B. Samuel Jacob
K. Ramani
V. Vinoth Kumar *Editors*

Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion

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ISBN 978-981-99-1178-3 ISBN 978-981-99-1179-0 (eBook)
https://doi.org/10.1007/978-981-99-1179-0

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Emerging pollutants sourced from both industries and anthropogenic activity have created havoc in recent years for public health and destruction of biodiversity at multiple levels. The alarming increase in the global population and rapid industrialization might aggravate the problems associated with these hazardous pollutants in the near future. Effluent from different industries may contain high amount of xenobiotic hazardous contaminants such as dyes, hydrocarbons, synthetic surfactants, and microplastics. Industries and public sewers handling such waste streams are facing a plethora of challenges in the effluent treatment and solid waste disposal due to various factors that start from production to adoption of appropriate technologies. Therefore, there is an immediate circumvention of bottlenecks through sustainable mitigation strategies.

Recent boom in circular bioeconomy have created an opportunity to consider the wastes as a resource for value-added products and fuel-similar chemicals. As developing countries are strongly dependent on second- and third-generation biofuels for future energy security, trends in decrease of cultivable land area and water scarcity have forced to depend on waste streams for biofuels and other green alternatives. Waste to wealth could be a sustainable option for the circular economy.

This book entitled *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion* encompasses the chapters that provide a deep insight into pollution abatements and energy production with biotechnological interventions that afford cost-effective technologies. To have a clear view from reader's perspective, the book chapters have been fragmented into two parts as follows:

Part I: Pragmatic treatment for hazardous pollutants

Chapter 1 focuses on principles and methods for the removal of microplastics in wastewater.

Chapter 2 elucidates the impacts of plastics on environmental sustainability and ways to degrade microplastics.

Chapter 3 provides an insight into the biosurfactants for plastic biodegradation.

Chapter 4 discusses the effluent xenobiotics and prospects of biogenic zinc oxide nanoparticles for the treatment of textile dye effluent.

Chapter 5 examines the significant advancements on biotechnological and microbial degradation of textile wastewater.

Chapter 6 emphasizes the emergence of antimicrobial resistance among microbiome in wastewater treatment plant and strategies to tackle their effects in environment.

Chapter 7 discusses the role of wastewater treatment technologies in municipal landfill leachate treatment.

Chapter 8 exemplifies the fungal bioremediation of soils contaminated by petroleum hydrocarbons.

Chapter 9 provides an overview on microbial biosurfactant in the removal of hydrophobic (oily) pollutants laden industrial wastes.

Chapter 10 examines hazardous organic pollutant contamination in Indian holistic rivers risk assessment and prevention strategies.

Chapter 11 details the marine wastes its source, production, disposal, and utilization.

Chapter 12 demonstrates a waste-to-wealth prospective through biotechnological advancements.

Part II: Waste to Energy—Bioconversion route

Chapter 13 explains the industrial perspectives of the three major generations of liquid and gaseous-based biofuel production.

Chapter 14 provides an insight into metabolic engineering approaches for bioenergy production.

Chapter 15 discusses exploitation of marine waste for value-added products synthesis.

This book will help to conceive and take up short term, small budget projects that instill confidence among the industry and academia personnel and promote the development of translational projects. More importantly, this would facilitate closer co-operation between industry and academia in the area of environmental cleanup and bioenergy.

Kattankulathur, Tamil Nadu, India

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Principles and Methods for the Removal of Microplastics in Wastewater

1

Muneeswari Rajasekaran, Swathi Krishnan Venkatesan, Thanmaya Mohan, Maseed Uddin, Ramani Kandasamy, and Sekaran Ganesan

Abbreviations

ESEM-EDS	Environmental scanning electron microscopy-energy dispersive X-ray spectroscopy
FT-IR-ATR	Fourier transform-infrared spectroscopy-attenuated total reflectance
py-GC-MS	Pyrolysis-gas chromatography-mass spectrometry
SEM	Scanning electron microscopy
SEM-EDS	Scanning electron microscopy-energy dispersive X-ray spectroscopy
TDS-GC-MS	Thermal desorption coupled with gas chromatography-mass spectrometry

1.1 Introduction

Plastic has become a necessary commodity in modern-day life. The manufacturing of plastic has been increasing substantially since 1950, and global production has reached 348 million tons in the year 2017 (Qi et al. 2018; Oliveira et al. 2019) As it is a lightweight, versatile, resilient, and inexpensive material, plastic has been

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_1

profoundly used as an essential constituent for a range of commercial and consumer products. Approximately 50% of plastics are used for single-use disposable products, and nearly 80% of the 8 billion metric tons of plastic produced to date are in landfills or accumulating in the environment (Wagner and Lambert 2018).

The injudicious plastic consumption and the poor management of plastic waste disposal around the world have led to high levels of pollution. Currently, small size plastic particles known as microplastics are widely studied as an emerging anthropogenic contaminant due to their detrimental biological effects on biotic life. The most critical concerns of microplastics are their harmful effects, which are often overlooked due to their microscopic size (Andrady 2017; He et al. 2019) The ubiquitous presence and persistence of these smaller sized microplastics in the environment pose a significant threat to all life on earth. They could physically and chemically harm a variety of exposed aquatic organisms ranging from zooplankton to mammals by blocking their digestive tract as well as providing a feasible pathway to transfer via the food chain and ultimately pose a hazard to human health (Alimi et al. 2018). Another issue concerning these particles is that they act as a vector for the transportation of toxic substances such as persistent organic pollutants, pharmaceuticals, or even heavy metals such as nickel or copper present in wastewater (Li et al. 2019; Naqash et al. 2020). Over the last 10 years, many studies have investigated the distribution and effects of microplastics within the aquatic environment, including ocean, a range of freshwater ecosystems worldwide, and even in Polar regions (Herbort and Schuhen 2017). Despite the contribution of several terrestrial sources of microplastics, wastewater treatment plants are suspected to be a primary point source for microplastics to enter the aquatic environment (Talvitie et al. 2017; Tofa et al. 2019). The origins of microplastics can be of both land and aquatic-based in which urban run-off and wastewater treatment plant effluent fall under land-based sources, while fragmented products of weathering, photolysis, and biodegraded products of macroplastics in the aquatic environment come under marine sources (Sun et al. 2019; Padervand et al. 2020). In several studies, smaller fragments of some conventional plastics including polyethylene and polypropylene beads and polyester, acrylic, polyamide, and nylon fibers were identified in the marine environment, and the researchers suggested that wastewater treatment plant effluent could be a leading source of these contaminants (Li et al. 2018a). This was confirmed by many researchers who have also witnessed a significant presence of microplastics in wastewater treatment plant effluent. For example, in a study conducted by Talvitie et al. (2017), microplastics extracted from the tertiary treated effluent of wastewater treatment plant in Finland and seawater from the Gulf of Finland were found to be similar (Ziajahromi et al. 2017). The extracted microplastics from the marine sediment and the wastewater treatment plant effluent were identical, which signifies that wastewater treatment plant effluent could be the main route for the entry of harmful microplastics into the environment. The sampling and the detection of microplastics in the aquatic environment is a significant challenge in the identification of point source for the release of microplastics into the environment (Song et al. 2015; Padervand et al. 2020). Apart from being microscopic, the analysis of the complex mixture of different plastics is even more

tedious. Due to the lack of standard methods for the investigation of microplastics, the development of reliable protocols for the sampling, identification, and characterization of microplastics present in the environmental samples has become the recent research focus among researchers (Godoy et al. 2019). Moreover, studies on the eco toxicological effects of microplastics on living organisms and the pervasive nature of microplastics in the environment emphasize the necessity of more research in this field (Cloutier et al. 2012).

This chapter discusses the environmental interactions of microplastics, different methodologies for the extraction of microplastics, analytical techniques for the characterization of microplastics, and also discusses the challenges and the possible mitigation strategies for the complete elimination of microplastics present in the environment.

1.2 Fate and Occurrence of Microplastics

1.2.1 Occurrence of Microplastics

Wastewater treatment plants can efficiently remove the microplastics in the wastewater but also may act as an entry point for microplastics to migrate into the aquatic environment (Prata 2018). The primary and secondary treatment processes of conventional wastewater treatment can eliminate microplastics from the wastewater by up to 99%. Despite the high removal efficiency, conventional wastewater treatment plants become the most crucial source of microplastics due to the discharge of huge volumes of effluent. Even though 95–99% of solid plastic particles settled with the biosolids, a tenfold increase in the microplastic concentration was observed in downstream of a wastewater treatment plant in the Chicago river (Mintenig et al. 2017). In Europe, it has been estimated that 520,000 tons/year of plastic waste is released in wastewater treatment plant effluent, despite that a substantial proportion of microplastics are suspected to be stuck in biosolids. Also, it is necessary to mention that the usage of wastewater treatment plant biosolids on cultivation lands also could be the potential source of microplastic contamination (Eerkes-Medrano et al. 2015). The schematic representation showing how the treatment plants become the major reservoir of microplastics and entry point for environmental contamination is given in Fig. 1.1.

Generally, microplastics are defined as human-made polymers of size less than 5 mm in diameter, and they are derived from a wide range of sources including textile fibers, pellets from plastic manufacturing and processing industries, and cosmetic industries and the breakdown of larger plastics due to mechanical abrasion and photochemical oxidation in the environment (Dris et al. 2015). Microplastics are found in different shapes such as fragments, foams, granules, and fibers. They are classified into primary microplastics and secondary microplastics (Meng et al. 2019).

1.2.1.1 Primary Microplastics

The primary microplastics are destined to be manufactured in a size >5 mm and are mostly found in clothing, pharmaceuticals, cosmetics like facial and body scrubs,

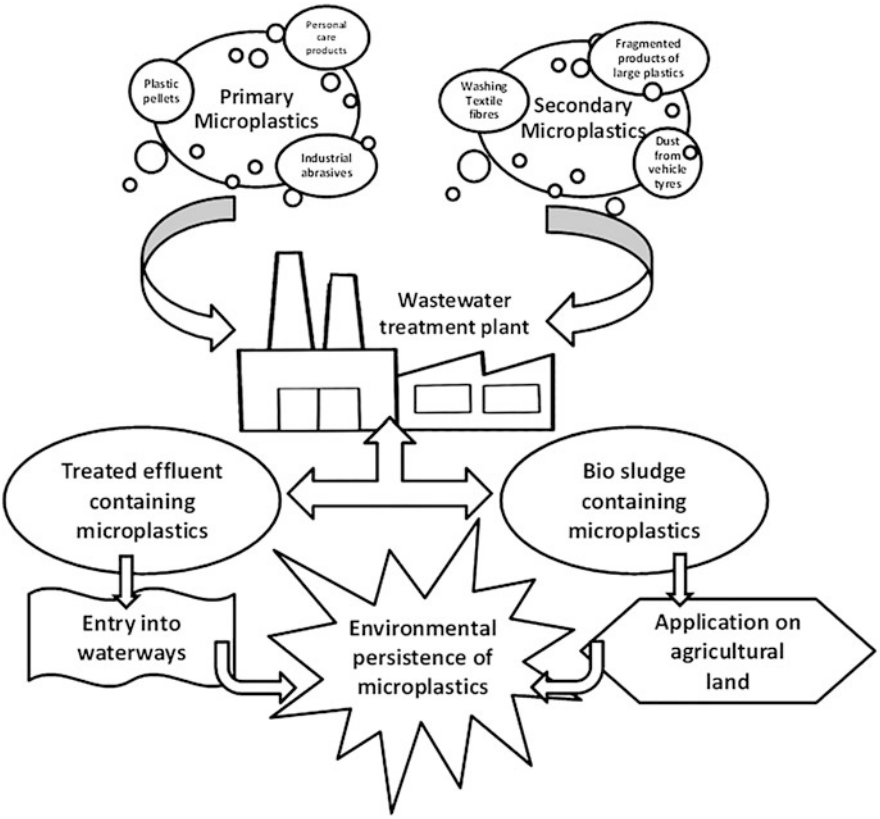

Fig. 1.1 Wastewater treatment plants—A major reservoir of microplastics. Microplastics of different origins discharged into wastewater treatment plants. While at treatment plants, these microplastics are removed via sequential treatment systems with varying efficiency and still large quantities of microplastics are yet again released into the environment through the discharge of treated effluent and biosludge

additives used to increase friction in consumer products, such as cosmetic and facial care products or hand-cleansers and toothpaste, medical supplies, such as grinding polishing agents used in dental teeth and capsules as vectors for inclusion of drugs, overflowing drilling fluid in oil exploration, industrial abrasives and air-blast cleaning media (Van Cauwenberghe et al. 2015). These primary microplastics can be transported by rivers, discharge from water treatment plants, and wind and surface run-off into either freshwater or seawater (Barboza and Gimenez 2015).

1.2.1.2 Secondary Microplastics

Secondary microplastics are the products formed by the fragmentation of large plastic particles due to photo-degradation, physical, chemical, and biodegradation during its stay in the environment (Yu et al. 2018). Fragmentation can occur during the use of materials like textiles, paint, and tires, or once the plastics have been

released into the environment. Most of the microplastics present in the environment are secondary plastics, and there would be an increase in the accumulation of secondary microplastics due to the unceasing disposal of plastics following the continuous transformation of secondary microplastics. Another concern arises since there is a higher probability of further breakdown of microplastics into nano plastics, which possesses environmental risks due to the nature of nano-sizes (Chen 2015).

1.2.2 Environmental Behavior of Microplastics

The increasing contamination of MP and its massive distribution in the environment becomes a potential threat to the lives of both terrestrial and aquatic systems. Microplastics are carcinogenic, genotoxic, teratogenic, and able to cause impaired reproductive activity, decreased immune response, and malformation in animals and humans (Ruimin et al. 2019). The number of research on the distribution and environmental effects of microplastics has been increasing recently. Microplastics are more readily consumed by organisms thus giving more chances for further exposure and subsequent effects compared to the larger plastic pieces (Toussaint et al. 2019). A diversity of organisms, including birds, fish, mammals, and aquatic invertebrates, has been shown to ingest microplastics, which has been related to many adverse effects. Furthermore, microplastics have been exposed as a vector for hydrophobic organic pollutants in the aquatic environment, increasing the accumulation of pollutants by marine organisms (Wang et al. 2019a).

1.2.2.1 Ecological Impacts: Interactions with Biotic Life

The migration of microplastics has been seen across all ecosystems in different trophic levels of both terrestrial and marine environments. Microplastics have entered the food chain: (1) animals including mammals, birds, amphibians, reptiles, and fish; (2) plants including algae of spore-producing plants and gymnosperms and angiosperms of spermatophytes; and (3) microorganisms including bacteria and fungi and ciliophoran, protozoa, and phylum. It has been found that three main factors, such as size, color and shape, and concentration, influence the consumption of microplastics by the organisms (Zhang et al. 2019). Mainly, upon contact with microplastics, either entanglement or ingestion by living bodies will occur, and it has been reported that over 200 marine species suffered from the entanglement and ingestion of plastic debris. However, the degree of the physical impact of microplastics on organisms remains unclear, and entanglement is frequently allied with relatively large animals and is observable when we compare it with consumption. Entanglement could cause severe impacts on aquatic species; they can even be lethal by means of drowning, suffocating, asphyxiating, or starving. The vulnerable species include sea turtles, mammals, seabirds, and crustaceans (Li et al. 2018a). Also, many studies evidenced that organisms at the bottom level of the marine food web ingest microplastic particles, which could lead to unintentional or deliberate consumption of these micro particles by the organisms as microplastics can be

flawed for food. Also, there arises a concern about potential dangers to organisms at the upper trophic level as microplastics ingested by zooplankton can be biomagnified to organisms at higher trophic levels including humans (Auta et al. 2017).

