2}), and bicarbonate (HCO_3). Besides, the quantity of available phosphorus (P) in the soil was measured, as this element is essential for the development of maize—the method used to this intent reported by Brazy and Kurtz based on NOM-021-RECNAT- 2000. Finally, soil enzymatic activity was determined using the Casida method, 1964, as a biological indicator of soil quality. All the experiments to edaphological characterization were by triplicate.

39.3 Discussion of Results

39.3.1 Germination of Maize Seeds (Zea mays L) during Electrofarming

Figure 39.2 shows that electrofarming using RuO_2-Ta_2O_5|Ti (Ru: Ta) showed the highest germination percentage compared against the results obtained using IrO_2-Ta_2O_5|Ti (Ir: Ta), and both results were higher than the control test after the sixth day of applying an electric field, showing 100, 94.2, and 82.86% of germination, respectively. In general, the three tests had high values of germination percentages (Fig. 39.2), which can be attributed to the ease of this type of plant to germinate due to the amount of starch present in its seeds' anatomical constitution and physiology.

When electrofarming was applied it showed an increase in the percentage of seeds. This result was, by mobilizing nutrients in the soil, reflected in the seed germination through the coleoptile's emergence. Thus, it was evident that in the case of cells treated with RuO_2-Ta_2O_5|Ti electrodes, the germination process began first and similarly ended earlier, thus occurring in the first 6 days of seed planting. The day and a half (36 h) was when the first coleoptile emergency appeared, which

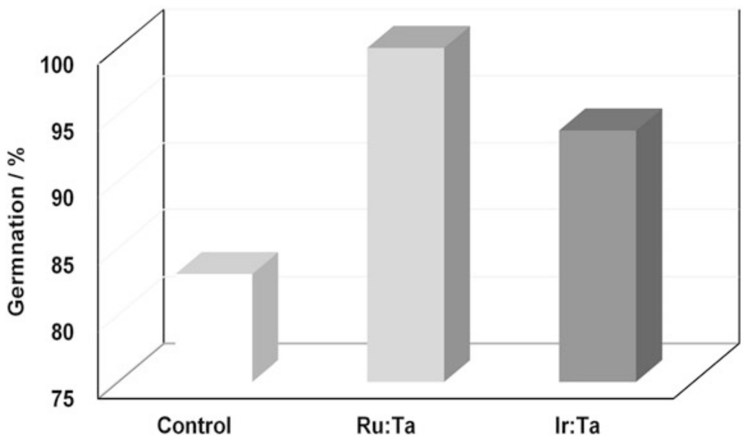

Fig. 39.2 Germination percentage after 14 days of electrofarming using IrO_2-Ta_2O_5|Ti (Ir: Ta) and RuO_2-Ta_2O_5| Ti (Ru: Ta) with respect to the control system

happened in all three cells. In the case of the control, the cell started with 12% germination.

In comparison, in the cell in which RuO_2-Ta_2O_5|Ti electrodes were used, the initial percentage was a 37% increase from the outset, compared to the 15% achieved on the cell IrO_2-Ta_2O_5|Ti electrodes. The electrofarming using RuO_2-Ta_2O_5|Ti reached 100% germination. In the other two tests germination was halted until that day, with a percentage of 94% in the case of IrO_2-Ta_2O_5|Ti and 83% for the control test, proving that the electrofarming treatment might enhance maize seed germination.

39.3.2 Maize Plant Growth and Development (*Zea mays* L)

Figure 39.3 shows photographic images of the monitoring of the growth of maize plants when undergoing electrofarming treatment. After 2 weeks of electrofarming treatment, the plants obtained from the treated maize seeds were harvested and measured as indicated in the methodology section to determine the plants' behavior when applying electrofarming. At the beginning of day 1, there was no change in any of the cells. After 36 h, some seeds broke the testa, and the coleoptile began to emerge. By the second day, the number of germinated seeds increased or when the plantlet started to appear in its lower part, giving way to forming the first roots, what happened on day 3, the same day that the coleoptile emerged almost entirely. On the other hand, Fig. 39.3 shows the germination process that took place daily for each of the treatments, indicating that it happens with the seed.

In Fig. 39.3, there is an evident difference between the analyzed groups, starting earlier in the cell treated with RuO_2-Ta_2O_5|Ti electrodes (day 3), then on day 4, the formation of the first leaves was observed, being higher in the cell of RuO_2-Ta_2O_5|Ti, following with the cell treated with electrodes of IrO_2-Ta_2O_5|Ti, and finally the control test, so it concluded that electrofarming technology affects plant development. The average height of the plants after applying 0.2 Vcm^{-1} using RuO_2-Ta_2O_5|Ti electrodes was higher than those observed when using IrO_2-Ta_2O_5|Ti electrodes and against the control test, as shown in Fig. 39.4.

Different plant measurements were made for length, stem, root, leaf, and stem (Fig. 39.4). For each of the cases, it is possible to appreciate that all the measured parameters increased for those plants treated with electrodes modified with RuO_2-Ta_2O_5|Ti (Ru: Ta), followed by the IrO_2-Ta_2O_5|Ti (Ir: Ta), suggesting that electrofarming is more efficient when using RuO_2-Ta_2O_5|Ti electrodes.

Concerning the stem and the radicle's dry weight, the leaf and radicle width results are shown in Fig. 39.5. In this way, the weight of the bare root (Fig. 39.6a) and radicle (Fig. 39.6b) was measured after 14 days of electrofarming using IrO_2-Ta_2O_5|Ti (Ir) and RuO_2-Ta_2O_5| Ti (Ru) close to the anode (CA), middle cell (MC), and close to the cathode (CC) with respect to the control (C) system. Analyzing the dry plant (Fig. 39.5), it showed that maize plants treated with RuO_2-Ta_2O_5|Ti (Ru: Ta) had a dry root weight gain of 3083 g compared to 2.85 g of IrO_2-Ta_2O_5|Ti (Ir: Ta) and 1.68 g of control test (Fig. 39.6a), which correlated with root coverage in the

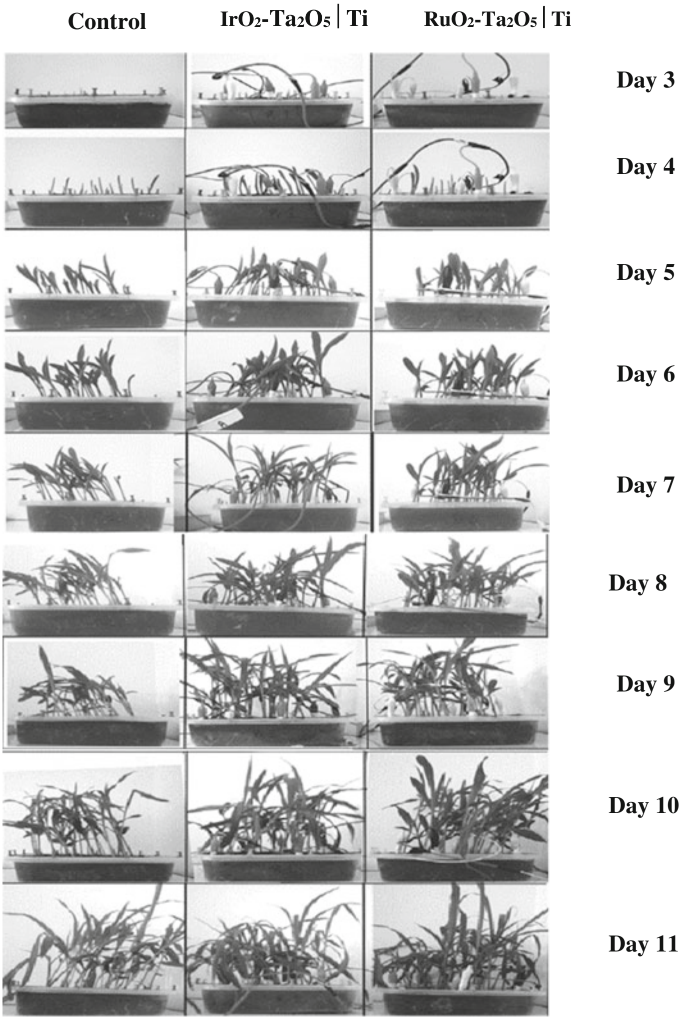

Fig. 39.3 Growth of maize plants after 3, 4, 5, 6, 7, 8, 9, 10, and 11 days of electrofarming using IrO_2-Ta_2O_5|Ti and RuO_2-Ta_2O_5| Ti with respect to the control system

soil. The same was the case for a dry stem, where a higher value was observed for plants whose land was treated with the electrofarming technology using RuO_2-Ta_2O_5|Ti (Ru) electrodes with 8.49 g distributed with 1.60 g CC, 3.13 g MC, and 3.76 g AC, followed by treatment with IrO_2-Ta_2O_5|Ti (Ir) with 7.31 g distributed with 1.09 g CC, 2.82 g MC, and 3.39 g CA.