1.2.2.2 Microplastics as a Chemical Threat: Interactions with Organic Contaminants

In recent times, studies on the toxicity of microplastics towards the aquatic ecosystem have also become the research focus. It has been found that microplastics could be a potential carrier for most of the environmental pollutants present in water systems (Li et al. 2019). Compared to freshwater environments, severe mechanical abrasion, and microbial function during the treatment processes in wastewater treatment plants might cause an improved effect on the physicochemical properties of the microplastics. Also, the physicochemical properties of microplastics present in biosolids were found to be influenced by the treatment. For instance, the microplastics are broken down into reduced sizes in lime stabilization, surface melting, and blistering were seen in thermal drying, and the microplastic concentration declined in anaerobic digestion. However, it is imprecise whether these surface alterations influence their adsorbing capacity (Auta et al. 2017). The adsorption and accumulation of several pollutants onto microplastics were widely studied, such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, antibiotics, and heavy metals. The larger surface area and the hydrophobic nature of the microplastics play a significant role in attracting hydrophobic organic pollutants (Guo et al. 2019). Many researchers also evidenced the presence of organic chemicals in a variety of microplastics. Yu et al. (2018) reviewed the behavior of adsorbed organic compounds on the microplastics in the aqueous environment. Previous studies reported that the size and hydrophobicity of Microplastics were the primary influence factors for plastic adsorption. In contrast, hydrogen bonding, hydrophilicity, and increasing specific surface ratio influenced the adsorption potential of aged microplastics. Besides, salinity and the pH of the water system also affect the sorption capacity of microplastics by altering the ionic nature of both microplastics and pollutants and lead to competing for adsorption (Chen 2015).

1.3 Sampling, Detection, and Extraction of Microplastics

1.3.1 Environmental Sampling of Microplastics

Though their search on microplastics has been increasing for years, no standard protocols for sampling, pretreatment, quantification, and identification are available. Also, a significant difference has been observed in previous research, which causes difficulty in developing solutions (Ziajahromi et al. 2017). For the sampling of microplastics from wastewater, two approaches are being followed: volume-reduced sampling and bulk sampling. Simple types of equipment like net-based devices (neuston or plankton nets) or a sieve are used for sampling and required no technical

assistance. The sieve mesh size of 300 μm is used worldwide. The neuston nets are highly recommended for bulk sampling in large rivers and lakes since the microplastics of all size ranges can be retained (Li et al. 2018b).

1.3.2 Extraction of Microplastics

Several separation techniques are being applied for the extraction of microplastics from the aqueous samples. Some studies included a single step, whereas some used a series of separation steps. Sieving, homogenization, concentration, digestion, and density separation are the commonly used extraction techniques (Ou and Zeng 2018). Still, no standardized protocol has been established for the extraction of microplastics from wastewater. More methodological research has to be performed on the extraction of microplastics and their fate during those procedures. Furthermore, the effects of operational parameters such as pH, presence or absence of Fenton's reagent, and temperature remain unknown. The physical characteristics of the microplastics such as size, shape, and density and the chemical attributes like the composition of wastewater (inorganic and organic matters) are the most critical factors to be considered during the separation process (Quinn et al. 2017).

Sieving is the widely used technique applied for the separation of microplastics from the wastewater samples. Density-based separation is another method used for the extraction of microplastics. Typically, salt mixtures are used in density-based separations to provide the buoyancy capacity to plastic particles. The selection of salt mixtures for the separation is made based on the recovery, operation cost, and environmental effect. Some of the generally used salt solutions are NaCl, $CaCl_2$, NaI, $ZnCl_2$, and Sodium polytungstate. However, this method is time-consuming and cannot distinguish the type of plastic. Additional measures like staining and alcohol burning could be used to achieve accuracy in the microplastic's characterization (Ou and Zeng 2018).

1.3.3 Detection of Microplastics

The identification of microplastics from various environmental samples can be performed by using different advanced instrumental analyses. The techniques applied for the detection of microplastics are categorized into physical and chemical characterization methods (Fig. 1.2).

1.3.3.1 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is broadly used for the physical characterization of microplastics. During analysis, the focused beam of electrons will be allowed to pass on the surface of microplastics which provides the morphological images of microplastics. SEM-energy dispersive X-ray spectroscopy (SEM-EDS), and environmental scanning electron microscopy-EDS (ESEM-EDS) could be additionally employed for the determination of elemental composition along with the surface

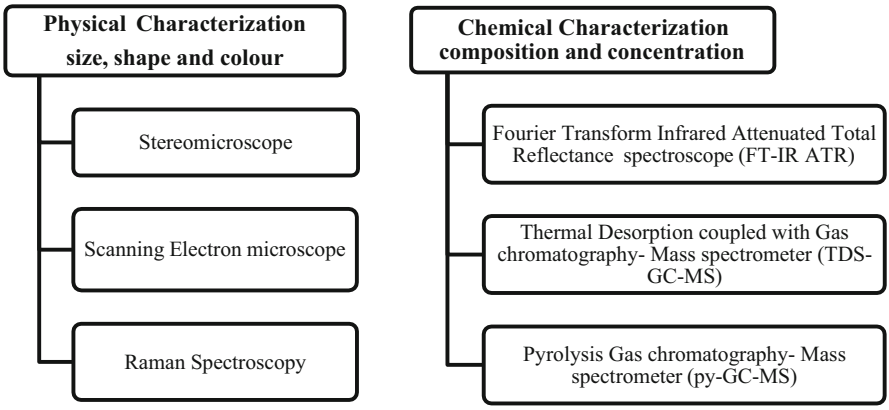

Fig. 1.2 Methods of characterization of microplastics. Analytical techniques available for the identification of microplastics are categorized accordingly

morphology of microplastics based on diffraction and reflection of emitted radiation from microplastics surface (Rocha-Santos and Duarte 2015; Talvitie et al. 2017).

1.3.3.2 Fourier Transform-Infrared Spectroscopy-Attenuated Total Reflectance

Fourier transform-infrared spectroscopy-attenuated total reflectance (FTIR-ATR) is the most frequently preferred method employed for the characterization of microplastics extracted from the effluent of wastewater treatment plants. Here, the infrared spectrum of microplastics will be analyzed for the characteristic peaks corresponding to the test sample with reference to the spectral library (Wang et al. 2019b; Zarfl 2019). During analysis, microplastic samples are exposed to the definite interval of infrared radiation and based on the composition. The molecular structure of the microplastic, excitation vibration spectrum will be derived. In attenuated total reflectance mode, larger microplastics of size more than 500 μ can be analyzed whereas, sizes lesser than 20 μm can be examined under FT-IR coupled with microscopy. These techniques are simple, fast, specific, reliable, well-established, and non-destructive (Song et al. 2015). The newly developed focal array plane-based micro-FT-IR imaging technique is highly effective in the quick acquisition of a broad spectrum in a short duration. The limitations associated with this methodology are (1) sample should be active in the infrared region; (2) Not suitable for non-transparent materials; (3) exorbitant and requires experienced personnel for handling equipment; (4) the detection and interpretation of data could be intervened by environmental matrices such as biofilm (Rocha-Santos and Duarte 2015).

1.3.3.3 Raman Spectroscopy

Raman spectroscopy is another frequently used spectroscopic method for the characterization of microplastics. The functional characteristics of the microplastics can be identified in the form of a vibrational spectrum based on the molecular vibrations

of the sample. This technique is highly sensitive towards nonpolar functional groups and is impervious to undesirable signals of water and atmospheric CO_2. Microplastics of particle size >1 μm can be analyzed using Raman spectroscopy coupled with microscopy, and it is the only technology existing for analyzing microplastics in the range of 1–20 μm.

However, this method is more susceptible to fluorescence intervention by biological, organic, or inorganic substances in samples. Hence, sample purification is necessary to avoid sample alteration before analysis. Some researchers used Nile red fluorescent dye for sample preparation for quick and precise analysis.

1.3.3.4 Thermal Desorption Coupled with Gas Chromatography-Mass Spectrometry

In Thermal desorption coupled with Gas chromatography-Mass spectrometry (TDS-GC-MS) analysis, the sample will be heated at high temperatures up to 1000 °C in a thermo-gravimetric balance; degraded products are allowed to adsorb onto the solid phase and then shifted to a thermal desorption unit. Then, the temperature will be raised to desorb the products, separated in the chromatography column, and finally analyzed by mass spectrometry. TDS-GC-MS is not suitable for qualitative analysis and is only preferred for samples of mass up to 100 mg (Ou and Zeng 2018; Nguyen et al. 2019).

1.3.3.5 Pyrolysis-Gas Chromatography-Mass Spectrometry

Pyrolysis-gas chromatography-mass spectrometry (py-GC-MS) is highly suitable for the characterization of microplastics of size >500 μm, which can be handpicked using tweezers. The analysis involves sample decomposition at elevated temperatures and separation of the gaseous products through the column of gas chromatography followed by mass spectrometric analysis. Reproducibility is challenging with py-GC-MS, as results are highly dependent on sample preparation, pyrolysis type, and pyrolysate transfer. Pyrolysis can be performed in three ways: (1) electrically heated filament pyrolysis, (2) furnace pyrolysis, and (3) curie point pyrolysis. Curie point pyrolysis is faster and more precise among the three methods, and quantification is possible since the temperature is high enough to avoid unpyrolyzed residue. When compared to TDS-GC-MS, py-GC-MS is highly specific and more suitable for the identification of small masses of particles (∼50 μg). The disadvantage of this technology is that the database is available only for selected polymers such as polyethylene and polypropylene (Ou and Zeng 2018; Toussaint et al. 2019; Zarfl 2019; Zhang et al. 2019).

1.4 The Fate of Microplastics during Wastewater Treatment

Understanding the transportation of plastic in wastewater treatment plants is challenging due to the complex nature of wastewater. The high concentration of microplastics is removed at the first screening operations, and microplastics removed during the secondary and tertiary treatment steps are stuck into biological sludge.

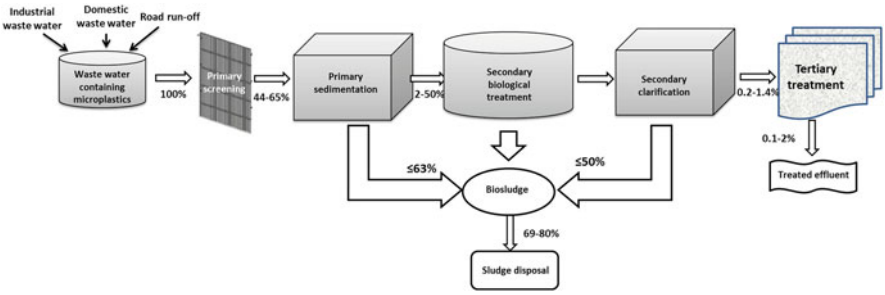

Fig. 1.3 Migration of microplastics during wastewater treatment—Microplastic removal efficiency is given for each stage in wastewater treatment plant

The treated wastewater of a single treatment plant releases around 105–10 numbers of microplastics per day into the environment since a massive quantity of treated effluent is being discharged from the wastewater treatment plants (Ou and Zeng 2018). The movement of microplastics through every treatment stage in a typical wastewater treatment plant was shown in Fig. 1.3.

The primary and secondary stages of the traditional wastewater treatment process can efficiently remove microplastics. However, many researchers suggested that wastewater treatment plants could be the potential sink for persistent microplastics due to the discharge of huge volumes of treated effluent and disposal of bio sludge bound with microplastics. Wastewater treatment plants have been incessantly operated to improve the quality of the effluent, but, there is no specific treatment technology available for the removal of microplastics from the wastewater. Conversely, some studies reported the improved removal efficiency of some unconventional final-stage wastewater treatment processes. Besides, more research have been performed to assess the stage-wise effectiveness of wastewater treatment plants in the removal of microplastics. It has been reported that most of the microplastics are removed during the primary stage itself and estimated that for every 1.14 thousand liters of discharge, an average of one microparticle was found.

Further, Talvitie et al. (2017) studied the efficiency of three different new tertiary treatment technologies: disc filter, rapid sand filtration, and dissolved air flotation in the removal of microplastics from the effluent of four wastewater treatment plants. Membrane bioreactor treating primary effluent and the tertiary treatment processes treating secondary effluent were included in the study. The membrane reactor removed 99.9%, rapid sand filter 97%, dissolved air flotation 95%, and disc filter 40–98.5% of the microplastics during the treatment. A recent study by Gies et al. (2018) conducted a study in secondary treatment plants in Vancouver, Canada. It estimated that 1.76 ± 0.31 trillion microplastics enter the wastewater treatment plant annually, with 1.28 ± 0.54 trillion microplastics settling into primary sludge, 0.36 ± 0.22 into secondary sludge, and 0.03 ± 0.01 trillion microplastics released into the receiving environment which corresponds to total retention of 99% microplastics in the wastewater treatment plant. Yang et al. (2019) evaluated the microplastic removal potential of China's largest water reclamation plant and

detected 18 different types of micro polymers of average size 1111 μm with microfibers as the dominant type. The influent concentration of 12.03 microplastics/L was reduced to 0.59 microplastics/L in the effluent after treatment, i.e., more than 95% of microplastics present in the influent was removed by the treatment. The treatment characteristics of the three different biological processes, such as anaerobic-anoxic-aerobic, sequence batch reactor, and media processes of the sewage treatment facilities in Korea were studied by (Lee and Kim 2018). All three examined methods efficiently removed the microplastics up to 98%, and individual efficiencies were found as 49.3%, 44.7%, and 49% for the anaerobic-anoxic-aerobic process, sequence batch reactor process, and media process, respectively. Also, it has been reported that in spite of the greater removal efficiency of biological processes, still more than 4 billion microplastics were discharged every year due to the large volume of effluent. Although all the treatment stages are undoubtedly removing the large concentration of microplastics from the effluent, still it remains a concern towards the complete mitigation to avoid the escape of microplastics from wastewater treatment plants into the environment (Gies et al. 2018; Lee and Kim 2018).

1.5 Perspectives

Since research on wastewater microplastics is in its beginnings, many questions remain unsolved, and more research is required in specific fields. The following areas have to be explored widely for a more profound understanding of the providence of microplastics in wastewater treatment plants. (1) the standard protocol for the surveillance of the entry of microplastics into wastewater treatment plants, (2) a valid methodology for the detection and quantification of microplastics in water environment; (3) a complete study on the migration and ultimate fate during treatment (4) assessment on the potential of water reservoirs to be a source of microplastics to the oceans; (5) evaluation and understanding microplastics interactions with biotic life; (6) influential study on the ecosystem and evaluate the concerns of microplastics towards humans (Barboza and Gimenez 2015; Eerkes-Medrano et al. 2015).