Additionally, the width of haulm and leaf (Fig. 39.7a) and the average of the number of leaf growth (Fig. 39.7b) were measured after 14 days of electrofarming using IrO_2-Ta_2O_5|Ti (Ir: Ta) and RuO_2-Ta_2O_5| Ti (Ru: Ta) with respect to the control system, with the fifth leaf at this time of evaluation (Fig. 39.7c).

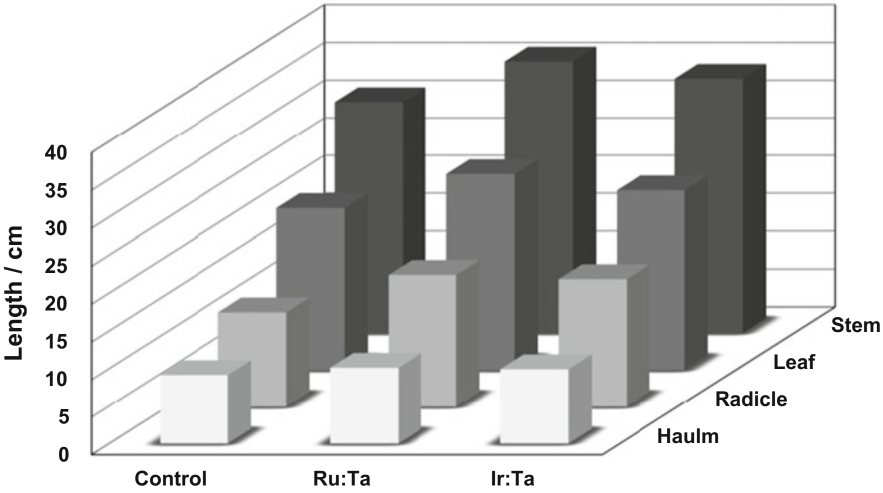

Fig. 39.4 The average length of the stem, leaf, haulm, and radicle after 14 days of electrofarming time using IrO_2-Ta_2O_5|Ti (Ir: Ta) and RuO_2-Ta_2O_5| Ti (Ru: Ta) with respect to the control system

In comparison, for the control test, a total of 6.23 g was obtained (Fig. 39.6b), which suggests an incremented metabolism of the treated plants. Another measurement was the width (cm) of the haulm and the leaves (using as a measuring instrument a vernier, Fig. 39.7a), presenting a more favorable value for plants treated with RuO_2-Ta_2O_5|Ti (Ru: Ta) with 1.15 cm of leaf diameter and 0.34 cm of stem diameter. Figure 39.7b shows that the average leaf size at the end of the experiments was higher for plants treated with RuO_2-Ta_2O_5|Ti (Ru: Ta) with the development of a fifth leaf at this time of evaluation (Fig. 39.7c), an event that did not occur in either of the other electrofarming tests using IrO_2-Ta_2O_5|Ti or the control group.

39.3.3 Edaphological Characterization

Table 39.1 shows the parameters that remained unchanged in the electrochemical cells before electrofarming and the control cells; in the case of texture, a higher percentage of clay with 49.49% was presented, as well as clay soil (> 30%) (Quiroz and Merchan 2016), and is characteristic of the *Vertisol pelic*.

Table 39.2 shows the physicochemical parameters before electrofarming, control systems, and reference values. The pH value of soil before farming was 7.297 ± 0.013, slightly increasing considered without change with a 7.450 ± 0.004, indicating a desirable value for crops as reported in the literature (Rodríguez-Fuentes 2011) which reported as 5.5–7.5 (Andrades and Martinez 2014).

Therefore, the availability of nutrients for our case study remained at their highest level of solubility, as the pH of the farming medium controls the chemical reactions that determine whether or not the nutrients will be available for absorption

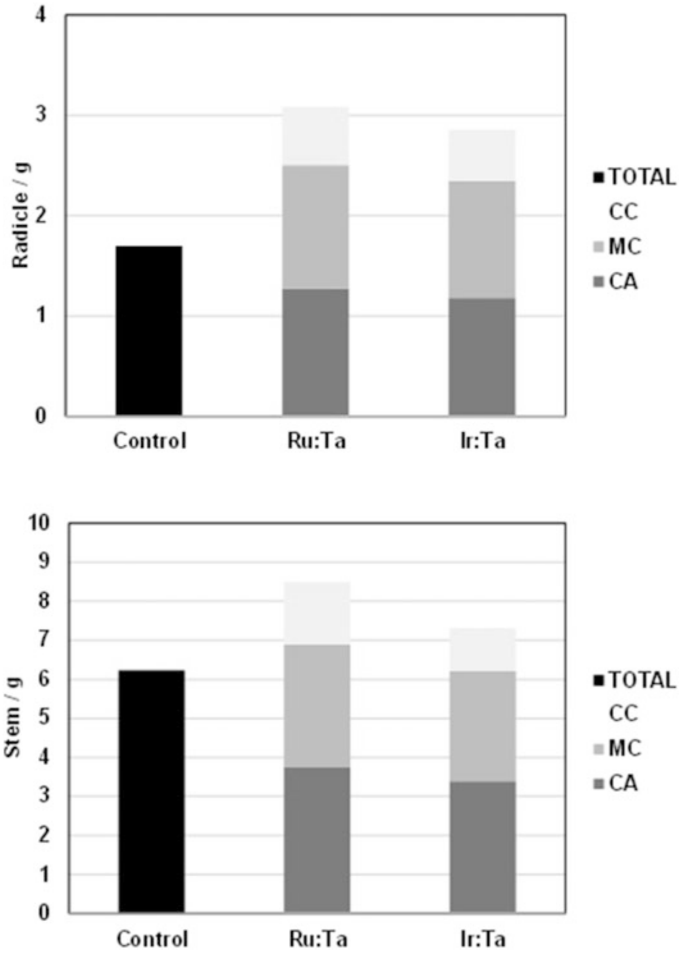

Fig. 39.5 Weigh of radicle and stem growth after 14 days of electrofarming using IrO_2-Ta_2O_5|Ti (Ir: Ta) and RuO_2-Ta_2O_5| Ti (Ru: Ta) close to the anode (CA), middle cell (MC), and close to the cathode (CC) with respect to the control system

(NOM-021-RECNAT- 2000; Barbaro et al. 2005); it can be affirmed that the treatment does not affect pH values which might affect the development of plants negatively. In the CEC case for soil before farming, 37.450 ± 0.346 $Cmol_c$ kg^{-1} was obtained and decreased to 35.50 ± 1797 $Cmol_c$ kg^{-1} after seed farming considering itself an abundant nutrition reserve.

According to the literature, CEC is found high when it is greater than 25 $cmol_c$ kg^{-1} soil, with a range of 24 to 40 $cmol_c$ Kg^{-1} soil (NOM-021-RECNAT- 2000). Furthermore, EC's value follows those reported previously in the literature (Barbaro et al. 2005). Its value was 0.170 ± 0.006 dS m^{-1} before farming and 0.242 ± 0.007 dS m^{-1} after it. This result negligible salinity effects on the soil, where the EC of the

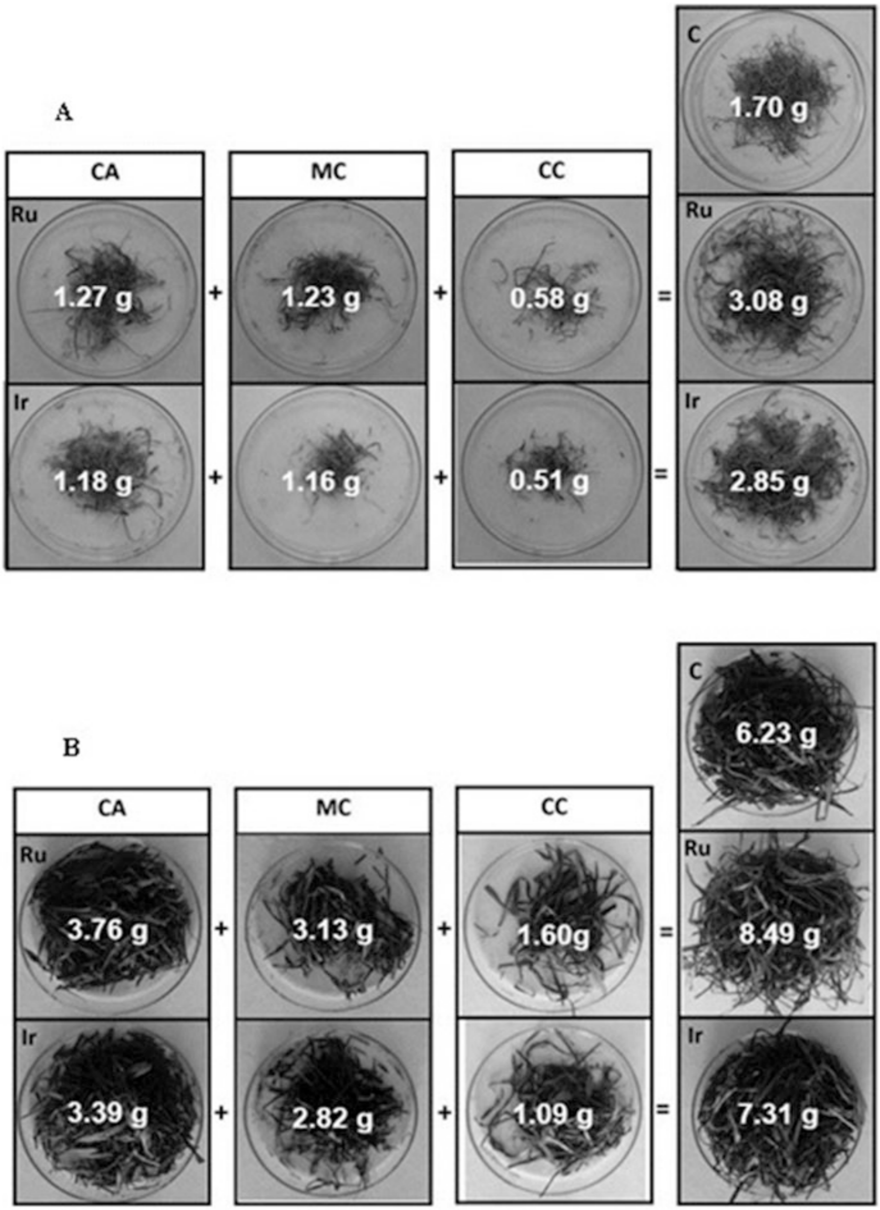

Fig. 39.6 Images and weigh of the dry root (**a**) and radicle (**b**) after 14 days of electrofarming using IrO_2-Ta_2O_5|Ti (Ir) and RuO_2-Ta_2O_5| Ti (Ru) close to the anode (CA), middle cell (MC), and close to the cathode (CC) with respect to the control (C) system

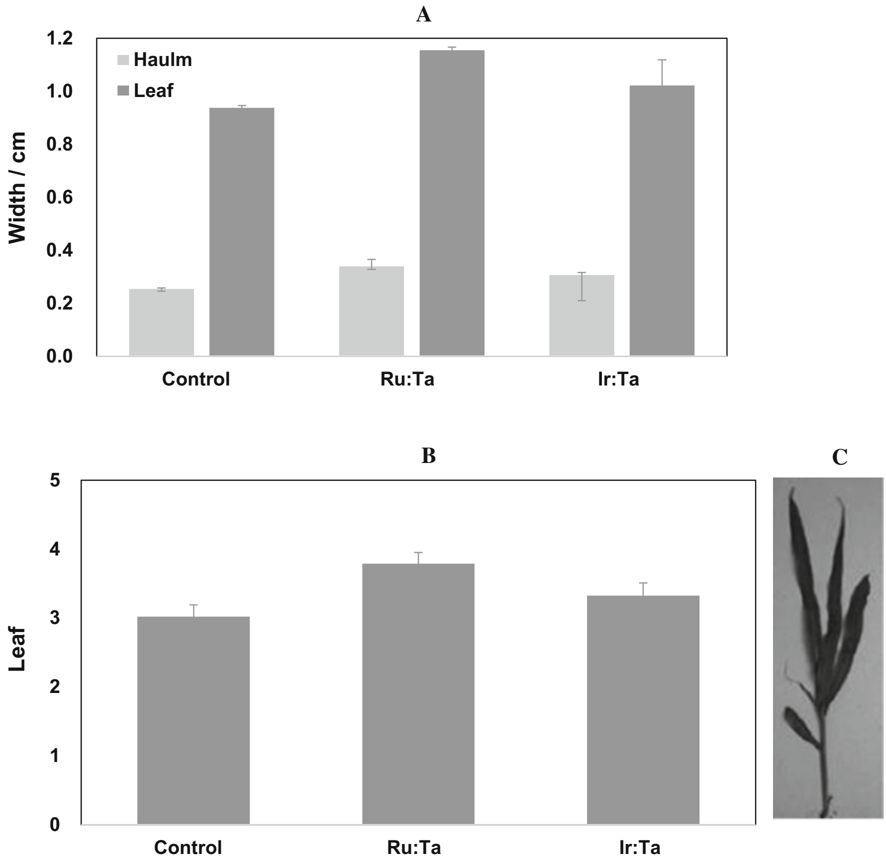

Fig. 39.7 Width of haulm and leaf (**a**) and the average of the number of leaf growth (**b**) after 14 days of electrofarming using IrO_2-Ta_2O_5|Ti (Ir: Ta) and RuO_2-Ta_2O_5| Ti (Ru: Ta) with respect to the control system, with the fifth leaf at this time of evaluation (C)

Table 39.1 Texture and humidity were measured before electrofarming and control systems

Parameter		Before electrofarming	Control
Texture	Clay (%)	49.49 ± 1.15	49.49 ± 1.15
	Sand (%)	34.51 ± 3.06	34.51 ± 3.06
	Silt (%)	16.00 ± 4.00	16.00 ± 4.00
Humidity (%)		5.26 ± 0.38	21.61 ± 3.04

substrate recommended must be less than one dS m^{-1} (Barbaro et al. 2005), this data reports that there is no salt accumulation on the soil due to evaporation processes related to the electrical activity of the treatment.

Before farming, the soil showed 4.503 ± 0.173% of SOM, and control soil after farming was 6.361 ± 0.362%. According to the classification for non-volcanic soils established in NOM-021-RECNAT- 2000, this places them at a high value (3.6 to

Table 39.2 Physicochemical parameters before electrofarming, control system, and reference values: pH_{water}, pH_{KCl}, cation exchange capacity (CEC), electrical conductivity (EC), soil organic matter (SOM), calcium (Ca^{2+}), magnesium (Mg^{2+}), carbonates (CO_3), bicarbonates (HCO_3^-), chloride (Cl^-), sodium (Na^+), potassium (K^+), phosphate (PO_4^{3-}), and enzymatic activity

Parameter	Before electrofarming	Control	Reference value
pH_{water}	7.297 ± 0.013	7.450 ± 0.004	5.5–7.5
pH_{KCl}	6.380 ± 0.036	6.530 ± 0.009	4.5–6.5
CEC ($Cmol_c\ kg^{-1}$)	37.450 ± 0.346	35.50 ± 1.797	25.0–40.0
EC ($dS\ m^{-1}$)	0.170 ± 0.006	0.242 ± 0.007	<10.0
SOM (%)	4.503 ± 0.173	6.361 ± 0.362	>6.0
Soluble cations			
Ca^{2+} ($mmol_c\ kg^{-1}$)	0.42 ± 0.002	0.28 ± 0.005	<2.0
Mg^{2+} ($mmol_c\ kg^{-1}$)	0.11 ± 0.001	0.22 ± 0.008	<0.5
CO_3 ($mmol_c\ kg^{-1}$)	0.000 ± 0.000	0.000 ± 0.000	<0.5%
HCO_3^- ($mmol_c\ kg^{-1}$)	9.500 ± 0.500	9.000 ± 0.000	2.2–15.0
Cl^- ($mmol_c\ kg^{-1}$)	6.500 ± 1.000	3.500 ± 0.447	5.0–8.0
Exchangeable cations			
Na^+ ($Cmol_c\ kg^{-1}$)	1.196 ± 0.049	0.490 ± 0.041	0
K^+ ($Cmol_c\ kg^{-1}$)	1.028 ± 0.038	0.873 ± 0.048	> 0.6
P_2O_3 ($mg\ kg^{-1}$)	9.020 ± 2.771	9.400 ± 0.778	5.5–11.0
Enzymatic activity (ppb)	6.097 ± 0.470	16.140 ± 1.38	0

6.0%) and very high SOM (> 6%), which corroborated in the same way with what was reported by Andrades and Martinez in 2014 for clay soils with a range value >3% which is considered as very high value (Andrades and Martinez 2014). The adequate proportion of SOM in the soil increases the total change capacity by favoring a good reserve of nutrients (NOM-021-RECNAT- 2000).

The concentration of the interchangeable bases of Ca^{+2} (0.042 ± 0.002 $Cmol_c\ kg^{-1}$) and Mg^{+2} (0.022 ± 0.008 $cmol_c\ kg^{-1}$) was very low, relative to the ranges reported in NOM-021-RECNAT-2000 (<2 and <0.5 $Cmol_c\ kg^{-1}$), respectively. As for the concentration of Na^+ ions in the initial soil which was higher (Na^+ was slightly above the expected mean concentration), this confirmed with the result obtained for the CEC that presented a top nutrition reserve. Besides, K^+ ions showed a high level value of 0.6 $Cmol_c\ kg^{-1}$ soil, with benefits for land before and after farming of 1.028 ± 0.038 and 0.873 ± 0.048 $Cmol_c\ kg^{-1}$, respectively, a high value which was ideal for growing maize as it is in the literature (Rodríguez-Fuentes 2011; Barbaro et al. 2005). There was an absence of carbonates (CO_3^{2-}) in soil, but a high presence of bicarbonates (HCO_3^-, 9 mmol L^{-1}) in the expected range as per NOM-021-RECNAT-2000 (2.2–15 mmol L^{-1}). P (mg kg^{-1}) a value was presented within the mean range (5.5–11) with 9.02 ± 2.771 before and 9.4 ± 0.778 mg kg^{-1} after farming, based on the amounts reported in NOM-021-RECNAT-2000, and it is considered a weak or scarce soil in this nutrient.