Also, the current wastewater treatment focuses only on removing the microplastics and not aiming for its complete degradation, which makes microplastics global pollutants. Their persistence continues to upsurge as they appear to be very difficult to remove physically because of their small size and less visibility. Also, the rate of the entry of microplastics into the environment surpasses the speed of its removal. Hence, the need for viable technology with the potential of eliminating these persistent pollutants from wastewater becomes mandatory. Recently, complete mineralization of plastics by some particular microbial strains has been reported by many researchers, and they have also attained prospective results in the biodegradation of these dangerous polymer substances. Many bacterial species have been found to have the potential of degrading plastic compounds. Singh et al. (2016) studied the efficiency of soil bacterial isolates

Staphylococcus sp., *Pseudomonas* sp., *and Bacillus* sp., on the degradation of polyethylene.

In the same way, Asmita et al. (2015) evaluated polyethylene terephthalate and polystyrene degrading potential of soil microbes including species of *Aspergillus niger*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Streptococcuspyogenes*. In addition, the biofilm-assisted polystyrene degradation was exhibited by abacterium *Rhodococcus ruber* in a study conducted by Mor and Sivan (2008). The use of plastic degrading microbes for the bioremediation of microplastics is considered an environmentally acceptable approach for the removal of microplastics during wastewater treatment (Deepika and Jaya Madhuri 2015). Also, *Pseudomonas putida*, *Brevibacillus borstelensis*, *Streptomyces* sp., *Pseudomonas stutzeri*, and *Alcaligenes faecal* were found as potential plastic degrader, and they produced enzymes for the breakdown of plastic polymers. Recently, a team of researchers identified the two enzyme systems PETase and MHETase, produced extracellularly by a bacterium *Ideonella sakiensis,* which degraded polyethylene terephthalate and terephthalic acid and ethylene glycol were released produced as the products (Yoshida et al. 2016). Hence, the application of these plastic assimilating organisms could be extended for the removal of microplastics from wastewater through biological interventions.

1.6 Conclusion

Being the major reservoir of microplastics, wastewater treatment plants become the essential point source of microplastic contamination in the environment. During treatment, microplastics are significantly removed stage-wise; still, an enormous amount of microplastics is being released into the environment via treated effluent and biosludge. Hence, more effort must be taken to mitigate the global rise of microplastic pollution. Although many policies are being proposed regarding the alleviation of microplastics, source elimination would be the best way to reduce microplastic pollution. In this way, the most efficient bioremediation approach could be employed to degrade the persistent microplastics, and further exploration in this field is compulsorily required.

Acknowledgements The authors are highly thankful to "The Department of Science and Technology, Ministry of Science and Technology, Government of India," for funding the project under the Scheme "Scheme for Young Scientists and Technologists (SYST)-Science for Equity and Empowerment Division (SEED)"; Project No: SP/YO/2019/1360(G). DST-INSPIRE Fellow Maseed Uddin [DST/INSPIRE Fellowship/2019/IF190897] acknowledge the Department of Science and Technology, Ministry of Science and Technology, Government of India for providing the fellowship and financial support to carry out the research.

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The Impacts of Plastics on Environmental Sustainability and Ways to Degrade Microplastics

2

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2.1 Introduction

Plastics are derivatives of petrochemicals and are usually synthesized of high molecular weight as a backbone and compensated with various complex chemical compounds. Plastics are derived from the polymerization of monomers, which are synthetic-based extracted from oil or gas. Due to various properties like easy manufacturing, flexibility, plasticity, toughness, durability, inert, corrosion-resistant, lightweight, sterile nature, comparative cost-effectiveness, and imperviousness to water**,** plastics have become one of the basic needs and most important requirement for everyone in daily life. But it triggers litter, harming nature, pollutes the environment, and reduction of valuable natural possessions on earth (Awasthi et al. 2017). Animals ingest plastic bags by thinking of their food, unfortunately, become sick and also cause death as it remains intact in their bodies and does not decompose even after their death (Puncochar et al. 2012). The polymer consists of non-renewable raw materials as well as renewable ones. These polymers are used in the industry, electrical appliances, transportation, construction, storing, and packaging purposes. (Eubeler et al. 2009). Polyvinyl chloride (PVC), Polystyrene (PS), Polypropylene (PP), and Polyethylene terephthalate (PET) are polymers that vary by their chemical structure, structural arrangement, physical properties, and their applications. When it gets discarded, it contaminates landfills, freshwater, damages ecological balance,

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_2

and causes negative impacts on the health of animals and human beings, generating different environmental problems (Aboulkas and Bouadili 2010).

The accumulation of plastic waste in landfills has increased due to the continuous demand for plastics which led to the depletion of petroleum as part of non-renewable fossil fuel since plastics were the petroleum-based material (Sharuddin et al. 2016). Around the world, plastic waste treatment has become a serious problem through landfill process, incineration, and dumping in the ocean. However, all these methods cause some environmental problems. The landfill method took up lots of space because of incompressibility, incineration method results in CO_2 emission which is considered as one of the greenhouse gases emitted from the combustion of polymer (Ferronato and Torretta 2019). Table 2.1 represents the chemical name and chemical structure of the polymer, their properties, application, drawback, and melting temperature. Plastic accumulation in the marine environment results in marine plastic pollution which causes significant environmental impacts and has a drastic effect on marine species (Thushari and Senevirathna 2020). Solid waste in municipal areas could be any kind of waste, for example, it can be semisolid, solid, non-biodegradable, or biodegradable. Pollution such as air, land, or water depends either on municipal or industrial waste (Banerjee et al. 2014). Research proved that plastic neither decomposes nor degrades instantly; it gets accumulated and could remain in the soil for about 300–400 years (Thompson et al. 2009).

The negative impacts of dumping plastic waste and its accumulation in the environment have been represented in Fig. 2.1.

2.1.1 Global Scenario of Plastic Pollution

Due to the vast application of plastics in many sectors, the production of plastics increased over the years globally. Annually, the world uses around 5 trillion plastic bags of which 13 million plastic wastes get accumulated in the seas and oceans. In the last decade, more plastic has been manufactured and used than in the whole last century. We use 50% plastic as single-use plastics or disposable and plastics waste makes up more than 10% of the total waste we generate daily (Ritchie and Roser 2018). Among the total quantity of plastics sent to landfills, 79% is transported to the oceans, less than 10% is recycled, and 12% is incinerated. About 25 trillion macro- and 51 trillion microplastics litter the oceans, of these, 269,000 tonnes float on the surface. This equates to 1345 blue whales and 500 times the number of stars in the Milky Way. Plastic has been found predominantly throughout the globe, including in remote and isolated locations where, plastic is expected to increase tenfold in the next 5 years (Costa et al. 2020).

2.2 Different Ways of Reducing Plastic Waste

According to science, everything is a waste, until we do not properly utilize them (Cauwenberghe et al. 2013). Pretreatment may be an alternative solution to reduce the problems associated with water litter and air trash includes some limitations (Brems et al. 2012).

Table 2.1 Representation of chemical name, the chemical structure of the polymer, their properties, and application

Identification Code	Type of Polymer	Chemical Structure	Properties	Applications	Melting and glass transition temp
	Polyethylene terephthalate (PET)	$(C_{10}H_8O_4)_n$	Hardness, clarity, strength, the barrier to gas, and moistness	Soft drink, water, salad dressing bottles, peanut butter and jam jars, and ice cream cone lids	$T_m = 130$, $T_g = -125$
	High-density polyethylene (HDPE)	$CH_2 = CH_2$	Porousness to gas, resistance to moisture, stiffness, Strength	Water pipes and gas line and fire pipeline	$T_m = 240$, Tg = 85
	Polyvinyl chloride (PVC)	$(C_2H_3Cl)_n$	Flexibility, toughness ease of amalgamation	Blister packaging for non-food items, Electrical cable insulation, rigid piping	$T_m = 240$; $T_g = 85$
	Low-density polyethylene (LDPE)	$(C_2H_4)_n$	Ease of sealing, the moisture barrier. Elasticity	Frozen food bags, squeezable bottles	$T_m = 120$, $T_g = -125$
	Polypropylene (PP)	$(C_3H_6)_n$	Resistance to heat, chemical greases and oil, versatile	Reusable microwaveable ware, kitchenware, yogurt containers, margarine tubs	$T_m = 173$, $T_g = -10$
	Polystyrene (PS)	$(C_8H_8)_n$	Easily formed, clarity	Egg cartons, packing peanuts, disposable cups, plates, trays, and cutlery	$T_m = 240$ (only isotactic) $T_g = 100$ (atactic and isotactic)

Fig. 2.1 The schematic flow diagram shows an accumulation of microplastics through plastic waste materials and its negative impact on fish and humans

Table 2.2 Types of recycling of plastics (Hopewell et al. 2009)

Types	Process of recycling
Primary recycling	Mechanical reprocessing of polymer waste into a product (equivalent to original product)
Secondary recycling	Mechanical reprocessing of polymer waste into a product (lower than the original product)
Tertiary recycling	Processes to recover chemical constituents from the polymer waste
Quaternary recycling	Waste to energy processes

2.2.1 Recycling of Plastics

Reusing is a process of waste recovering without its biological, physical, or physicochemical transformation, whereas, recycling involves a change in its biological, physical, or physicochemical properties (Burlakovs et al. 2019). Reprocessing plastic waste is a fascinating idea due to the diversity of the material and its chemical composition. Bioplastics would act as another option that is made using renewable feedstock. Table 2.2 describes types of recycling of plastics.

The recycled plastic product would be favorable to the environment by saving energy and CO_2 also avoids the process of plastic manufacturing, oil extraction, and reduction of dumping in landfills (Briassoulis et al. 2013). The recycled product can

be added for an application like furniture, food packing, and domestic product, and also might be used for various applications (Muhammad et al. 2015). With the help of the European Union (EU), reprocessing of plastics comprehensively decreases the waste in the environment that would avoid polluting soil, air, ocean, and spreading of diseases. Recently proposed amendments of directives on wastes (Costa et al. 2020) are planned for 65% of municipal waste and 75% of packaging waste, including plastics, to be recycled by the year 2030. Reusing plastic waste is undoubtedly a waste-management process, as well as can apply in trade and environmental science, considered as natural ecology (Punkkinen et al. 2017).

2.2.2 Degradation of Plastics

The global impact on plastic wastes has created a fondness towards the area of degradation of plastics (Ren and Nick 2021) Degradation is defined as any physical or chemical changes in polymer with environmental factors, such as light, heat, moisture, chemical conditions, or biological activity. Degradation of plastics has been resulting in changes of material properties such as optical, mechanical, or electrical characteristics in cracking, crazing, discoloration, erosion, and phase separation. The extensively utilized plastics do not naturally degrade when it gets released into the environment. The property of high stability and durability of the polymer was one of the major reasons for the popularity and widespread application of many polymers. In the environment, the degradation of plastics happens by four mechanisms such as thermo-oxidative degradation, photodegradation, hydrolytic degradation, and biodegradation by microorganisms (Webb et al. 2013).

2.2.3 Reuse of Plastics Waste

Reuse is desirable when compared to recycling, as it required more energy and reserve than reuse, either for its inventive determination or to accomplish a similar purpose. Recycling is a method of breaking down raw materials and making a new product (Grause et al. 2011). In terms of wide-ranging commercials, it can be made as a classic product and offered to the market, the organization creates jobs and commercial activities which would increase to grow the economy. There are two most uncertain blocks such as substantial capriciousness and the charges accompanying recognizing and separating plastic waste into identifiable grade range. It depends on material information and proposal of reuse and recycles that puts into valuable reuse applications (Tangri and Wilson 2017).

In the emerging world, a high level of reused plastic waste is challenging and try not to make plastic waste if it is reusable, nevertheless, growing, earning, or subsequent consumer mandate for the convenience of disposable items and make the reuse of very low-value item (Chen et al. 2011). Recently, eco-friendly awareness is progressively moving towards attitudes and guidelines. The novel packing regulation will gradually reverse the condition. The best example of conservative use

is the delivery of milk in glass bottles instead of plastic packs, usage of distribution boxes instead of fluted fiberboard boxes, usage of returnable and reusable plastic boxes (Caruso 2015). The applications of the low-value plastic waste blend can be reused as a low-value commercial recycle waste fiber. Historically, the difficulty in the effective introduction of FPCs in the European country is of high costs because virgin polymer or single grade causes the highest value of recycles. The low-value plastic waste is found from a mass handling of non-segregated domestic black bag waste (Wei and Zimmermann 2017). The reuse of plastic materials should be technologically advanced to construct low-volume roads in different regions across India, if the constituents could be properly applied in the construction of highways, then the problem of disposing of plastic waste and the pollution in the environment must be gradually reduced. There are various advantages in terms of road making with the help of plastic waste. Roads made from plastic waste would be stronger than normal roads, decreases the cost of road construction, have less melting during summer, resistance towards the formation of potholes caused due to rainfall, and reduce the usage of bituminous (Samolada and Zabaniotou 2014). There are various advantages and disadvantages to reuse plastic waste. Raw material and energy savings from exchanging numerous single uses of products as a reusable product reduce the process of manufacturing which is the most important advantage of reusing the waste, while the disadvantage is products like television or second-hand automobiles can be harmful or less energy efficient if it is reused continuously (Georgiev and Mihailov 1992). Many technologies are developed to simplify the mixed plastic waste into the segment for removing the impurities that result in valuable products (Yuan et al. 2020). The recent development in bulk scale and highly efficient procedure for handling non-segregated plastic waste could be the change of these economic or constant plastic waste. This will be a key challenge to understand all the plastic waste would be an energy resource however the development of advanced techniques and processes helps to convert these waste plastics into high-value products. (Eriksen et al. 2014).

2.3 Microplastics

Microplastic (MPs) is a very small particle of plastic with a size less than 0.5–10 mm (according to different studies) including plastic debris and those not visible to the naked eye which causes pollution in the environment (Dümichen et al. 2017). Chemicals like polybrominated diphenyl ethers (PBDEs) and phthalates tetrabromobisphenol (TBBP) are considered microplastics (Lambert and Wagner 2018). Microplastics consists of two categories, i.e., primary microplastic, which is present in the environment or directly enters into marine water and secondary microplastics, which are generated through the breakdown or leaching of larger plastics that transformed into microparticle (Ghosh et al. 2019). MPs certainly exist in the environment for many decades. Researchers had reported on marine plastics in the 1970s, but they have not studied extensively. Due to its small size and adhering properties, it gets incorporated into the food, and its accumulation introduces toxins

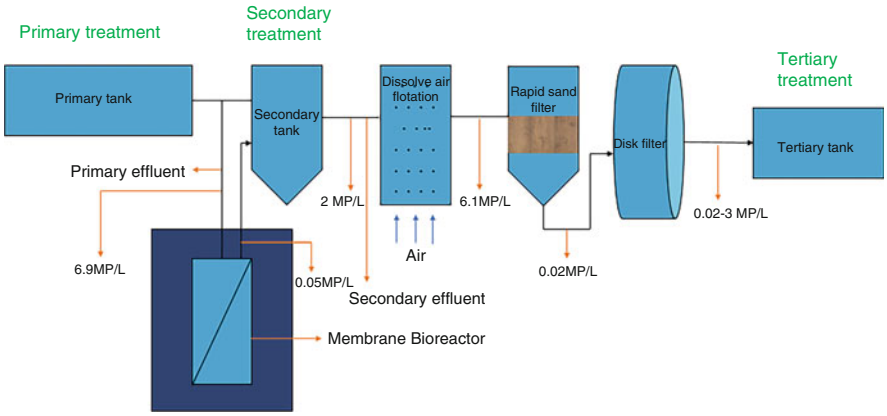

Fig. 2.2 Schematic diagram shows the removal of microplastics by the treatment of wastewater

in the food chain. Figure 2.2 explains the schematic flow of microplastic involved in wastewater treatment (Dümichen et al. 2017).