A significant increase was observed for the enzymatic activity after the seed farming, increasing from 6.097 ± 0.47 to 16.14 ± 1.38 μg mL^{-1} TFF. This value permits us to conclude that electrofarming can favor soil microorganisms, since this enzyme (dehydrogenase) is usually found in soil organisms' living populations, indicating microbial activity elevation. The data obtained from a comparison between the control soil used in seed farming and electrofarming-treated land using RuO_2-Ta_2O_5|Ti and IrO_2-Ta_2O_5|Ti electrodes are in Table 39.3, where the control soil and the soil under electrofarming had a similar pH (both in the current and potential, ranged from 7.4 to 6.6, respectively), indicating that a suitable solubility would be ions to promote plant growth.

On the other hand, there was an increase in CEC in the soil treated with electrofarming concerning the control soil (35.50 ± 1.797 $Cmol_c\ kg^{-1}$), which is very similar for Ru and Ir in each section (3.960 $Cmol_c\ kg^{-1}$), so electrofarming improves CEC of the land as in ion exchange. In electrofarming, an increase showed for the control group (0.242 dS m^{-1}), mainly in the area close to the anode for the case of Ir with 0.355 dS m^{-1} and in the middle cell area for Ru with 0.343 dS m^{-1}, so electrofarming (by electrolyte measurement) depends on the total concentration of ions present and the mobility of each of the dissolved ions (Barbaro et al. 2005). In this way, in the case of cells where electrofarming was applied, there is greater mobility and availability of ions than expected by favoring the different transport phenomena during the electric field soil electromigration.

Also, in the case of SOM, a significant increase in soil treated with electrofarming was observed. The SOM increased similarly in the three zones (CA, MC, CC) between 7.2 and 7.6% in the presence of Ir; in contrast, in the case of Ru, there was a more significant presence of SOM in the middle cell area with 7.612% followed by the area close to the cathode with 7.312%, which is to the electromigration of nutrients that occurs when applying the electric field (Pérez-Corona et al. 2013).

In addition, the concentration of Ca^{+2} ions for the control soil was 0.028 $Cmol_c\ kg^{-1}$, showing a slight decrease in soil subjected to electrofarming, in the case of Ir with 0.27 $Cmol_c\ kg^{-1}$ for each section and in Ru with 0.26 $Cmol_c\ kg^{-1}$ (MC) > 0.25 meq 100 g^{-1} (CC) > 0.24 meq 100 g^{-1} (CA). In contrast, in Mg^{+2}, a slight increase was observed, which has no statistical significance, without any change for all cells. Ca^{+2} and Mg^{+2} are absorbed in the plant during its growth, which can happen with maize (Correndo and García 2017).

HCO_3^- concentration increased in the cell where Ru was with 15.5 mmol L^{-1} CA followed by 12.50 mmol L^{-1} in MC and CC. In the case of Ir, there was a decrease to 8.0 and 8.5 mmol L^{-1} CA and CC sections, respectively, while in MC, it remained the same to the control soil with 9 mmol L^{-1}. According to Lemus et al. (2010), root and microbial respiration involves absorption of O_2 and expulsion of CO_2, which dissolves in the soil solution ($CO_2 + H_2O \rightarrow H_2CO_3$), generating carbonic acid that is unstable in alkaline pH and breaks down to bicarbonate ($H_2CO_3 \rightarrow H^+ + HCO_3^-$); for this reason, the presence of carbonates (CO_3^{2-}) remains absent due to breathing processes on the ground. Also, according to Comninellis and Chen (2010), Ru generates more O_2 during electrofarming, so it may be possible to increase HCO_3^- using RuO_2-Ta_2O_5|Ti higher than in the presence of IrO_2-Ta_2O_5|Ti.

Table 39.3 Physicochemical parameters after electrofarming using IrO_2-Ta_2O_5|Ti and RuO_2-Ta_2O_5| Ti (CA = close to the anode, MC = middle cell, CC = close to cathode) with respect to the control system: pH_{water}, pH_{KCl}, cation interchange capacity (CIC), electrical conductivity (EC), soil organic matter (SOM), phosphate (PO_4^{3-}), enzymatic activity, calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), potassium (K^+), carbonates (CO_3), bicarbonates (HCO_3^-), and chloride (Cl^-)

	After electrofarming						
		RuO_2-Ta_2O_5|Ti			IrO_2-Ta_2O_5|Ti		
Parameter	Control	CA	MC	CC	CA	MC	CC
pH_{water}	7.450 ± 0.004	7.440 ± 0.203	7.355 ± 0.099	7.375 ± 0.014	7.405 ± 0.007	7.393 ± 0.009	7.363 ± 0.047
pH_{KCl}	6.530 ± 0.009	6.600 ± 0.100	6.600 ± 0.100	6.600 ± 0.100	6.415 ± 0.029	6.415 ± 0.031	6.415 ± 0.030
CIC (meq 100 g^{-1})	35.50 ± 1.797	38.313 ± 1.317	39.85 ± 4.045	39.920 ± 4.179	39.600 ± 1.544	39.600 ± 1.565	39.600 ± 1.565
EC (dS m^{-1})	0.242 ± 0.007	0.243 ± 0.047	0.343 ± 0.069	0.303 ± 0.007	0.355 ± 0.009	0.301 ± 0.009	0.263 ± 0.032
SOM (%)	6.361 ± 0.362	6.550 ± 0.767	7.612 ± 0.308	7.312 ± 0.739	7.552 ± 0.214	7.638 ± 0.348	7.261 ± 0.200
PO_4^{3-} (mg kg^{-1})	9.400 ± 0.778	11.720 ± 2.715	10.820 ± 0.665	8.190 ± 4.045	10.310 ± 0.056	10.780 ± 1.952	10.630 ± 4.264
Enzymatic activity	16.14 ± 1.380	24.790 ± 4.520	11.080 ± 2.440	20.660 ± 6.610	16.990 ± 4.300	11.660 ± 2.580	9.670 ± 3.514
Ca^{2+} (meq 100 g^{-1})	0.028 ± 0.005	0.024 ± 0.004	0.026 ± 0.003	0.025 ± 0.003	0.027 ± 0.002	0.027 ± 0.002	0.027 ± 0.003
Mg^{2+} (meq 100 g^{-1})	0.022 ± 0.008	0.022 ± 0.002	0.024 ± 0.002	0.024 ± 0.002	0.021 ± 0.007	0.020 ± 0.007	0.021 ± 0.007
Na^+ ($Cmol_c$ kg^{-1})	0.490 ± 0.041	0.424 ± 0.228	0.595 ± 0.143	0.555 ± 0.076	0.678 ± 0.187	0.551 ± 0.116	0.436 ± 0.174
K^+ ($Cmol_c$ kg^{-1})	0.873 ± 0.048	0.853 ± 0.074	0.838 ± 0.073	0.709 ± 0.260	0.998 ± 0.188	0.875 ± 0.051	0.725 ± 0.101
CO_3 (mmol L^{-1} de CO_3^{2-})	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
HCO_3^- (mmol L^{-1} de H_3^-)	9.000 ± 0.000	15.500 ± 1.643	12.500 ± 1.643	12.500 ± 7.120	8.000 ± 0.000	9.000 ± 2.191	8.500 ± 0.548
Cl^- (mmol L^{-1} de Cl)	3.500 ± 0.447	7.000 ± 0.707	7.500 ± 0.707	7.750 ± 0.524	7.250 ± 1.440	6.500 ± 0.707	9.250 ± 0.524

Chloride (Cl^-) content increased in the cells where electrofarming was used compared to the control soil, which can function as a disinfectant near the plants' root, promoting the more exceptional approach of nutrients in the case with the presence of $^{\bullet}OH$. Cl^- compounds are highly soluble in water. They persist in dissociated form as anions, with positively charged cations (such as Na^+). The data was obtained from Na^+ and K^+ determination, where greater availability was cations in electrofarming-treated cells.

Regarding the Na^+ concentration, a higher value of this nutrient was observed in the Ir and Ru cells, having a greater exchange in CA areas with 0.678 and 0.551 $Cmol_c$ kg^{-1} in MC for the case of Ir and in CC areas with 0.555 and 0.595 $Cmol_c$ kg^{-1} in MC for Ru. There was a decrease in CA areas of Ru with 0.424 $Cmol_c$ kg^{-1} and CC of Ir with 0.436 $Cmol_c$ kg^{-1} to the control soil with a value of 0.490 $Cmol_c$ kg^{-1}. Andrades and Martínez (2014) reported an increase in Na^+ resulting in deficiencies of Ca^{+2} and Mg^{+2}.

As for the K^+ content, it had a high value in all cells, increasing only in the CA area of Ir with 0.998 $Cmol_c$ kg^{-1} and decreasing to 0.709 and 0.725 $Cmol_c$ kg^{-1} for CC of Ru and CC of Ir, respectively, while in the remaining areas, it remains similar to control soil with a value of 0.873 $Cmol_c$ kg^{-1}. For maize growth, K^+ is one of the essential nutrients, as it contributes to the overall development of the plant, mainly in leaf (Kant and Kafkafi 2002; SAGARPA 2016). It may be happening that these cations are more active at exchange sites due to the electrical field applied.

The phosphorus concentration increased for electrofarming-treated cells to soil control for most areas, except for the CC area of Ru, from 9.4 (control soil) to 8.19. The highest concentration was also detected for Ru cells in the CA area with $11.72 > 10.82$ (MC of Ru) > 10.78 (MC Ir) > 10.63 (CC of Ir) >10.31 (CA of Ir), suggesting that it contributed to the increase of stem and leaf in these areas mostly. On the other hand, to verify the ratio of the soil parameters measured in the soil under electrofarming to Ir and Ru compared against the control group, the data obtained from Table 39.3 were used to perform a correlation analysis between them, separated by treatment: control, $RuO_2\text{-}Ta_2O_5|Ti$ and $IrO_2\text{-}Ta_2O_5|Ti$.

CEC is related to pH by the ionic load of the particles of soil, where there are some functional groups ^-OH on its surface, which can absorb protons. EC was higher by the concentration of salts in the solution, which depends on the pH. In this way, it recommended that the electrofarming of a substrate below, as far as possible less than 1 dS m^{-1}. EC was low and facilitated the management of fertilization and avoids problems by phytotoxicity in the crop (Barbaro et al. 2005), so the value obtained in this research work was 0.242 dS m^{-1} in control soil and 0.343 dS m^{-1} on the electrofarming-treated ground, indicating that soil fertility was favored because the EC was less than 1 dS m^{-1}.

These experiments probed that SOM % was related to pH, as SOM had the potential to provide nutrition to crops such as N, P, and S, among others. It provided the soil with the buffering capacity to resist pH or salinity variations because the soil humus fraction contains reactive groups with H^+ in their structure, which is released to the ground in the decomposition process (Rodríguez et al. 1993). Also, pH ($\cong$7.4) and SOM (>6%) were obtained in the results of edaphological characterization, which are ideal for maize growth as reported in the literature (NOM-021-RECNAT-2000; Andrades and Martinez 2014).

Ca^{+2} is governed by cation exchange phenomena like the other cations and remained attached as an interchangeable Ca^{+2} on the surface of negatively charged colloids. Generally, Ca^{+2} is the dominant cation in the soil as observed in this study, even at low pH values, hence their ratio, because the available H^+ ions were placed in negatively charged spaces in the soil, thus displacing nutrients such as Ca^{+2}.

CIC is the number of cations that can be retained by soils, and one of those cations is K^+ (Rodríguez et al. 1993), hence their relationship in this study, which favored the growth of maize plants when applying the electrofarming treatment, and by showing a good content of K^+, a high value was presented in all electroculture cells. For maize growth, K^+ is one of the essential nutrients, as it contributes to the overall development of the plant, mainly in the leaves (Kant and Kafkafi 2002; SAGARPA 2016).

Na^+ was related to the soil EC, according to the EC analysis in these soils was made to establish whether the soluble salts (Na^+, K^+, $Ca^{+2,}$ and Mg^{+2}) were distributed in adequate quantities needed to affect normal seed germination (without treatment), whether in the plant growth or plant water absorption. In this way, Na^+ was employed in small amounts in the plants, as micronutrients, and helps in the metabolism and synthesis of chlorophyll in maize and was even a partial substitute for potassium and was useful in opening and closing stomata, which helped regulate the internal water balance.

EC increased mainly close to the anode of IrO_2-Ta_2O_5|Ti and in the middle cell area when RuO_2-Ta_2O_5|Ti was used. EC (by electrolyte measurement) depends on the total concentration of ions present and each of the dissolved ions' mobility. In the cells where the electrofarming developed was using RuO_2-Ta_2O_5|Ti anodes, there is greater mobility and availability of ions than when the IrO_2-Ta_2O_5|Ti anodes were (Table 39.3).

Na^+ showed greater availability in the cells containing the anodes of IrO_2-Ta_2O_5|Ti compared to those containing the anodes of RuO_2-Ta_2O_5|Ti, having greater availability in CA and MC areas for the case of IrO_2-Ta_2O_5|Ti and CC and MC areas of RuO_2-Ta_2O_5|Ti. Na^+ ion results generated Ca^{+2} and Mg^{+2} deficiencies, which explains that the Ca^{+2} concentration was lower in IrO_2-Ta_2O_5|Ti cells than in those of RuO_2-Ta_2O_5|Ti, as the Na^+ content was excessive. EC measures represented the number of salts present in solution, which refers to the inorganic constituents of the soil soluble in water, like Na^+, K^+, $Ca^{+2,}$ and Mg^{+2}.

K^+ governed by the phenomena of cation exchange just like the other cations remains attached as an interchangeable K^+ on the surface of the loaded colloids, as well as is one of the elements which is fundamental for the development of plants, being essential in the germination process, as it was in the case of maize. K^+ acts as a regulator of the element nitrogen and other elements for the first development of plants, such as maize. The absorption of nutrients by the plant begins before it emerges from the soil. The amounts of nutrients absorbed by seedlings during the early stages of development are not large, yet the nutrient concentration should be high near the root of the developing seedling. The absorption of K^+ by the plant stops practically after the grains' appearance, but other nutrients such as nitrogen and phosphorus continue to vary close to the plant's maturity stage.

Some nitrogen and phosphorus, and other nutrients essential for the plant, move from the plant's vegetative parts (leaves and stem) to the developing grains during the final stages of maize growth. This translocation can result in leaf underdevelopment unless adequate amounts of nutrients are available to the plant during these plants' development period. Nitrogen and phosphorus that the maize plant absorbs are in the grain. Most of the absorbed K^+ returned to the soil in the leaves, stems, and other parts of the plant. Even though the amount of nutrients absorbed is relatively small, the final size of the leaves, stream, cob, and other parts of the plant depends heavily on the adequate availability of nutrients during this initial period.

In the cells where IrO_2-Ta_2O_5|Ti was, SOM's presence increases similarly in the three zones (CA, MC, and CC). Therefore, OM's increased presence was in the MC area, followed by the CC area, which could be due to the electromigration of nutrients, as reported in the literature (Acosta-Santoyo et al. 2018).

Most of the plant's dry matter consists of organic carbon materials that result from photosynthesis, and subsequent processes require 16 chemical elements for the plant to develop and be productive. The supply of each nutrient's right amount during each plant development stage is essential for optimal crop development. In IrO_2-Ta_2O_5|Ti electrodes, they are suitable radical generators of $^{\bullet}OH$ (Volkov 2012; Aladjadjiyan 2012) and in contact with the soil may favor the increase of oxygen, which in turn helps in the germination of the seeds. For their part, electrodes modified with Ru are suitable oxygen generators and favor photo-oxidation and water reduction.

39.4 Conclusions

RuO_2-Ta_2O_5|Ti (30:70) electrodes in electrofarming favored seed germination and growth rate of maize plants compared to IrO_2-Ta_2O_5|Ti (70:30) electrodes and the control test with no electricity. Also, RuO_2-Ta_2O_5|Ti (30:70) electrodes generated better plant growth, and they tended to grow more uniformly in the treated maize plants and increased the appearance of leaves compared to IrO_2-Ta_2O_5|Ti (70:30) electrodes and the control test. Thus, the edaphological properties are positively affected by electrofarming treatment.

On the other hand, the results showed no soil pH change during electrofarming, but there was an increase in CEC and EC. Treated soil presented simultaneous rise of SOM close to the anode, close to the cathode, and middle cell using IrO_2-Ta_2O_5|Ti (70:30) and RuO_2-Ta_2O_5|Ti (30:70), in the central cell area followed by the area close to the cathode.

Finally, electrofarming using RuO_2-Ta_2O_5|Ti (30:70) favors greater mobility and availability of ions like Mg^{+2}, as well as an increase in HCO_3^- due to the production of oxygen by RuO_2_Ta_2O_5|Ti (30:70), which generated 100% seed germination, higher than that obtained with IrO_2-Ta_2O_5|Ti (70:30), where the germination percentage was 94.29%.