2.3.1 Route and Discharge of Microplastics

The soil and water bodies like lakes, rivers, sea, and oceans get polluted with microplastics through direct and indirect ways such as contact with clothing fibers, cosmetic beads, industrial, domestic waste draining systems, fishing and aquaculture activities, also due to fragmentation of macroplastic into microplastics in form of degradation (Siddique et al. 2008). Other indirect sources such as thin films of plastic used for crop production during frost season and other agricultural emissions of plastics that contaminate soil as well as water bodies, winds, tides, etc. (Bartsev and Gitelson 2016).

2.3.2 Environmental Resolution and Degradation

The current development in the degradation of unused polymer is procured in the laboratory, for example, biodegradation. The prediction of the fragmentation rate of plastic is not an easy process, whereas the kinetic fragmentation model is investigated in maths and physics literature and polymer kinetics are investigated in macromolecular science and literature (Sgier et al. 2016). MPs disintegrate progressively into very tiny particles as nanoplastics which are smaller than 1 μm (Jiang et al. 2020). Nevertheless, diverse groups of microorganisms already exists in nature may already be offering embryonic exploitable solutions for harmless degradation and bioremediation of waste plastics (Karn and Jenkinson 2019).

2.3.3 Microplastics and their Characteristic Ecotoxicology Test

Marine plastic pollution has a negative social, ecological, and economic impact. Plastic pieces accumulated in the ocean can be ingested by marine organisms, such as turtles, fish, birds, and mammals, that create indigestion, malnutrition problem, and also cause lethal wounds and respiratory impairment by getting entangled in drift nets, synthetic ropes and lines, and plastic debris. Toxicity issues caused by plastics that contain chemical contaminants get leached into the surrounding environment (Yuan et al. 2020). Moreover, in all the levels of the food chain, plastics have been detected which cause problems in human health. The plastic in the environment would block water drainage and also provide a breeding ground for mosquitoes that leads to the spread of diseases and interferes with the regular ecosystem's function (Curren and Leong 2019). There is various advantage of ecotoxicology test, the main advantage is of applying standardized tests that show various benefit as reproducible tests and acts precisely in inter-laboratory. Whereas, the widespread knowledge has been implemented from the area of testing the properties of chemicals on particular model organisms and is considered most advantageous in standardized ecotoxicity tests. The disadvantage of these tests has occurred in the testing of small particles (Klein et al. 2018). Two standard tests were established by the organization of economic cooperation and development (OCED), the first one is an acute immobilization test (48 h) (OECD TG 202) and a chronic reproduction test (21 days) (OECD TG 211) is the second one. For the determination of soluble chemicals, these tests have been followed. The particle expression shows very diverse activities towards insoluble chemicals and gets stimulated to apply the same set-ups of test nevertheless, some studies used to consider the effect of microplastics by following these standard tests. Casado et al. (2013) showed an acute immobilization test with 55 and 110 nm polyethyleneimine PS beads and reported EC50 values of 0.8 mg/L and 0.7 mg/L, respectively. They conduct a similar test with 1 μm PE beads gets an EC50 value of 57.4 mg/L. The massive difference shows the resulted plastics are of different sizes and shapes would be specified that mortality is not a complex biological response when it comes to polymer occurring in tiny particles (Arias-Villamizar and Vázquez-Morillas 2018).

2.3.4 Analysis of Microplastics

At an early age, microplastics have been identified visually. Heavy elements can be seen and identified with the help of the naked eye, whereas tiny or microparticle/microplastics are recognized with the help of scanning electron microscopy (SEM) or binocular microscopes (Yuan et al. 2020). The visual inspection of the sample was followed by the initial investigation of microplastics such as particle productivity, size, shape, and particle treatment. Therefore, additional spectrometric or spectroscopic methods are required to ensure whether the particles are made up of synthetic plastics (Briassoulis et al. 2013). This study can be done by using Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy by the interaction of

Table 2.3 Identification of microplastics using analytical techniques (Shim et al. 2017)

Methods	Analytical technique
Physical characterization	– Polarized microscopy – Dissecting microscope – Atomic force microscopy – Scanning electron microscopy – Fluorescence microscope – Fourier transform infrared spectroscopy – RAMAN spectroscopy – Energy dispersive X-ray spectroscopy
Chemical characterization	**Thermal analysis** – Differential scanning calorimetry **Pyrolysis and thermal desorption** – Gas chromatography/Mass spectrometry

chemical bonds and functional groups. The transmission of FTIR can be measured between 10 μm and 20 μm size of microparticles. On the other hand, divided particles are viewed in the rigidity test report, with the help of needles or tweezers pressure gets applied to the particle (Jungnickel et al. 2016). Most specific methods for the recognition of microplastics are designated by Sgier et al. (2016).

Microplastics are comprised of various sizes, shapes, and polymer types that are difficult to identify as it is completely reliable from complex environmental matrices using a single analytical method (Jungnickel et al. 2016). Therefore, the combination of more than two analytical techniques has been widely used. For confirmation of plastics, physical characterization of potential plastics (e.g., microscopy) followed by chemical characterization (e.g., spectroscopy) is used. Each method and various combinations have both advantages and limitations. Table 2.3 summarized the analytical technique used for the identification of microplastics.

2.3.5 Ecological Degradation of Synthetic Plastics

Conventional plastics and their high resistance to oppose ecological impact is most considerable which takes long residence time as well as slow degradation rate after they enter into the environment (Zheng et al. 2005). The subsequent diverse mechanism depends on the variation of physical, chemical, or biological influences. During the process of degradation, plastic waste is transformed into very tiny molecules like microplastics such as chemically modified forms, oligomers, or monomers (Chen et al. 2011). Table 2.4 describes various factors involved in polymer degradation.

2.4 Biodegradation of Microplastics

In the last few decades, many researchers and organizations came forward to reduce plastic waste by improving conventional methods such as a physical method that includes physical stress, UV treatment and chemical method includes methanolysis,

Table 2.4 Various factors involved in polymer degradation routes (Shah et al. 2008)

Factors (Requirement/ Activity)	Thermo-oxidative degradation	Photodegradation	Biodegradation
1. Requirement of heat	Higher than the ambient temperature required	Not required	Not required
2. Active agent	Heat and oxygen	UV-light or high-energy radiation	Microbial
3. Other consideration	Environmentally not acceptable	Environment friendly if high-energy radiation is not used	Environmental friendly
4. Rate of degradation	Fast	Initiation is slow, but propagation is fast	Moderate

hydrolysis, oxidants, results in the alteration of their physiochemical properties and fragmented to smaller fragments called meso- and microplastics and further to nanoplastics (Rist and Hartmann 2018). These processes need too much energy, cost, and man power and also produce toxic gases that damage the environment and harm the health of living beings (Dris et al. 2018).

To avoid such problems, researchers have started working to improve techniques of biodegradation in which living organism or their by-product is used to degrade the plastic without harming the environment by conducting two main processes, i.e., direct action, in which, several microbial florae can be directly used to deplete the plastic waste while microbial product is used to degrade the waste plastic through indirect mode or action (Green et al. 2016). Another alternative solution for reducing plastic waste is the manufacturing of biodegradable plastics such as bioplastic, compostable hydrodegradable, and biocomposite. The concept of biodegradation has its limitation over biodegradability and environmental impact (Folino et al. 2020). Table 2.4 explains the factors involved in the degradation of plastics.

2.4.1 Stages and Processes Involved in Biodegradation of Plastics

The notable thing is most of the biodegradable plastics are water-insoluble solid materials that are made up of macromolecules. Microorganisms cannot directly pass through the cell membrane due to the large size of the molecule. The first stage of biodegradation occurs on the surface by extracellular enzymes that erode the surfaces of solid materials (Tosin et al. 2019). The stages of biodegradation are explained in Table 2.5.

During the process of biodegradation, living organisms are broken down into organic substances.

The biodegradation process involves three processes:

1. **Aerobic process**—Presence of oxygen
2. **Anaerobic process**—Absence of oxygen
3. **Semi-aerobic process**—with or sometimes without oxygen

Table 2.5 Stages involved in the process of biodegradation

Stages	Process
Stage 1: Depolymerization	The process in which the polymer gets converted into a monomer and oligomer. Polymer ⟶ Monomers + oligomers
Stage 2: Uptake and metabolism	The process of immediate assimilation of monomer by microbes. Monomers + oligomers ⟶ Biomass
Stage 3: Mineralization	The mineralization of Organic carbon into CO_2 and H_2O. Biomass + O_2 ⟶ CO_2 + H_2O

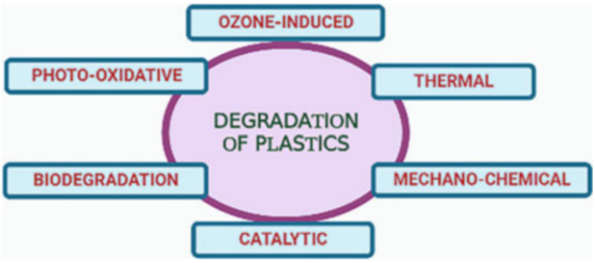

Fig. 2.3 Different types of degradation of plastics

2.4.2 Bacterial Degradation of Microplastics

Many bacterial species are available for the degradation of plastic waste. But, only few of them are effective and eco-friendly species such as *Corynebacterium* spp., *Streptomyces* spp., *Arthrobacter., Pseudomonas, Rhodococcus,* and *Micrococcus* spp. These species of bacteria are generally found in areas like low-temperature or cold environments (Oehlmann et al. 2009). Some microbes are found within the biofilm, which utilizes these waste polymers as the energy source (carbon source) and performs the hydrolytic process. This kind of species can act as hydrocarbon-degrading heterotrophs, autotrophs, symbionts, and even predatory bacterial species. An eco-friendly bacteria provides a solution for the mitigation of plastic pollution. For example, the bacterium *Pseudomonas putida* degrades polyvinyl chloride (PVC) and *Rhodococcus ruber* degrades polystyrene (PS) (Rehse et al. 2016). Research has found that some microorganisms have adapted or evolved to clean up the ocean by reducing plastic waste and researchers are finding ways for it. Types of degradation of plastics are explained in Fig. 2.3.

2.4.3 Fungi-Mediated Microplastics Degradation

Biodegradation of plastic waste is an eco-friendly plan, which provides a great and novel opportunity in plastic waste management with no harmful effects (Rehse et al. 2016). Fungi have been investigated that can be able to use plastics as a sole source of nutrients in the solid matrix. For example, soil and compost. The study shows that fungi can degrade with the help of agricultural soil (Casado et al. 2013). Scientists

believed that a species of fungus called *Aspergillus tubingen* could help to break down the microplastic in the environment (Eriksen et al. 2014). Subsequently, *Penicillium* and *Aspergillus* species are specified in most of the degradation studies whereas, bacteria have the capability for the formation of biofilm with the help of fungal species. The polyethylene had undergone advanced deterioration in hydrophobicity of the surface. Many species of *Aspergillus* have the potency to degrade polyethylene however, the dilapidation capacity of *Aspergillus niger* tends to degrade plastic by up to 38% and 31% by *Aspergillus lavus* in 60 days, respectively. *Phanerochaete chrysosporium*, a commonly used fungi called white-rot fungus, could degrade the very high range of persistent pollution or xenobiotics in nutrient-limited conditions.

2.4.4 Enzymatic Degradation of Microplastics

Extracellular enzymes and intracellular enzymes such as lipase, proteinase K, and dehydrogenases can be used for the degradation of plastics (Patricia et al. 2020). The activity of these enzymes depends on several factors such as the presence of oxygen, temperature, and UV radiation. Other factors include the presence of a functional group in the polymer chain to oxidize with the help of reduction of the molar mass of the polymer as well as provide an effective platform for the bacterial action (Nisha et al. 2020). For example, an enzyme produced by the bacterium *Pseudomonas chlororaphis* is used to degrade polyester. According to Kawai et al. (2019), one of the most highly produced synthetic polymers is Polyethylene terephthalate (PET), which is used in textile and for packaging purpose that gets accumulated in the environment in high amount. They categorize the enzymes into two groups: PET surface-modifying enzymes and PET hydrolases enzymes that can significantly degrade the block of PET. Cutinases, Lipases, and Carboxylesterases are the enzymes involved in the degradation which are isolated from *Thermobifida*, *Saccharomonospora*, and *Thermomonospora* species (Cedervall et al. 2012). The enzymatic degradation of plastics is explained in Fig. 2.4.