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40 Recent Advances in Biotechnology for Generating Yellow Mosaic Disease Resistance in Mungbean (*Vigna radiata* L. Wilczek)

Sanjeev Kumar, Yuan-Yeu Yau, Mona Esterling, and Lingaraj Sahoo

Abstract

Yellow mosaic disease (YMD) affects several types of vegetables and leguminous crops, including the mungbean (*Vigna radiata* L. Wilczek). Mungbean is known as a rich source of digestible protein widely cultivated across Southeast Asia. Plant YMD is characterized by a bright yellow mosaic pattern on leaf surfaces with severe infections also presenting on stems and pods. These infections result in tremendous yield losses (70–100%) with seed quality deterioration in survivors. YMD is not limited to mungbean, as it infects collateral and alternate hosts through the vector whitefly. In the last decade, advancements in molecular detection techniques have identified a wide range of YMD-causing begomoviruses in several legumes, including mungbean. Three major begomovirus species impacting mungbean are *Mungbean yellow mosaic India virus* (MYMIV), *Mungbean yellow mosaic virus* (MYMV), and *Horsegram yellow mosaic virus* (HgYMV). Legume is also an important crop in fixing atmospheric nitrogen through symbiotic association with *Rhizobium* bacteria to improve soil health. Yield loss evaluation of these short-duration crops due to YMD is needed. This chapter focuses on major discoveries related to combating YMD in mungbean, including identifying YMD-causing viruses, describing viral

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vectors, molecular breeding with genetic engineering approaches using pathogen-derived resistance (PDR), RNAi, and CRISPR/Cas-based genome-editing techniques. All of these advances can be used to develop high-yield YMD-resistant mungbean varieties.

Keywords

Begomovirus · Genome editing · Molecular breeding · Mungbean · Transcriptomics · Yellow mosaic disease

40.1 Introduction

Mungbean (*Vigna radiata* L. Wilczek) is a vital grain legume cultivated mainly in South and Southeast Asia. Mungbean is popular due to its short life cycle, intercropping capability, high growth rate, and common use in recipes (Singh et al. 2016). Mungbean cultivation requires low input and fixes atmospheric nitrogen through a symbiotic association with *Rhizobium* and *Bradyrhizobium* bacteria. This benefits soil fertility to enhance yield in subsequently planted crops (Jat et al. 2012). Mungbean seeds are rich in protein (24%) with low flatulence, rich in iron (40–70 ppm), and benefit health, especially in developing countries facing malnutrition challenges (Vairam et al. 2016). Besides seeds, it's sprouts, which contain high vitamin C, are also a popular ingredient used in many Asian cuisine. In 2019, global mungbean cultivation was>7.0 million hectares with an annual yield of 3.5 million tons primarily from Asian countries (Nair et al. 2019). India is the world's largest producer of mungbean with an annual grain yield of 2.17 million tons from 4.32 million hectare, with an average reduction in productivity of ~502 kg/ha (Singh 2011). Mungbean cultivation and production challenges include both biotic and abiotic stressors causing severe yield loss (Ali et al. 2010).

Yellow mosaic disease (YMD) is the primary biotic production constraint of mungbean and grain legumes in the Indian and other South Asian countries. The virus-causing legume YMD carries at least seven distinct species of sap-sucking whitefly (*Bemisia tabaci*). These whiteflies are native hosts to *Begomovirus*, a genus of family *Geminiviridae*. Of the seven best-known viral species, *Mungbean yellow mosaic virus* (MYMV) and *Mungbean yellow mosaic India virus* (MYMIV) are the leading infection-causing agents in mungbean. Both species contain bipartite genome organization and have a very narrow host range. For example, viral strain MYMV or MYMIV majorly infects mungbean. They usually don't infect cereal and vegetable crops. Based on genetic factors involved in mungbean resistance and viral infection stage, yield losses per field vary between 10 and 100%. Infection at early mungbean growth stages increases yield loss and the likelihood of 100% yield loss (Kitsanachandee et al. 2013). Viral infection inhibits photosynthetic efficiency and plant growth by structural changes to plant leaf tissues that ultimately lead to significant yield loss (Sudha et al. 2013a, b). Several approaches have been adopted to manage YMD through vector control of whitefly, involving application of insecticide sprays and various plant extracts. However, effective vector control at the field level remains elusive. Due to wide YMD host range, mutation rate of the viral genome, and quantitative inheritance, durable breeding of YMD-resistant mungbean cultivars presents a challenge. Deployment of resistant genotypes is one promising way to mitigate YMD disease. This chapter systematically deals with recent scientific developments regarding YMD strains infecting mungbean globally, by describing challenges for YMD management including host pathogen resistance, breeding, advanced genetic engineering approaches, and other genomic-based approaches.

40.2 Genome Organization of YMD-Causing Begomoviruses

The family *Geminiviridae* contains nine genera, viz., *Begomovirus*, *Capulovirus*, *Becurtovirus*, *Curtovirus*, *Mastrevirus*, *Topucovirus*, *Eragrovirus*, *Turncurtovirus*, and *Grablovirus*. Viruses are attributed to each respective genus by host, vector, and genome arrangements (Varsani et al. 2017). *Begomovirus* is the largest genus within family *Geminiviridae*, comprising nearly 88% of all geminivirus species (https://talk.ictvonline.org/taxonomy/). Genus *Begomovirus* contains nearly 322 species and more than 500 isolates, which impacts several economically important dicot crops (Varsani et al. 2014, 2017). Begomoviruses contain twinned quasi-icosahedral particles (20 × 30 nm) encapsulating circular single-stranded DNA (ssDNA) genome. *Begomovirus* is known mainly to infect legume crops and vegetables. There are two genetic classifications of begomoviruses, bipartite (containing two DNA components, DNA-A and DNA-B, with size of ≈2.7 kb for each DNA component) or monopartite (ssDNA-A-like component with ≈2.7 kb in size) (Briddon et al. 2010). Their replications are based on "Rolling-Circle Replication" (RCR) model, which was first described nearly half a century ago. DNA-A component contains genes involved in its replication, transcription activation, and encapsidation, overcoming host defense. DNA-B encodes two proteins, nuclear shuttle protein (NSP; ~33.1 kDa) and movement protein (MP; ~29.6 kDa). These two proteins involve in virus inter- and intracellular movement and yellow mosaic symptom development (Singh et al. 2007). Begomoviruses generate geminate (twin) particles measuring 18–20 nm in diameter, apparently consisting of two incomplete icosahedra particles. These twin particles join together to form 22 pentameric capsomeres and 110 identical protein subunits. They are capable of multiplying through whitefly membrane-based barriers, including midgut epithelial cells, apical plasmalemma of these epithelial cells, and basal lamina of primary salivary glands (Hogenhout et al. 2008). Molecular analysis of YMD-causing viruses reveals high levels of relatedness between four bipartite viruses *Mungbean yellow mosaic India virus* (MYMIV), *Mungbean yellow mosaic virus* (MYMV), *Dolichos yellow mosaic virus* (DoYMV), and *Horsegram yellow mosaic virus* (HgYMV). Among these, MYMV, MYMIV, and HgYMV are primary causative agents of YMD in mungbean and other *Vigna* crops within India's subcontinent (Malathi and John 2008).

40.3 Molecular Characterization of YMVs

YMV-causative agents are characterized through complete sequencing of virus isolates or by sequencing of DNA-A and DNA-B components. Early reports indicated 97% sequence similarity for coat protein (CP) and 94% for nuclear shuttle protein (NSP) among MYMIV isolates (Islam et al. 2012). Phylogenetic analysis of legume-infecting begomoviruses revealed MYMIV strain-A as native to Indonesia and MYMV strain-B as native to Vietnam (Tsai et al. 2013). Study of strains across Pakistan involved sequencing of 44 viral DNA components (23 DNA-A, 19 DNA-B, and two beta-satellite DNA) from a variety of *Legume yellow mosaic viruses* (LYMVs) revealing MYMIV of two distinct types (Ilyas et al. 2010). Molecular analysis of both blackgram-infecting begomovirus and cowpea-infecting begomovirus showed both to be strains of MYMIV (Naimuddin et al. 2011a, b; Kumar et al. 2017a, b). Characterization of viral coat protein (CP) genes revealed considerable genetic variability in MYMV isolates from blackgram, mungbean, and cowpea samples (Maheshwari et al. 2014). In 2018, blackgram YMV occurring in Andhra Pradesh (India) were identified as MYMIV isolates. However, MYMV was found to cause YMD disease in the neighboring state of Tamil Nadu (India). Recently, a new isolate of MYMIV (Mg-mungbean-1) was reported from Meghalaya (India) containing recombinant DNA-B. In support of this finding, DNA-A phylogeny confirmed this novel isolate to be MYMIV (Banerjee et al. 2018).

40.4 Host Range and Evaluation of Disease Symptoms

Diverse YMD host range has been reported in MYMV, MYMIV, and HgYMV. They are able to survive on a variety of alternate and collateral hosts. Alternate hosts of MYMV include pigeon pea (*Cajanus cajan*) (Deepa et al. 2017; Sai et al. 2017), urdbean (*Vigna mungo* L. Hepper) (Ramappa et al. 2017; Sai et al. 2017), soybean (*Glycine max* (L.) Merr.) (Ramappa et al. 2017; Sai et al. 2017), common bean (*Phaseolus vulgaris*) (Ramappa et al. 2017; Sai et al. 2017), horsegram (*Macrotyloma uniflorum*) (Ramappa et al. 2017; Deepa et al. 2017), cowpea (*Vigna unguiculata*) (Deepa et al. 2017), tobacco (*Nicotiana benthamiana*) (Deepa et al. 2017), *Croton bonplandianum* (Deepa et al. 2017), *Euphorbia geniculata* (Deepa et al. 2017), *Parthenium hysterophorus* (Deepa et al. 2017), *Malvastrum cormandelianum* (Deepa et al. 2017), *Acalypha indica* (Deepa et al. 2017), and *Alternanthera sessilis* (Deepa et al. 2017).

MYMIV is known to infect cowpea (*V. unguiculata*) (Kumar et al. 2017a, b), common bean (*P. vulgaris*) (Shahid et al. 2012), lima bean (*P. lunatus*) (Shahid et al. 2012), pigeon pea (*C. cajan*) (Biswas et al. 2008), soybean (*G. max*) (Marabi et al. 2017a, b, 2018), urdbean (*V. mungo*) (Chakraborty and Basak 2018; Marabi et al. 2017a, b), wild mungbean (*V. radiata* var. *sublobata*) (Naimuddin et al. 2011a, b), wild urdbean (*V. mungo* var. *silvestris*) (Naimuddin et al. 2011a, b), cucumber (*Cucumis sativus*) (Shahid et al. 2018), *Ageratum conyzoides* (Marabi et al.

2017a, b), *Corchorus olitorius* (Marabi et al. 2017a, b), and *A. sessilis* (Marabi et al. 2017a, b).

HgYMV hosts include common bean (*P. vulgaris*) (Prema and Rangaswamy 2017), pole bean (*Phaseolus coccineus*) (Prema and Rangaswamy 2017), soybean (*G. max*) (Prema and Rangaswamy 2017), lima bean (*P. lunatus*) (Prema and Rangaswamy 2017), rice bean (*Vigna umbellata*) (Prema and Rangaswamy 2017), and moth bean (*Vigna aconitifolia*) (Qazi et al. 2007; Prema and Rangaswamy 2017).

Visible symptoms of YMV infection are (a) appearance of yellow and green irregularly shaped areas on the leaves of infected host plant, (b) reduction of leaf size resulting in complete yellowing and drying at late stage, (c) bidirectional leaf curling, (d) necrotic patches visible on late-stage infected leaves, and (e) overall stunted growth of infected plants (Fig. 40.1). Some other appearance changes in infected plants are reduced number of flowers and pods containing small, shriveled seeds (Poehlman 1991).

40.5 Breeding for Enhancing YMD Resistance

Mungbean cultivation is affected by three major begomoviruses MYMV, MYMIV, and HgYMV, with MYMV reported to be the most destructive. Presently in India, susceptibility to mungbean yellow mosaic viruses is ubiquitous across mungbean varieties with rates of field infection reported between 10% to upto 100% (Nene 1972). A recent study reports that genetic similarity between MYMV-mungbean strain and MYMV-urdbean strain were found dominant in northern India. *MYMV-Vigna* was found to be predominant in southern India, while *Mungbean yellow mosaic India virus* (MYMIV) strains predominate in eastern India (Nair et al. 2017). Mungbean genotypes resistant to MYMD are potential donors for breeding programs to create mapping populations for development of MYMD-related potential markers. Researchers have deployed traditional plant breeding and disease screening methods to develop mungbean-resistant lines. Marker-assisted selection (MAS) is the most promising technique for developing disease-resistant cultivars. Linked markers for identifying R gene and quantitative trait loci (QTL) help construct maps with the potential to broaden MYMD resistance breeding programs (Sudha et al. 2013a, b). In this context “VMYR1,” a yellow mosaic virus linked marker, has been developed in mungbean (Basak et al. 2004).

During mungbean cultivation season, plant breeders usually use linked-marker “genotyping” result as indicators for absence/presence of viral disease. As opposed to genotyping, phenotyping is a complex, time-consuming process and the data might not be as accurate as molecular data. So, it is important to introduce molecular breeding to reduce labor and time required during introgression of MYMD resistance. New donors from interspecific sources have also been identified to aid the development of MYMD resistance (Nair et al. 2017).

40.6 Pathogen-Derived Resistance and RNAI-Based Strategy

Pathogen-derived resistance (PDR) refers to ectopic expression (RNA or protein) of viral genomic sequences against homologous or heterologous viruses. Sequences for viral coat protein (CP), transcriptional activator protein, transcriptional enhancer protein, membrane protein (MP), replicase, and others have been attempted to impart YMD resistance in legumes and other plants. Similar genomic sequences have been used for RNAi-based gene-silencing technology (Karthikeyan et al. 2014). For developing YMD resistance against geminiviruses, coat protein (CP) and Rep protein (AC1) are primarily used for PDR (Kunik et al. 1994). However, lack of reports in mungbean PDR might be due to *Agrobacterium*-mediated transformation recalcitrance.

Shivaprasad et al. (2006) demonstrated MYMV-gene-based PDR using DNA from Rep-sense, Rep-antisense, CP, movement protein (MP), nuclear shuttle protein (NSP), and T-Rep in tobacco leaf disc assay. Similarly, hpRNA technology with AC4-sense and AC4-antisense was also employed to evaluate MYMV DNA accumulation in tobacco. This reveals potential for AC4 hpRNA gene to confer YMD resistance (Sunitha et al. 2013). However, lack of resistance was observed in blackgram, when a MYMV-derived DNA-A bidirectional promoter was used to activate post-transcriptional gene silencing (PTGS) against YMD resistance (Pooggin et al. 2003). In another mungbean study, plants were inoculated with infectious *MYMIV* clones containing complementary-sense gene (*AC1*) encoding Rep and showed infection rates of 64% infection (Haq et al. 2010), but co-inoculation with anti-Rep construct reduced both symptom severity and infection rates. Haq et al. (2011) reported deletion of MYMIV CP amino acid sequences at N0 (75 and 150) to change pathogenicity and systemic spread of viral particles, while agroinfiltration of the RNAi-based CP-hairpin construct prevented viral pathogenesis in mungbean (Kumari and Malathi 2012).

Kumar et al. (2017a, b) also demonstrate RNAi-derived resistance to MYMIV in cowpea. MYMIV agroinfection of transgenic cowpea lines expressing RNAi-*AC2*- and RNAi-*AC2* + *AC4* constructs showed almost complete resistance to MYMIV. Transgenic cowpea lines were also observed for higher accumulation of transgene-specific siRNAs and low level of viral DNA titers. PDR has become a realistic method for YMD management in a variety of *Vigna* species.

40.7 Genome Editing for Developing YMV Resistance

Since the genome-editing technologies are available, several research groups have successfully developed various viral resistance in different plant species. These genome-editing tools include the most popular clustered regularly interspaced short palindromic repeat (CRISPR) system. Historically, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were used for genome editing before discovery of the CRISPR/Cas-based genome-editing system. The early versions of genome-editing tools (TALEN and ZFN) require a new nuclease pair for every genomic target region. It is less flexible, and the design was complicated. Due to its simplicity, CRISPR technology has rapidly become the most popular genome-editing approach, as its use is widely accepted and some of its products are considered "non-GMO." The CRISPR/Cas system contains two components: a single guide RNA (sgRNA) and a CRISPR-associated endonuclease (Cas protein). Researchers can modify a genomic target by simply designing a new sgRNA for the target sequence. Several recent reports have successfully demonstrated generating virus resistance via genome editing in plant species using CRISPR technology. sgRNAs designed to target genomic regions to generate resistance for more than one strain of *Begomovirus* were also reported (Zaidi et al. 2016).Viral DNA targets, including intergenic region (IR), coat protein (CP), and Rep genes, were used for CRISPR-Cas9's system to impart resistance to *Beet severe curly top virus* (BSCTV) in transgenic tobacco (*Nicotiana benthamiana*) and *Arabidopsis thaliana* (Ji et al. 2015). Ali et al. (2015) generated resistance against multiple strains of geminivirus, viz., *Beet curly top virus* (BCTV), *Tomato yellow leaf curl virus* (TYLCV), and *Merremia mosaic virus* (MeMV), using CRISPR/Cas9 technology in tobacco (*Nicotiana benthamiana*), by deployment of sgRNA specific to the conserved sequence (TAATATTAC) of the viral intergenic region. CRISPR/Cas9-mediated genome editing has been recently reported in other legume crops, including cowpea (*V. unguiculata*), for disruption of the symbiotic nitrogen fixation gene. This study targeted symbiosis receptor-like kinase gene with high mutagenic efficiency (~67%), resulting in complete blockage of nodule formation (Ji et al. 2019). The utility of CRISPR/Cas9 in a *Vigna* species is expected to promote functional genome analyses for other traits, including YMD resistance in other *Vigna* species.

CRISPR-associated endonuclease variants are currently available (i.e., Cpf1 apart from Cas9). This results in higher efficiency and lower off-target effects (Ji et al. 2019). Potential for the development of YMD-resistant strains of *Vigna,* including mungbean, is growing with the help of genome editing. Simultaneously, the CRISPR/Cas9 genome editing system can help identify vital host factors involved with plant resistance control through targeted mutagenesis (Zaidi et al. 2016).