2.5 Thermal Processing of Plastic Waste

Thermal processing of plastic parts has been employed for numerous decades. The dumping of miscellaneous garbage in dumping sites, backyards, landfills, burning directly and energy recovery leads to loss of resources that causes health problems. The long-chain backbone of the polymer components could begin separating at a very high temperature that reacts with the properties of the polymer due to overheating (Bartsev and Gitelson 2016). The manufacturer of the plastic industry are confident that it must be recycled, which can reduce pollution economically, globally, and environmentally. In the market, very high demand of used high-density polyethylene (HDPE) and polyethylene terephthalate (PET) bottles and containers would increase their number of groups whereas, Canadian Plastics Industry

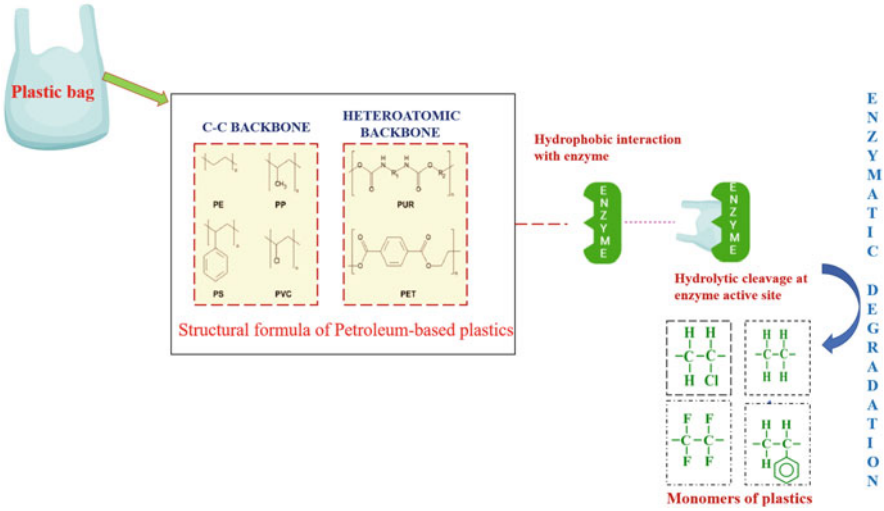

Fig. 2.4 Diagrammatic representation shows the enzymatic degradation of plastics

Association (CPIA), Environment and Plastics Industry Council (EPIA), sponsored and stay to the promotion for fulfilling the demand in the market (Pivnenko et al. 2016). Generally, the high yield is recovered by poly (tetrafluoroethylene), polystyrene (methyl methacrylate), and trace chemicals of plastics. Plastic waste is divided into two main categories, Municipal plastic waste (MPW) and Industrial plastic waste (IPW). About, 65–70% of waste is produced by packaging materials. Polystyrene (PS), polypropylene (PP), low-density polyethylene (LDPE), high-density polyethylene (HDPE), and very low amount of PVC attains structural changes by various kinds of the reactor through thermal processing of microplastic and waste plastic such as fluidized bed reactors and rotary kilns. A continuous tank reactor is considered as an inappropriate heat transfer, resulting in the formation of char or chemical process (Zheng et al. 2005). Dehydrochlorination of PVC is typically degradative extrusion. Two key features are important for selecting a suitable reactor, i.e., heat and mass transfer. In a low heat transfer of plastic materials, heat circulation is inhomogeneous for the melting of the plastic. This resulted in improper thermal degradation of plastic or distribution and their requirement of alkenes from the fragmentation tends to be chemically unstable (Karn and Jenkinson 2019). Consequently, the aim of the catalytic process is the removal of heteroatoms and the hydrogenation of alkenes, for instance, halogen, oxygen, and nitrogen, etc. (Nizzetto et al. 2016). Figure 2.5 shows the thermal processing of plastic waste and its products.

Tertiary recycling results in the production of oil or gas that could be recycled at a petroleum refinery resulting in the production of plastics, chemicals, or liquid fuels (Kawai et al. 2019). The thermal cracking occurs in virgin or discarded plastics such as polypropylene (PP) and low-density polyethylene (LDPE) in a semi-batch reactor,

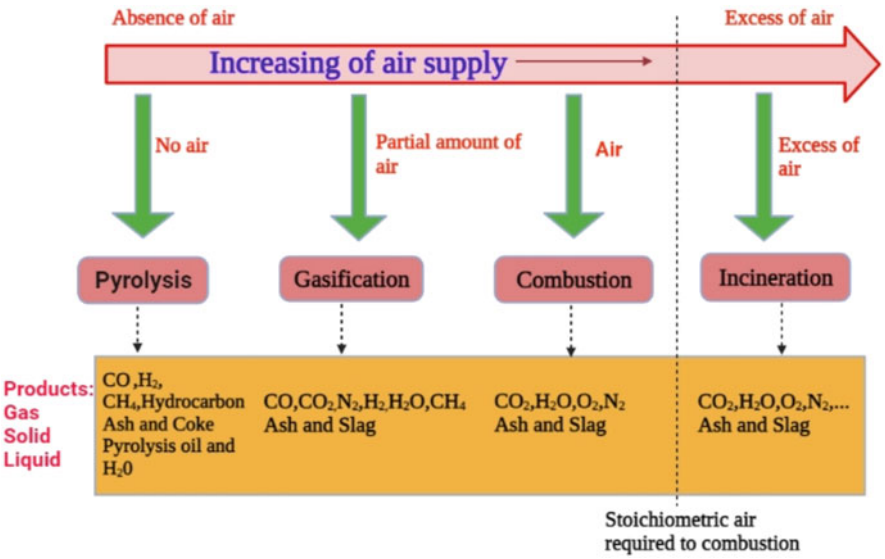

Fig. 2.5 Flow chart describes the thermal processing of plastic waste and their products

under atmospheric pressure at 460 °C. The high yield of liquid (84%) is given by the thermal pyrolysis of PP and LDPE at 460 °C (Friedman 1964). By the analysis of gas chromatography, production of oil from LDPE during the pyrolysis process confined about 21% of oil in the gasoline range of hydrocarbon whereas PP contains 58% of oil in the gasoline range of hydrocarbon (Ghosh et al. 2019). Plastic or plastic to liquid (PTL) pyrolysis is a thermal breakdown of waste with the help of free oxygen in the environment that produces liquid. Plastic is comprised of excess watercourses covered by several resins, which degrades the widespread range of materials from a monomer with a mix of olefins, paraffin, and waxes.

2.5.1 Incineration

Incineration is a process of treating waste by burning carbon-based substances present in waste material. Every day, the use of non-biodegradable (plastics could stay as long as 4500 years on earth as per current studies) products is increasing rapidly (Acomb et al. 2013). Due to the high rise in discardment of plastic waste, could leads to spread various diseases like reproductive problems in humans and animals, breast cancer, skin infection, genital abnormalities, etc. Incineration with energy recovery is one among the several technologies involving waste conversion to energy (Tangri and Wilson 2017), for example, anaerobic digestion. Incinerator decreases solid mass in left-over residue by 80–90% or by 95–96% (volume), which completely depends on the reprocessing rate of the material like metal recovery (Hopewell et al. 2009). The major drawback associated with the incineration of solid

waste is releasing hazardous compounds, such as dioxin. Nonetheless, up-to-date incinerator plants use filters to trap hazardous gases and particulate dioxin.

2.5.2 Pyrolysis

Pyrolysis has the capability of converting solid material into valuable products yet conservatively leads to a broad variety of pyrolysis products, which is problematic to detached and exploited. In the Incineration process, the plastic waste would be processed for energy recovery, where, energy can be significantly used for power, heat, and electricity generation. Energy retrieval processes are emerging methods for extremely assorted polymer waste, for example, electrical and electronic waste from developed as well as developing countries. Polymer waste treatment is very significant nowadays; all carbon-based compost for plastic and used rubber litter are characterized from collected garbage that could act as a valuable latent secondary raw material (Curren and Leong 2019). There are various types of incineration processes such as specialized incineration, fixed grate, rotary kiln, moving grate, and fluidized bed.

Thermal plasma technology and development are well-recognized for material synthesis and structural processing, etc. Undoubtedly, the thermal plasma waste procedure is the utmost significant application in devasted and harmful waste (Gong et al. 2018). Pyrolysis or gasification processes are energy-intensive that challenge to decrease the volume of garbage and produce oil or synthetic gas with the help of incineration. The classification of waste gasification form of incineration by the US Environmental Protection Agency and European Union (USA 40 CFR §60.51a; EU Directive 2010/75/EU Art 3.40) combines, most cases lead to the explosion of the subsequent gases in waste treatment through a thermal process. Samolada and Zabaniotou (2014) established the flow of plasma heated laminar or entrained-flow reactor to find out the evaporation features of husk particles in flash burning rates (Wei and Zimmermann 2017). The solid waste of urban areas and municipal areas contains commercial and domestic garbage that is collected by the municipality in particular allotted areas (Danso et al. 2019).

2.6 Conclusion

The public opposition is against disposing of waste in available sites because it creates a crisis in waste treatment. For the recycling of waste, proper management to outrange the services time and the residual waste disposal site is need of the hour. The thermochemical process converts the carbon-based elements to flammable gas such as methane, hydrogen, carbon monoxide, and carbon dioxide that can be used for power generation, lighting, and heating. The breakdown of polymers and plastic structures (such as uncontrolled dumping and landfilling) is unconditionally disagreeable due to its potential and actual ecological pollution with microplastics. While comparing the disintegrating methods of plastic waste treatment, gasification,

incineration, and pyrolysis resulted in life cycle assessment (LCA). Among them, pyrolysis and pyro-gasification are considered to be the most desirable methods. Because of its slow degradation rate, synthetic polymer biodegradation has not been implemented on a market level until now; however, substantial research is still underway in the field of biodegradation. Initially, acquiring active plastic enzymes and using them in the production of real biopolymers is a very rewarding research task and would significantly reduce our global plastic problem.

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Biosurfactants for Plastic Biodegradation

3

Matthew L. Smith and Pattanathu K. S. M. Rahman

3.1 Introduction

Biosurfactants are biological molecules with the ability to reduce the surface and interfacial tensions of polar substances such as oil and water. They exhibit an incredible structural diversity which accounts for their ability to mediate a vast amount of industrial reactions, also behaving in a cell-specific nature, allowing them to also interact directly with many biological mechanisms. This structural diversity, with their inherent amphiphilic nature, make biosurfactants a clear source for identifying novel ways to tackle ecological issues such as plastic pollution. The devastating impacts of plastic pollution have sparked several strategies which aim to reduce its overall environmental harm. Natural biodegradation is heavily reliant on a microorganism's ability to form a stable biofilm across plastic, biosurfactants will be tested in their ability to improve biofilm formation and hence aid biodegradation.

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_3

3.2 Biosurfactant

The first biosurfactant, surfactin, was identified in 1968 as being a potent anti-coagulant produced by *Bacillus subtills*. After additional study, researchers found surfactin to be composed of both hydrophobic fatty acid and hydrophilic protein components. This unique structure promoted an incredibly strong surface active nature which resulted in the new classification of the lipopeptide biosurfactant (Arima et al. 1968). The discovery of such a valuable microbial product triggered a vast amount of research which lead to the discovery of a wealth of novel biosurfactant products, each providing us with new and interesting properties which have various applications across the industry. Subsequently, a number of new biosurfactant groups were classified, each type being a product of the species genotype, environmental factors, fermentation conditions, and nutrient availability (Rahman and Gakpe 2008).

3.3 Biosurfactant Classification

There is a range of ways in which biosurfactants may be classified, one is to group them based on charge, at which point they may be anionic, cationic, non-ionic, or amphoteric. Another common method for their characterization is to group them based on their molecular weight. Low molecular weight biosurfactants will exhibit lower surface active tendencies and higher molecular weight biosurfactants which have a greater emulsification efficiency at lower concentrations (Ron and Rosenberg 2002; Rahman and Gakpe 2008). Chemically synthesized surfactants, often petroleum derivatives are usually classified based on their charge, while microbially produced biosurfactants are typically classified based on other properties, including their molecular weight or chemical composition. General classes of biosurfactants which have been identified through previous research include glycolipids, phospholipids, lipoproteins/lipopeptides, and polymeric biosurfactants, each of which exhibits their own structural properties hence affecting their use and potential application across a number of industrial and ecological processes (Vijayakumar and Saravanan 2015). Since their discovery, biosurfactants have been characterized as being amphiphilic moieties which reduce the surface and interfacial tensions of two immiscible liquids by interacting with both simultaneously, often forming micelles. For micelles to form, the concentration of surfactants must be above that of the critical micelle concentration (CMC). This is a crucial surfactant property and is often used as an indicator of biosurfactant efficiency. A low CMC will mean they have the same emulsification properties but at lower concentrations thus increasing their efficiency (Pacwa-Płociniczak et al. 2011).

3.4 Biosurfactant Properties

There is often hesitation throughout literature to claim that the exact ecological role of biosurfactants is known however it can often be assumed through the direct comparison of the advantageous traits biosurfactants may bring a population when compared to their microbial competitors. One such advantage is the ability to emulsify, disperse, and/or adsorb immiscible substrates which otherwise would not be able to act as a suitable carbon source (Healy et al. 1996). Other advantages of microbial surfactants in nature include their ability to exhibit antimicrobial properties, mediate cell–cell communications, facilitate biofilm formation, and facilitate water channel formation, all of which induce evolutionary pressures which explain their development throughout natural populations (Abdel-Mawgoud et al. 2010; Dusane et al. 2013). The rapidly changing and harsh conditions found across natural habitats explain the prevalence of biosurfactant diversity. This is an ecological adaptation which ensures bacterial survival as well as yields many varied products of economic value within the industry and scientific research.

3.5 The Value of Biosurfactants in Research

Biosurfactants have sparked the interest of scientists across many research areas, specifically their use to relieve ecological environments of anthropogenic contamination. The interest in using biosurfactants in natural systems comes partly as a result of their ability to emulsify substances which are otherwise insoluble but is also a result of their low ecotoxicity, tolerance to environmental extremes, and their ability to biodegrade (Fenibo et al. 2019). The use of petroleum-based surfactants is common throughout the industry however their toxicity and their tendency to persist in environments make their use counterintuitive when we consider our attempts to relieve environments of pollutants, increasing the desirability of microbial-based surfactants in this area of research (Araújo et al. 2019). This is particularly significant when considering the use of biosurfactants directly in ecological bioremediation and clean up (Mulligan 2004). The need to move away from chemically synthesized surfactants in all areas of industry has been acknowledged yet the economic undesirability of using biosurfactants is still a prevalent concern which prevents their current use and advancement (Fiechter 1992).

3.6 The Barriers Facing Research

In order to overcome the issues associated with biosurfactant use, research should be focused on studying screening methods previously evaluated in past studies and adapting them to identify biosurfactants produced by a range of species. Some studies have focused on the study of microbial surfactant producers in marine environments but not enough to represent the true potential of the microbial flora available in the marine hydrosphere (Satpute et al. 2010a, b). It would therefore be

advantageous to expand on current research and study more marine sites that generate a selective pressure for biosurfactant production such as those contaminated by crude oil as this would give us an increased opportunity to study the rich fauna which could be holding novel compounds. Current research should also focus on optimizing biosurfactant biosynthesis by identifying techniques used in the past and not only fine-tuning them, but also adapting them in novel ways to test for increased rates of production. This should be paired with the biotechnological aim of genetically altering strains and engineering their products to further increase the reaction rates to reduce overall production costs.

3.7 Microplastics

The industrial significance of plastics has increased exponentially since their initial discovery and their presence has been propelled across all areas of modern life. It is therefore no surprise that they have expanded beyond modern use, becoming ubiquitous across and within global ecosystems (Eriksen et al. 2014; Wilcox et al. 2015). Microplastics are microscopic plastic particulates which may be formed through the slow natural degradation of macroplastics, often via mechanical and photo-oxidative mechanisms, here being referred to as secondary microplastics. They possess the same properties as their larger counterparts but experience a drastically increased surface area to volume ratio while working across a much smaller plane, something which causes them to impact ecosystems in a way which is both novel and damaging, increasing the negative pressures such ecosystems will experience. Microplastics can originate from countless sources, with many being made directly in industry to be used across areas such as cosmetics, these are therefore known as primary microplastics (Mason et al. 2016). The development of microplastics from so many different primary sources results in many of them being washed away into drain systems, causing their increased concentration within water treatment facilities.

Water treatment facilities are hotspots for both the accumulation of pathogenic bacteria and the development of antimicrobial resistance (Mamoon and Maimun 2019). Having microplastics with such a high surface area for such species to form stable biofilms before releasing them into the environment brings risks which surpass that of individual components themselves. We must consider the negative effects that microplastics bring but we must also do this within the context of the toxic environments provided by wastewater treatment facilities. It is for this reason that the removal or at least detoxification of such particulates should be of great concern to researchers within this area.

3.8 Plastic Biodegradation

Biosurfactants could play a significant role in the study of plastic biodegradation, their emulsification and amphiphilic properties allow them to behave in a way which could impact the chemistry of the plastic surface itself, microbial biofilm formation,

or even the enzymes' ability to interact with the plastic. Previous research has shown biosurfactant production to be positively correlated with the concentration of contaminants (He et al. 2019). In this way, we should aim to study biosurfactant production in relation to plastic-contaminated water treatment facilities and how this relates to any of those features which could impact the way in which microorganisms and plastics behave. For this reason, future research should aim at increasing our knowledge of the interaction between microbial communities with plastics in this complex environment. This will provide us with insight into what effect this type of interaction may be having on the substrate itself which could furthermore lead to the successful degradation of the plastic or at least its detoxification via biofilm disruption.

3.9 Biosurfactant Screening Techniques

Screening methodologies are imperative in the study of biosurfactants as they are the first stage in identifying surfactant-producing microorganisms which therefore may lead to the discovery of novel products/species. This has resulted in an interest in research which has encouraged the development of countless screening technologies, each bringing its own unique advantages (Table 3.1). The type of screening method used is often specific to the conditions of the investigation; however, research has continuously indicated that regardless of the methods used, multiple should be used in conjunction with one another to ensure correct conclusions should be made (Yalcin et al. 2018; Eldin et al. 2019). There is an incredibly broad range of microorganisms capable of synthesizing biosurfactants, owing to a great deal to both chemical and functional diversity. This highlights the importance of using multiple screening methods, as one method alone may not be able to identify emulsification activity across the range of potential biosurfactants which may or may not be present in the sample (Satpute et al. 2008).

3.9.1 Drop-Collapse Test

From all the methods employed in biosurfactant screening, there is a number which stand out across the literature. One being the drop-collapse test which has been used repeatedly in the screening of biosurfactant producers from natural environments (Jain et al. 1991; Bodour and Miller-Maier 1998; Youssef et al. 2004). This method may be used to generate both qualitative and quantitative information on the presence of biosurfactants and is used as a result of being sensitive, easy, and often high-throughput (Youssef et al. 2004). The method can be conducted with the use of a 96 microwell plate, this allows for 96 simultaneous tests which contribute to the high-throughput nature of the methodology. Oil is placed into each well, followed by a droplet of the sample being investigated. The behavior of this droplet after 1 min will indicate whether or not biosurfactants are present. If the droplet disperses, it is likely a result of emulsification activity, suggesting biosurfactant presence. However, if the drop remains beaded then the opposite is

Table 3.1 A selection of biosurfactant screening methods used in the identification of biosurfactant producing isolates, identified from past literature

Screening method	Details of protocol	References
Drop-collapse test	Qualitative method: 1.8 μL Pennzoil is added to a microwell. Followed by a 5 μL droplet of the sample. Surfactant presence is indicated by the drop collapsing. Quantitative method: 2 μL mineral oil is added to a microwell, followed by a 5 μL sample droplet. The diameter is measured with a dissecting microscope after 1 min, there is a negative correlation between droplet size and surfactant activity	Bodour and Miller-Maier (1998)
Oil-displacement test	10 μL crude oil is placed on top of 40 μL water to form an oil film. 10 μL sample is gently placed into the center of the oil, the area of displaced oil is a measure of surfactant activity	Morikawa et al. (2000), Nwaguma et al. (2019)
Haemolytic activity assay	Isolated strains are cultivated on agar containing 5% blood, this is incubated at 28 °C for 48 h. A clear zone around the colony indicates haemolytic activity	Mulligan et al. (1984), Carrillo et al. (1996)
Hydrocarbon-overlay agar selection medium	Mineral agar plates are coated with 100 μL oil (e.g., diesel). Plates will be inoculated using the pour plate method and will be incubated at 30 °C for 48–72 h. A clear halo around the colonies will represent emulsification activity	Cipinyte et al. (2011)
Cetyl tri ammonium bromide (CTAB)–Methylene blue agar selection medium	Agar is prepared by adding 0.2 g CTAB, 0.005 g methylene blue and 15 g agar into mineral salt medium. Inoculate the plates and incubate at 37 °C for 48 h. The development of dark blue halos is proportional to the amount of anionic biosurfactant being produced	Lin et al. (1998)
Axisymmetric drop shape analysis (ADSA)	Cells are suspended in a liquid broth culture, droplets are then placed on a fluoroethylenepropylene surface, and the droplet shape is analyzed via a computer for up to 2 h. Biosurfactant producing cultures will change the drop profile due by lowering the surface tension	Satpute et al. (2010a, b)

(continued)

Table 3.1 (continued)

Screening method	Details of protocol	References
Emulsification index (EI) test	4 mL water and 6 mL kerosene is added to 1 mL culture supernatant and vortexed 2 min before being allowed to stand for 48 h. EI is calculated by dividing the height of the emulsion layer by the total height and dividing this by 1000	Satpute et al. (2008), Nwaguma et al. (2019)
Tensiometeric measurements	A tensiometer is used to measure the surface tension of a sample over time, a reduction in surface tension will indicate biosurfactant production	Satpute et al. (2010a, b)
High-performance liquid chromatography (HPLC)	Samples are spotted on thin-layer chromatography (TLC) plates and are developed in a solvent which contains chloroform, methanol, and water (65:25:4 ratio). A densitometric analysis will then develop a chromatogram to allow for biosurfactant quantification.	Das et al. (2008)
Matrix-assisted laser desorption/ ionizationtime-of-flightmass spectrometry (MALDI-TOF MS)	Extracted cultures are suspended in 1 mg/mL ethyl acetate this will be measured by MALDI-TOF MS using 2,4,6-trihydroxyacetophenone as the matrix	Saika et al. (2019)

likely to be true. The quantification of biosurfactant concentration from this method can be used by measuring the droplet diameter after 1 min and comparing this to the droplet diameters of surfactants of known concentration which can then be represented graphically (Bodour and Miller-Maier 1998).

3.9.2 Oil Displacement Test

Another method which is commonly used throughout the literature to identify biosurfactant production is the oil-spreading assay also known as the oil displacement test. This involves adding water to a petri dish before adding a thin layer of oil on top of it to form an oil membrane, the sample will then be dropped into the center of this membrane to begin the analysis. The presence of emulsification activity will be indicated by the formation of a clear zone in the oil, the concentration of biosurfactants present in the sample positively correlates with the diameter of the zone of clearance in the oil which allows the test to generate both quantitative and qualitative data (Morikawa et al. 2000; Nwaguma et al. 2019). This method allows for the direct analysis of biosurfactant activity and much like the drop-collapse test, only requires small amounts of the sample being tested. The evaluation of such screening methods is crucial in the accurate identification of biosurfactant producers,

such evaluation has led to the increased reliability of both the drop-collapse and the oil-spreading assays in both their identification of biosurfactant producers and the quantification of their products (Cipinyte et al. 2011).

3.9.3 Using Screening Methods Correctly

There is a plethora of methods which may be utilized in the screening of biosurfactant production (Table 3.1) so it is important that an investigator chooses a method which will reliably detect the presence of emulsification activity. Failing to do so will generate anomalous data resulting in the exclusion of potential biosurfactant producers therefore hindering progress in the field, something which may have already impacted previous research. Initial investigations will likely be performed on a small scale which will limit the volume of biosurfactants removed from each sample, preventing wastage which will therefore enable numerous repeats. For this reason, it is also a benefit for the screening technique to have the capacity for generating quantitative data in regard to the microbial surfactant being tested as this will reduce the volume of the sample required for future testing.

3.9.4 Modern Screening Technologies

In addition to the chemical tests shown in Table 3.1, there is a number which may be employed to measure biosurfactant production in a way that will generate direct quantitative results. Examples of such methods include tensiometric measurements, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). These methods have been praised for their accuracy and their ability to generate precise measurements which allow for a more accurate account of biosurfactant characterization. The implications of using these are often associated with their cost which is significantly higher than that of the other methods shown in Table 3.1, they also require a greater deal of training which often limits the amount of people that are able to utilize them within their research (Satpute et al. 2010a, b; Das et al. 2008). Additionally, methodologies such as TLC have been found to be accurate in detecting well-known biosurfactants such as mannosylerythritol lipids (MELs) and sophorolipids but may overlook novel biosurfactants which have different molecular structures. This once again indicates the importance of using multiple screening methods while also aiming to employ new and unique strategies which may be able to identify these unpredictable novel structures, such as matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI—TOF MS) which is a high-throughput means of identifying novel compounds with a wide variety of mass-to-charge ratios, increasing its ability to identify a greater range of structures (Saika et al. 2019).

3.10 Optimization of Biosurfactant Synthesis

The environmental benefits of biosurfactants have been proven across the literature with the benefits of their large-scale use being increasingly apparent (Marchant and Banat 2012). The main obstacle preventing their use across the industry is their incredibly high production cost, mostly due to our limited understanding of the biosynthetic pathways and how the cells are interacting with their abiotic environments. Many studies often focus on the optimization of an individual pathway, with much research being focused on the production of rhamnolipids, a type of glycolipid biosurfactant (Maqsood and Jamal 2011; Chen et al. 2018). It is important to consider that the necessary conditions for different biosurfactant producing organisms are possibly going to vary, meaning we must build on research which is specific to the individual pathways being tested. An issue here is that there are many studies which focus on the optimization of biosurfactant production and emulsification activity but are not specific in identifying what pathways they are studying (Maneerat and Phetrong 2007; Dehghannoudeh et al. 2019). This makes building upon these findings difficult meaning research should clarify the specificity of the biosurfactant producing mechanisms being optimized to allow for a greater understanding of the processes themselves (Banat et al. 2010).

3.10.1 Traditional Optimization Strategies

Focusing on specific biosurfactant pathways in research will allow for the use of precise conditions within a bioreactor which have been known to match that which is being tested. This will be based on previous findings, which will initially generate the greatest production rates which have already been achieved through past studies. Providing us with a starting point from which we can begin to push the reaction conditions in new ways as a means of further increasing production. In this way, future research should be maximizing the benefit of past studies to increase the overall yield as we progress through the area, instead of continuously repeating past experiments with ambiguity as to what is actually being studied.

In order to maximize biosurfactant biosynthesis it is imperative that we understand the key parameters which influence both the microbial growth and the maintenance of the biosurfactant producing mechanisms. One such factor is the carbon source on which the strains are reliant, research often aims at studying the impacts of renewable feedstocks which maintains the environmental viability of the process. One strategy would be to use waste material as a substrate, allowing for the removal of waste to form a valuable product that would benefit multiple areas of industry therefore increasing the attractiveness of biosurfactants (Makkar et al. 2011). One such example is the use of waste kitchen oil which allows for rhamnolipid production in *Pseudomonas aeruginosa*. In addition to this being environmentally sustainable, it was also found to increase biosurfactant production when compared to other carbon sources (Chen et al. 2018). Other examples include the use of lactic whey and olive oil which also fared well against their nonrenewable counterparts (Rodrigues

et al. 2006; Abouseoud et al. 2008). Issues in using such waste substrates often come from their lack of homogeneity, it can also prove difficult to generate waste which contains the right nutrient balance to maximize growth however, due to the potential value of the area, it is one which deserves continued attention from research (Makkar et al. 2011).

We must also consider the significance of other abiotic factors which should be maintained within a bioreactor to maximize the potential yield. Such conditions will relate to the specific strain of microorganism in question but the literature in this area had often found a maximum yield within the temperature ranges of 25–37 °C, a pH range of 6.5–8, and cells are typically incubated on a rotary shaker at 220 rpm to maximize the oxygen uptake (Guerra-Santos et al. 1984; Rodrigues et al. 2006; Abouseoud et al. 2008; Das et al. 2008). When studying cells in the future it would be of interest to the researcher to begin within these boundaries but studying a range, to find the initial highest production conditions. Furthermore, research should aim to find gaps in the reaction conditions being tested in previous studies to test for new ways in which these may be influencing the mechanisms of biosurfactant biosynthesis.

3.10.2 Biotechnological Strategies

Traditional methods of increasing yield by altering the reaction conditions are crucial but will be limited in the production rates that they would be able to offer, even when they have been fully optimized. Biotechnological engineering offers advantages to biosurfactant production which surpass the abilities of traditional techniques and is something that must be focused on to truly increase the industrial validity of biosurfactants (Geys et al. 2014). Issues in this area of research are mainly a result of the lack of understanding of the gene clusters which contribute to the production of each biosurfactant type. As these begin to be resolved we will increase our ability to manipulate these genes and subsequently engineer strains to increase their rates of production. Some pathways, such as the synthesis of sophorolipids in *Starmerella bombicola,* have been resolved which has allowed for the engineering of the process in previous research (Van Bogaert et al. 2013). The genetic modification of microorganisms also allows us to increase our understanding of how the production of biosurfactants impacts cells, helping us draw conclusions as to what their natural purpose is and increasing our understanding of how they may be used throughout the industry. In addition to increasing yield, biotechnological engineering allows us to target areas of biosurfactant research that would be untouchable with traditional methods alone. This includes modifying the products to increase their efficiency, generating novel products, and transferring the mechanisms to more appropriate strains which may then make the processes even more cost-effective. To achieve this research will likely be focusing on areas such as modifying transcriptional regulatory genes, genomic analysis, promoter engineering, promoting efflux systems, and combinatorial biosynthesis (Hu et al. 2019).

3.11 Plastic Biodegradation

Plastic accumulation is becoming a greater burden to society and the increasing amount of research into this area simply deepens the concern we have for its impacts on our biosphere. Many strategies have been employed to understand the chemical nature of plastics which prevents their natural degradation and what can be done to accelerate this process industrially (Sivan 2011). One of the greatest obstacles in the biodegradation of plastic is the hydrophobicity of the substrate, an obstacle which may potentially be overcome with the use of amphiphilic biosurfactants, other issues include plastic's high molecular weight, crystallinity, and a lack of functional groups (Urbanek et al. 2018). One avenue for plastic biodegradation is the engineering of plastic-degrading enzymes such as PETase. This was initially isolated from *Ideonella sakaiensis* and was additionally modified to enhance its ability to degrade plastic (Austin et al. 2018). This is an important step forward in understanding how we may be able to break down plastic in controlled environments and minimize the downstream negative effects on ecology.

3.12 Influence of Biosurfactants on Plastic

Biosurfactants have been found to increase the ability of certain microbial species to utilize plastic as a sole carbon source thus highlighting their benefit to us within degradation strategies (Vimala and Mathew 2016). This provides a solid foundation for future research to deepen this understanding with the basis that there is in fact an initially positive association. The broad range of biosurfactants synthesized by different microbial groups opens up the potential for many different types of strategies when considering the removal of microplastics from wastewater treatment facilities. One example would be manipulation of the amphiphilic nature of biosurfactants, if they were able to form micelles around a particular type of microplastic then this may lead to a strategy which could help us extract them from wastewater, especially when we consider the use of charged moieties which could then be extracted with the use of electrostatic interaction. This method would utilize the very properties of plastic which make them so difficult to degrade to our own benefit and is just one of many examples which suggest the massive potential of using biosurfactants in solving issues related to plastic pollution. This type of interaction does however suffer from a lack of research which means many of these claims are currently shrouded in assumption. As the success of biosurfactants in research continue and the sustainability of their production becomes more viable, this will no doubt change, meaning a greater understanding of how they can be manipulated in dealing with plastic pollution.