40.8 Transcriptomic Approaches

Due to vast amount of data currently available from genomic tools, researchers have shifted toward a transcriptome approach to better understand mechanisms behind biotic and abiotic stress tolerance. Expression profiling allows for acquisition of quantitative information regarding the transcriptome of research sample, allowing for the study of differential gene expression. Some reports of differentially expressed transcripts have been made available in *Vigna* spp. against YMV. In 2018, Chakraborty and Basak used suppression subtractive hybridization to report transcriptional modifications in *Vigna mungo* by comparing samples of both MYMIV resistance and susceptibility. They identified 145 differentially expressed transcripts in resistant plants and 109 differentially expressed transcripts in susceptible plants.

Interestingly, roughly 43% of the unique, expressed sequence tags (ESTs) shared homology with *Glycine max* sequences. Only 14%, 13%, and 9% of ESTs were homologues of *Vigna radiata*, *Vigna angularis*, and *Phaseolous vulgaris*, respectively. Upon MYMIV infection, gene expressions in resistant genotypes underwent major shifts in jasmonic acid biosynthesis, signal transduction pathways, cell wall modifications, and other metabolic pathways. Their study provided functional validation of 12 genes for differential expression including PR protein, ROS regulatory transcripts, phytohormone, jasmonic acid, detoxification proteins, and several metabolic pathways. This work is foundational understanding of plant's MYMIV systemic-resistant response.

Similarly, Kundu et al. (2015) observed 345 candidate genes illustrating differential expression during compatible or incompatible interactions. ESTs were categorized into nine functional groups based on probable function, as they were known to play major roles in various metabolic pathways such as glycolysis where it showed upregulation in their expression. Certain EST upregulated the genes involved in glycolytic pathway. In transcriptome sequencing, ESTs cannot provide exact quantitative estimates of gene expression. Researchers use quantitative real-time PCR (qPCR) to provide more exact information. Kundu's group also documented a remarkable change in differential expression of mitogen-activated protein kinase (*AtMAPK6*) in *Arabidopsis thaliana*. They observed a ninefold change at 12 h post-inoculation in resistance to VMR84 strains, which is a significant increase in post MYMIV inoculation, suggesting salicylic acid (SA) signaling participation.

Using a transcriptomic approach, Kundu et al. (2015) identified 20 genes related to R protein. They were heat shock protein 90 (*HSP 90*), suppressor of G2 allele of skp1 (*SGT1*), systemic acquired resistance (SAR) indicator gene, pathogenesis-related (PR) genes, genes related to reactive oxygen species (ROS) homeostasis, ascorbate peroxidase (*APOX*), superoxide dismutase (*SOD*), metallothionein (*MET*), thioredoxin (*TRX*), some signal transduction genes such as calmodulin (*CAM*), and other genes such as phenylalanine ammonia lyase (*PAL*), glutathione S transferase (*GST*), ubiquitin ligase, cysteine protease (*CSP*), tryptophan synthase (*TS*), MADS box protein (*MADS*), rubisco activase (*RuAc*), oxygen-evolving complex (*OEC*), auxin response factor (*ARF*), and *WRKY*, at different time point intervals in both *MYMIV*-susceptible and *MYMIV*-resistant cultivars of *Vigna mungo*. In this context, non-TIR-NBS-LRR (a subfamily of plant *R* genes), encoding candidate gene *CYR1*, was characterized and found to be tightly associated with MYMIV resistance in urdbean (Maiti et al. 2012). Similarly, the *LRR LIKE-PROTEIN KINASE* gene was found to be involved in soybean MYMIV resistance (Yadav et al. 2015). Studies have shown that most of the *R* genes encode proteins with nucleotide binding site (NBS) and leucine-rich repeat (LRR) region. Disease resistance genes from NBS-LRR class are identified for conferring multi-virus resistance in chilli (*Capsicum annuum* L.) and have been characterized (Naresh et al. 2017). This indicates in-depth analysis of NBS-LLR candidates in mungbean is needed for mungbean YMD resistance. Candidate genes may also aid the development of gene-specific markers for use in resistance breeding.

40.9 Conclusion and Future Prospect

Since the mid-1990s, mungbean crop diversity has increased. Unfortunately, MYMV-affected areas have gradually increased. These can both be attributed to intensive mungbean farming. YMD resistance is not governed by a single factor but a range of factors including plant genotype, YMV strains, ambient weather conditions, and presence of alternate hosts. YMD management challenges include (i) development of multiple lines resistant to specific viral strains, (ii) reduction of threshold vector population under field conditions, and (iii) lack of precise molecular level information regarding YMV infection mechanisms. The primary reason YMD-durable-resistant mungbean strains have not emerged after four decades of screening is due to the natural existence of a wide variety of begomovirus species. This is compounded by the presence of cryptic species of whitefly in mungbean fields (Nair et al. 2017). Therefore, any efficient *Vigna* YM -management strategy must include study of various YMV strains, distribution of whitefly biotypes, distribution of specific biotypes in target areas (Nair et al. 2017), and artificial screening through forced feeding and agroinoculation (Mohan et al. 2014).

A few studies have suggested more aggressive recombinant strains of legume-infecting begomoviruses have appeared, leading to widespread crop loss (Ilyas et al. 2010). An urgent need exists for an exhaustive genomic database containing a variety of begomovirus isolates responsible for affecting crops worldwide. Detailed phylogenetic information about MYMV and other isolates infecting grain legumes should be included in this database. Such data could facilitate identification of strategies for developing resistance with the help of resistance gene(s) (Prema and Rangaswamy 2018). Coordinating information could assist in appropriate judgments concerning many preventive and control measures, like spray schedules, to reduce YMD incidence.

Mungbean's small genome size makes it a suitable candidate for fast tracking a genome-assisted breeding program for developing YMD-resistant varieties. The rapid advent of low-cost RNA-seq technologies can assist gene mapping for QTLs and MAS. Various markers linked with a YMD resistance gene have been previously reported, and some are population specific and not yet validated across mungbean genotypes. There is a need to identify a large number of SNP markers linked with YMD resistance (Maiti et al. 2012). The omics-based studies involving the transcriptome, proteome, interactome, and degradome of *Vigna* species provide insight into host cell changes that ultimately lead to viral infection. This data may identify functional components involved during both compatible and incompatible interactions.

Due to small genome size, large carrying capacity, and wide host range, geminivirus offers great prospects for various novel applications using RNAi, ZFN, and CRISPR/Cas system for genome modification. Sustainable YMD management may deploy various novel and advanced biotechnological approaches, especially gene editing, QTL-Seq, and RNA-Seq to better understand this vital disease in mungbean (Fig. 40.1).

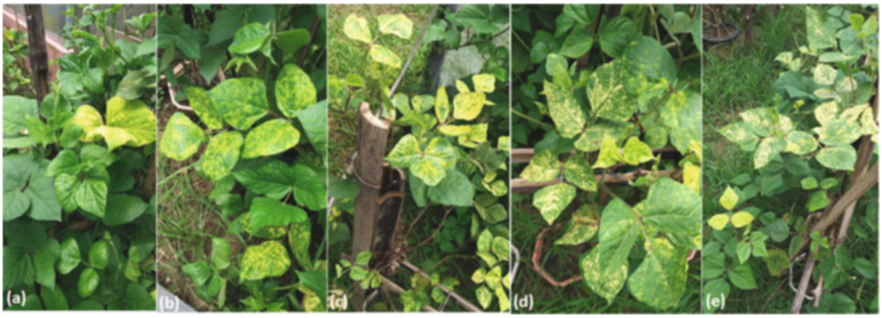

Fig. 40.1 Various stages of MYMIV infection in mungbean plants

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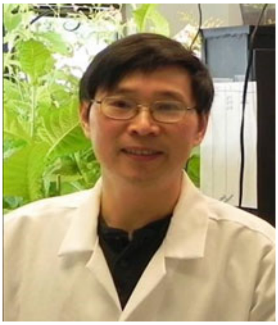

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Appendix

This timely book is a compilation of edited articles by distinguished international scientists discussing the different trends of Environmental Biotechnology in the given two different sections of the book. Scientists have been developing environmental biotechnology as a solution to environmental problems. Coordination of science and engineering expertise relating to the use of microorganisms and their degraded post-treatment products in the prevention of environmental degradation through the biotreatment of waste and the biomonitoring of the ecosystem and treatment processes is provided by environmental biotechnology.

The book also consists of information about Environmental Sustainability using Green Technologies, and new developments in Environmental biotechnology incorporate emerging technologies for the treatment and management of environmental contaminants.

It outlines the requisite biotechnological applications, techniques and processes to ensure sustainable growth and to ensure long-term success in the future. In addition, the book contains new information as well as future directions for biotechnology science. Today biotechnological approaches blended with artificial intelligence and machine learning are helping to develop cutting-edge technology for cleaning up of contamination and restoration of a pollution-free ecosystem.

This book appeals to policymakers, universities, and research institutes by providing a thorough, state-of-the-art assessment of present and future environmental biotechnology expectations.

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