3.13 Biosurfactants in Biofilm Formation

One crucial aspect to focus on in particular would be how natural microbial populations are responding to the plastics imposed on their habitats, understanding how biosurfactants may impact biofilm formation across plastics would bring us one step closer to doing this. Recent literature has focused on the degradation of plastics using modified enzymes and the effects that biosurfactant pretreatments may be having on this process (Austin et al. 2018; Artham and Doble 2008). However, little research has focused on the impacts such surfactants may be having on the initial attachment of microbes to plastics and their effects on their natural degradation. A key factor in complete plastic biodegradation, resulting in mineralization, is the ability to form a biofilm across its surface, which highlights the need for research to focus on the impacts biosurfactants may have on initial biofilm formation (Mor and Sivan 2008).

Previous literature has shown that biosurfactants, in many instances, are able to inhibit biofilm formation, a characteristic which validates their potential antimicrobial properties. They can do this by interacting with the bacterial cell wall hydrophobicity and inhibiting biofilm forming machineries (Salman et al. 2014; Kulper et al. 2004; Kiran et al. 2010). However, much of this research is conducted by medical journals to assess the ability of biosurfactants to be used as a treatment against pathogens, there is therefore a bias to study instances where the surfactants are going to be having a negative impact on biofilm formation, a phenomenon which would be beneficial against such microorganisms. When looking deeper into the literature, it can be seen that there is a more complex relationship between biosurfactants and biofilm forming behaviors. Therefore, it may be the case that the impacts they have on biofilms may be a lot more varied than originally found, as a result of a circumstance which is something indicated by the discrepancies in their effects across the literature (Mehdi and Giti 2008). One study shows an example of this by investigating the effects of rhamnolipids on the formation of a monospecies biofilm in *Pseudomonas aeruginosa,* this found that although the surfactants did not help in initial biofilm formation, they were necessary for the maintenance of nutrient and water channels which is significant in biofilm development (Davey et al. 2003). It would therefore benefit researchers to spend more time studying the interesting relationships between microbial surfactants and the biofilms they form, especially when grown with plastics. Once bacteria have been able to establish a stable biofilm across plastic, their enzymes will be in much closer proximity to the substrate, increasing any rate of reaction which may be able to subsequently degrade the plastic, increasing its potential for its use as a carbon source. Promoting this within water treatment facilities may therefore mean the successful degradation of microplastics either before or shortly after being released into a natural environment.

3.14 Biosurfactants in Microplastic Detoxification

The release of microplastics into a natural water source is something that carries many risks, the greatest of which is arguably the ability of the microplastics to concentrate toxic contaminants on its surface. This could be in the form of harmful biofilms which are easily introduced through the presence of coliforms in wastewater facilities or may even be an accumulation of other harmful pollutants, such as herbicides (e.g., glyphosates), which can be known as persistent organic pollutants (POP's) (Andrady 2011). Due to their small size, microplastics will be easily ingested by organisms that will subsequently receive highly concentrated doses of these toxic compounds which are then easily passed up through food chains, resulting in bioaccumulation (Fig. 3.1).

For this reason, if the microplastics found in wastewater treatment facilities cannot be completely removed, it would be within our best interest to ensure that they are at least being detoxified of any harmful pathogens, which in this environment, would also be likely to be carrying some form of antibiotic resistance determinants. This may be one example where the biofilm-disrupting properties of biosurfactants may be of use, disrupting any biofilms would not only detoxify the plastics but it would also increase the ability to reduce the toxicity of the overall final effluent of the wastewater treatment facility, something which consistently calls for improvement.

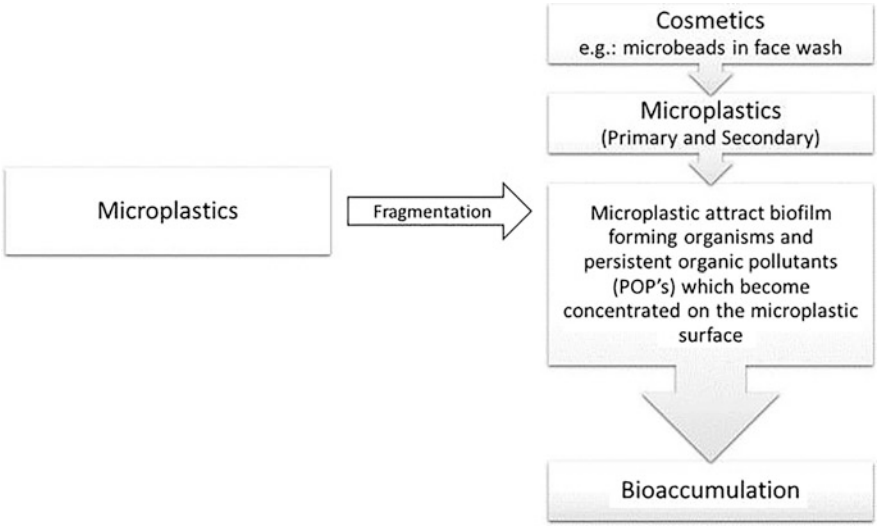

Fig. 3.1 Demonstrating the key processes in primary and secondary microplastic formation and the link to the subsequent bioaccumulation of either organic pollutants or harmful biofilm forming bacteria (modified figure from Andrady 2011)

3.15 Conclusion

Biosurfactant research has come a long way since their initial discovery in 1968, with their range of influence across our industry increasing the more that we are able to uncover about both their structure and their function. This has led to the development of a wealth of screening strategies aimed at the discovery of novel products, strategies which have been incredibly successful thus far but still require further development. The issues associated with using biosurfactants industrially are often a result of the high production costs of the process, something which is caused by a lack of knowledge in the area. Many traditional studies have focused on the optimization of the natural pathways, this has had little success rates with a limit to the productivity it has achieved. Recent research has shown that a greater emphasis should be placed on biotechnological approaches to biosurfactant production. This is a fairly new area but offers a lot of promise where we consider the impacts of gene editing on cells which we could then pair with the traditional methods of optimizing the conditions. Overcoming the issue of production costs would propel the use of biosurfactants across the industry, a result of their positive impacts on downstream ecologies, in addition to their ability to make modern processes both more renewable and economical.

There are many ways in which microbial surfactants may be used to solve environmental concerns. This review has focused mainly on the use of biosurfactants to relieve ecosystems of microplastic pollution from the final effluent of wastewater treatment facilities. Research has focused on the impacts such surface-active agents are having on the enzymes which interact with plastics; however, it should also be of great concern for us to understand how they are able to interact with cells in relation to biofilm formation. This plays a key role in the ability of cells to be able to interact with plastics in a way that would be able to lead to their subsequent biodegradation. An area which has experienced little study and is one that should be focused on in future research.

Pinnacle research has been identified which has contributed to our current understanding of biosurfactants but has also been able to identify gaps in said research which have resulted in a lack of understanding in areas which could have the potential to greatly improve the way in which we can use microbial surfactants. Future studies to resolve these unanswered questions will hopefully lead to an increased ability to manipulate both biosurfactants and their microbial producers which could hopefully then relieve many of the environmental concerns that we are currently facing.

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4 Effluent Xenobiotics and Prospects of Biogenic Zinc Oxide Nanoparticles for the Treatment of Textile Dye Effluent

K. P. Anjali and Susmita Dutta

4.1 Introduction

Textile industries are one of the oldest industries, utilizing multifaceted technologies and chemical demand among all other industries. A significant share of the global economy lies in the textile and garment industries and is a principal source of employment worldwide (Verma et al. 2012). The textile industries manufacture a broad spectrum of products essential for our daily life, ranging from door carpets to the most modern outfits. The modifications in the standard of living and attitude of the people have affected their fashion trends, and, hence, there is a growing demand for textile products according to the changing trends. With the escalation in the number of textile manufacturing units, there is a proportional increase in the generation of industrial effluent. The contaminants present in the textile mill effluent pose a serious threat to the environment and human health, particularly causing severe damage to the water bodies. Textile mill effluents were considered as one of the most polluted wastewaters containing dangerous dye pollutant (Khan and Malik 2014). Furthermore, textile industries consume vast amounts of chemicals and are also a vital contributor to potable water contamination. Also, textile industries consume a massive amount of water at the beginning and end of every step of textile processing and can be considered as one of the most significant consumers of water (Kant 2012; Yaseen and Scholz 2019).

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_4

4.2 Dyes

The dye is the principal constituent to impart color to any fabric, which makes it more attractive. A dye compound consists of two parts; one is the component responsible for the dye color (chromophores), and the other helps to strengthen the dye color (auxochrome). The interaction of the dyes with the fabric depends on the chemical composition of the dye, and it could be due to van der waals forces, hydrogen bonds, and hydrophobic interactions. Dyes are chosen based on the diversity in color shades, easy applicability, quick wetting, and least energy consumption (Phugare et al. 2011). Moreover, the dyes should be intended to resist fading upon contact with sweat, light, and water and also to prevent oxidation and bacterial attack (Odjegba and Bamgbose 2012).

Dyes can be classified as natural and synthetic dyes based on the source of manufacture. Natural dyes were extracted from vegetable and animal sources and were commonly used until the discovery of synthetic textile dyes in 1856. Another classification based on the general structure of the dye is anionic, cationic, and non-ionic. The anionic dyes include direct, acid, and reactive dyes, cationic dyes comprise of azo basic, anthraquinone disperse, and reactive dyes, and the disperse dyes belong to non-ionic dyes (Yaseen and Scholz 2019).

4.2.1 Hazards Associated with Dye Effluents

Textile mill effluents consist of complex chemicals and colored compounds generated during the dyeing processes. A single dyeing process utilizes multiple dyes from different class of chemical compounds. Furthermore, the chemical composition and the nature of the textile mill effluents vary depending on the type of fabric, methodology, and type of industry (Chung 2016). The treatment of textile effluents must involve intricate processes due to the color intensity, pH, suspended solids, temperature, biological oxygen demand, chemical oxygen demand, metals, and salts (Daniela and Carmen 2012).

The chemical composition of the textile mill effluents contains numerous harmful contaminants, including heavy metals used in textile processing. The contamination of the water bodies caused due to industrial operations poses a severe threat to aquatic life as the dye chemicals present block the passage of light and reduces the dissolved oxygen drastically. Around 40–50% of the colorants used in textile mills contain organically bound chlorine, which is highly dangerous, owing to its carcinogenic nature (Chung 2016). These chemicals, when evaporate, get mixed with the air and are inhaled or absorbed through the skin causing irritation, allergic problems, and respiratory diseases. Furthermore, the antimicrobial agents used in the garment industry make the effluents resistant to biodegradation. The azo dyes, the main class of dyes used in the dyeing industry, were found to be highly toxic due to the azo linkage itself and most resistant to biological degradation (Khan and Malik 2014).

The organic dye, malachite green is also used as an anti-parasite in aquaculture; however, long-time exposure at high concentrations and temperature impose

potential health risks including multi-organ tissue injury, carcinogenic, mutagenic, and developmental abnormalities (Rovira and Domingo 2018). The treatment of dye effluent in an eco-friendly manner would be the safest way of disposing of the dyes containing wastewater to the environment.

4.2.2 Treatment Methods

Figure 4.1 shows various techniques used for the treatment of textile mill effluent. The activated sludge treatment, along with coagulation, was one of the oldest methods followed to remove dyes and other organic chemicals present in the wastewater. In subsequent years, membrane technology gained momentum in the efficient removal of dyes using bioreactors. However, the regeneration of the membranes and the cost-effectiveness were the challenges associated with the technology. Later, bioremediation using plants and microbes turned out to be the environmental friendly method for the treatment of dye effluents. Uysal et al. reported the treatment of textile wastewater using a pond vegetated with duckweed, *Lemna minor L*, which was found to be a simple and efficient method with maximum removal of color obtained in a contact time of 3 days (Uysal et al. 2014).

Ultrafiltration and nanofiltration were also employed for the removal of color and organic pollutants present in textile mill effluents (Aouni et al. 2012). Syafalni et al. demonstrated the treatment of dye effluents for the removal of color, ammonia, and chemical oxygen demand using an adsorption column and found that the best results were obtained when zeolite was used as a filter in the top layer and granulated activated carbon in the bottom layer of the column (Syafalni 2012). The benefits of

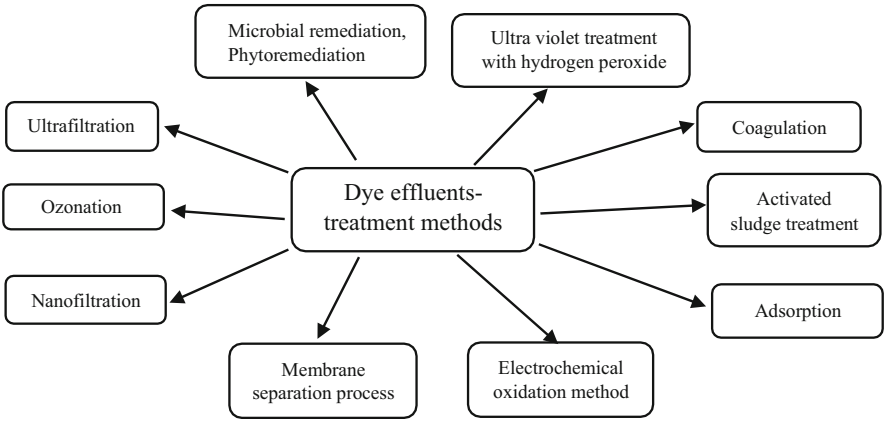

Fig. 4.1 The conventional methods used in the treatment of dye effluents encountered some shortcomings in terms of efficiency and cost-effectiveness. The recalcitrant nature of the dyes' complex structure employed in the textile industries is difficult to disintegrate. Photocatalytic degradation using nanomaterial exhibited better decolorisation efficiency owing to the properties of nanomaterial

zinc oxide photocatalysts were best utilized in the remediation of dye effluents, and the treated water was found to be suitable for domestic and irrigation purposes (Hussein 2013).

4.2.3 Nanotechnology in the Treatment of Dye Effluents

The implementation of nanotechnology in effluent treatment tremendously enhanced the performance of the existing systems. The properties of the nanomaterials that make them suitable for water treatment include high surface area and associated active sites for adsorption, high (photo) catalytic activity, antimicrobial properties, paramagnetic property for particle separation, optical and electronic properties for water quality monitoring (Alvarez 2013; Villasenor and Rios 2017; Xue et al. 2017). For instance, nano-adsorbents elevated the rate of adsorption due to their high surface-to-volume ratio and associated active sites, small intraparticle diffusion distance, and controllable pore size and surface chemistry (Qu et al. 2013; Shanker et al. 2017).

Textile mill effluents contain complex chemicals, dyes, pigments, and other organic contaminants, which are difficult to degrade even after treatment. The qualities of nano photocatalysts have aroused the interest of the research community for the remediation of dye effluents owing to their ability to dissociate complex organic compounds (Mekasuwandumrong et al. 2010; Alvarez 2013). A good photocatalyst should be capable of absorbing light/photons, preferably in the visible or near UV region, initiate the redox reaction to generate free radical ions, and disintegrate the complex molecules into simpler and nontoxic components. The photogenerated electrons and holes are capable of degrading virtually all types of organic, inorganic, and microbial contaminants (Sudrajat and Babel 2016; Khalafi et al. 2019). The nanostructured metal oxide semiconductors such as titanium dioxide, zinc oxide, and tungsten oxide effectively removed both chemical and biological contaminants (Baruah et al. 2016). In the context of finding out an environmental friendly cost-effective method for the treatment of dye effluents, photodegradation using nanostructured catalysts would be the best option owing to the properties of the nanomaterials (Boon et al. 2018).

4.3 Zinc Oxide Nanoparticles: Synthesis, Properties, and Applications

Zinc oxide nanoparticles, an inorganic semiconductor material, are nontoxic and biocompatible with excellent thermal and photostability, fast electron transport, wide bandgap (~3.37 eV), and high oxidation resistance (Diallo et al. 2015; Ahmed et al. 2017). Being a promising material with versatile properties, extensive research were carried out in various fields of applications like biosensing, biomedical, pharmaceutical, drug delivery, photocatalysis, and optoelectronics (Khan et al. 2011; Talebian et al. 2013; Madhumitha et al. 2016; Mirzaei and Darroudi 2017; Vishnukumar et al.

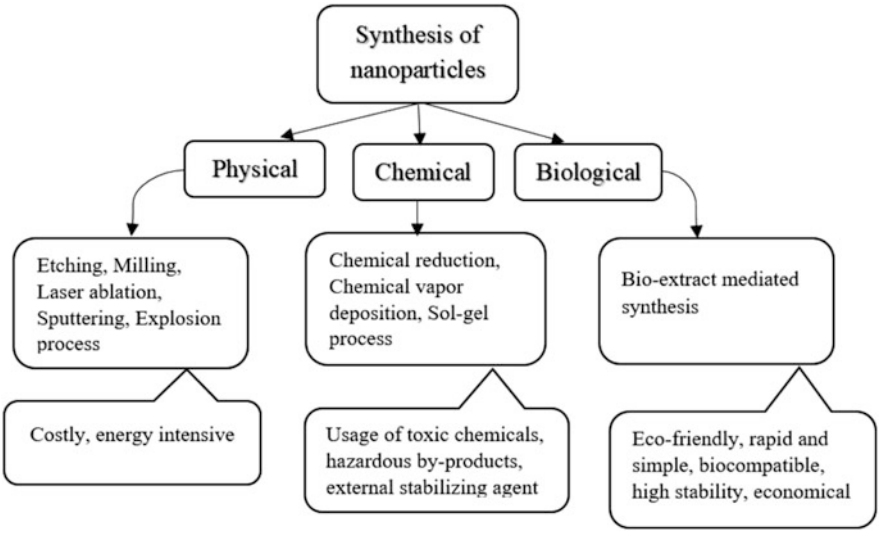

Fig. 4.2 Comparison of different methods of nanoparticle synthesis. The problems associated with the handling and disposal of harmful chemicals in the chemical methods of nanoparticles synthesis were alleviated with the introduction of biological resources as reducing agents

2018). Studies proved that ZnO nanoparticles could be one of the best and cost-efficient alternative for TiO2 in photocatalytic applications; only 1/4th of the costs of production of TiO2 nanoparticles (Boon et al. 2018; Yang et al. 2018). Furthermore, nanostructures with different morphologies can be fabricated using relatively simple and easy methodologies.

Different methods of synthesis were followed to obtain nanoparticles with controlled size and morphology as most of the applications were size-dependent (Kumar et al. 2013). Until a decade before, nanoparticles were synthesized via physical and chemical methods. Even with the successful protocols, there were some shortcomings, concerning the usage of toxic chemicals, hazardous by-products, high energy consumption, and costs (Sharma et al. 2015). Particularly in chemical methods, an additional chemical was used as an external capping/stabilization agent in regulating the characteristics of the particles. The complete removal of these chemicals and the recovery of nanoparticles have become a significant concern, and this has triggered researchers to employ eco-friendly reducing/capping agents (Kharissova et al. 2013; Makarov et al. 2014). Figure 4.2 illustrates the different approaches to synthesize nanoparticles.

4.4 Biological Synthesis of Zinc Oxide Nanoparticles

The development of environmentally beneficial biosynthesis methods has taken a big leap over the physical and chemical methods as it is clean, safe, cost-effective, and biocompatible. Subsequently, researchers are more inclined towards the

development of natural sources to function as reducing agent in the synthesis of nanoparticles. Also, the biological synthesis methods provide easy fabrication of nanoparticles. Various biological resources, including plants, microorganisms, and marine sources have been successfully used in the synthesis of ZnO nanoparticles (Nagarajan and Kuppusamy 2013; Ahmed et al. 2017; Fawcett et al. 2017). Table 4.1 shows various biological species used in the green synthesis of zinc oxide nanoparticles and the corresponding properties and applications of the nanoparticles.

The general scheme of the biological synthesis of zinc oxide nanoparticles is shown in Fig. 4.3. The flower extracts of *Nyctanthes arbor-tristis* effectively reduced zinc acetate dehydrate to form ZnO nanoparticles having a notable antifungal activity (Jamdagni et al. 2018). The inherent medicinal properties of *Nyctanthes*, including antimicrobial, antiviral, anticancer, anti-allergic, could have been transported to the obtained nanoparticles through the light capping layer formed on the surface of the nanoparticles.

The phytochemicals, such as polyphenols and flavonoids, play a crucial role in the biological reduction process. Consequently, the aqueous extract of *Cassia fistula*-generated zinc oxide nanoparticles have excellent bactericidal activity obtained from zinc nitrate hexahydrate (Suresh et al. 2015b). Similarly, the flavonoids found in the roots of the Chinese medicinal plant *Scutellaria baicalensis* actively assisted the reduction reaction of zinc nitrate to form ZnO nanoparticles (Chen et al. 2019). The flavonoids-like compounds such as polyphenols were the active compounds found in the extract of bark of *Kalopanax septemlobus*, which promoted the formation of ZnO photocatalysts (Lu et al. 2018).

The phenolic compounds, terpenoids, and proteins present in the aloe vera extract played a significant role in stabilizing the ZnO nanoparticles, making them more dispersible (Ali et al. 2016). The zeta potential noted for ZnO nanoparticles prepared using *A. occidentale* reflected the capping of phenolic compounds by the negative value (−53 mV), and the obtained nanoparticles showed extraordinary cytotoxic potential against the pancreatic cancer cells (Zhao et al. 2018).

The synthesis pathways alter the morphological behavior of the ZnO nanoparticle. The *Pedalium murex*-mediated ZnO nanoparticles synthesis followed two different protocols; microwave heating and sol-gel method. The former method resulted in the formation of nanoparticles and the latter generated nano-sheets (Babitha et al. 2019).

4.5 Biosynthesized ZnO Nanoparticles in the Treatment of Dye Effluents

Semiconductor particles in their nanoscale can act as active photocatalytic agents due to increased surface-to-volume ratio and better electron transfer capability for the degradation of organic pollutants under the irradiation of sunlight or UV-light (Saikia et al. 2014; Shim et al. 2018; Boon et al. 2018). Zinc oxide nanoparticle is an excellent semiconducting material with comprehensive bandgap energy and

Table 4.1 Biological sources used for the synthesis and the properties and applications of ZnO nanoparticles

Biological species	Source of Medium	Shape and size	Applications	Reference
Aloe vera	Leaves	Spherical, oval, hexagonal 8–18 nm	Antibacterial applications	Ali et al. (2016)
Vitex negundo	Leaves	Spherical 60 nm	Antibacterial applications	Ambika and Sundrarajan (2015)
Phyllanthus niruri	Leaves	Rectangle, triangle, hexagonal, spherical 25.61 nm	Photocatalytic application	Anbuvannan et al. (2015)
Lycopersicon esculentum	Fruits	9.7 ± 3 nm	Photocatalytic application	Castro-beltr (2017)
Scutellaria baicalensis	Roots	Spherical 50 nm	Photocatalytic application	Chen et al. (2019)
Hippophae rhamnoides	Leaves	Flower shape 20.17 nm	Photocatalytic application	Hee et al. (2018)
Lemon	Fruit	Spherical 21.5 nm	Photocatalytic application	Davar et al. (2015)
Trifolium pratense	Flower	60–70 nm	Antibacterial applications	Dobrucka (2016)
Chelidonium majus	Aerial part	Spherical 8 nm	Cytotoxic and antimicrobial applications	Dobrucka et al. (2018)
Vitex trifolia L.	Leaves	Spherical 15–46 nm	Antimicrobial and photocatalytic applications	Elumalai et al. (2015)
Artocarpus heterophyllus	Leaves	Spherical 10–30 nm	Photocatalytic applications	Vidya et al. (2017)
Allium sativum (garlic), Allium cepa (onion), Petroselinum crispum (parsley)	Root bulbs Root bulbs Leaves	Spherical 14 nm 21 nm 70 nm	Photocatalytic applications	Stan et al. (2015)
Prosopis farcta	Aerial part	Hexagonal 40–80 nm	Fungicidal and cytotoxic applications	Miri et al. (2019)
Mirabilis jalapa	Leaves	Needle shaped 12.9 nm	Antimicrobial applications	Nadeem et al. (2019)
Scutellaria baicalensis	Roots	Spherical 50 nm	Photocatalytic applications	Chen et al. (2019)
Abutilon indicum	Leaves	Spheroid, rod 16.72 nm	Biomedical applications	Khan et al. (2017)
Trianthema portulacastrum	Aerial part	Spherical 25–90 nm	Biological applications	Khan et al. (2019)

(continued)

Table 4.1 (continued)

Biological species	Source of Medium	Shape and size	Applications	Reference
Kalopanax septemlobus	Bark	Flower-like 500 nm	Photocatalytic applications	Lu et al. (2018)
Plectranthus amboinicus	Leaves	Rod 50–180 nm	Photocatalytic applications	Fu and Fu (2015)
Hydnocarpus alpina	Aerial part	Spherical 38.84 nm	Antioxidant, antimicrobial, photocatalytic applications	Ganesh et al. (2019)
Euphorbia jatropha	Latex	Hexagonal 18 nm	–	Geetha et al. (2016)
Sargassum wightii	Aerial part	Spherical 40–50 nm	Biological applications	Ishwarya et al. (2018a, b)
Aeromonas hydrophila	Microbial culture	Spherical, oval 57.72 nm	Antimicrobial applications	Jayaseelan et al. (2012)
Rambutan	Fruit peel	Spherical 25–40 nm	Photocatalytic applications	Karnan et al. (2016)
Tabernaemontana divaricata	Leaves	Spherical 20–50 nm	Antimicrobial and photocatalytic applications	Raja et al. (2018)
Microalgae *Chlorella*	Powder	Spherical 20–50 nm	Photocatalytic applications	Khalafi et al. (2019)
Moringa oleifera	Leaves	Spherical 16–31.9 nm	–	Matinise et al. (2017)
Sargassum myriocystum	Aerial part	Spherical 36 nm	–	Nagarajan and Kuppusamy (2013)

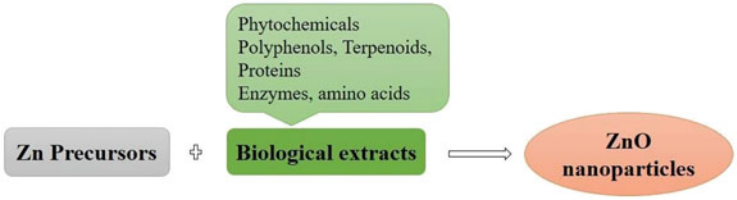

Fig. 4.3 Green synthesis of ZnO nanoparticles. The bioactive chemicals present in the natural resources assisted the quick and easy fabrication of nanoparticles

immense photocatalytic activity. The native surface defects such as the zinc interstitials and the oxygen vacancies allow the quick and easy generation of electron/hole combinations and enhance the absorption of visible light (Zhu et al. 2019). When a light source with energy equal to or greater than the bandgap energy of the semiconductor material falls on the surface of ZnO nanoparticles, electron-

hole pairs are generated in the conduction and the valence bands. Some of these pairs migrate to the photocatalytic surface, the H^+ reacts with water to form hydroxyl radical ($OH^·$), and the e^- reacts with oxygen to produce superoxide O2 radicals. Thus, formed reactive oxygen species (ROS) have strong oxidizing power to disintegrate the organic pollutants including the dye contaminants making them a potential candidate to use in dye effluents treatment (Saikia et al. 2014; Atchudan et al. 2017; Achouri et al. 2018).

The particle size of nanoparticles influences the photocatalytic property of ZnO nanoparticles. The surface-to-volume ratio is higher when the size of the particle is smaller, providing more active sites on the surface of the photocatalyst. The synthesis of ZnO nanoparticles using garlic extract resulted in the formation of smaller size particles, whereas the protocol using parsley extract created larger particles with reduced bandgap and lesser surface defects (Stan et al. 2015). Consequently, the highest percent removal of methylene blue was exhibited by the nanoparticles prepared using garlic extract. The photocatalytic efficiency of biosynthesized ZnO nanoparticles was evidenced in the tests conducted for the degradation of methylene blue dye solution; the deep blue solution turned colorless with the addition of ZnO nanoparticles (0.05 mg/mL) under UV irradiation, and the percent degradation was estimated to be 98.6 in 210 min (Chen et al. 2019).

The morphological behavior tends to have an impact on the photodegradation capacity of ZnO nanoparticles. The variation in the concentration of bio-extract in the reaction mixture resulted in the generation of zinc oxide nanoparticles with different shapes such as bullets, buds, cones, and bundles (Madan et al. 2015). The bullet-shaped nanoparticles in 4 mL of neem extract outperformed the other shaped particles in the photocatalytic degradation of methylene blue under sunlight, and UV irradiation and the maximum percent decolorization noted was 92% under sunlight for 120 min. Babitha et al. assessed the degradation capacity of two different ZnO nanostructures, nanoparticles, and nanosheets and reported 96.24% removal of methylene blue in the presence of UV light using nanoparticles and 91.36% using ZnO nanosheets (Babitha et al. 2019).

The pronounced light retention capacity enhances the photocatalytic potential of ZnO nanoparticles for the degradation of organic pollutants. The viable source of light available in nature is solar irradiation and was found to be most effective in the photodegradation of dye contaminants when compared with other sources of light (Nethravathi et al. 2015; Yang et al. 2020). Sai Saraswathy et al. reported the eco-friendly, cost-effective method of degradation of methyl orange under solar irradiation with a percent removal of 93.4 in 100 min (Sai Saraswathi et al. 2017). The degradation of methylene blue using ZnO nanoparticles prepared using *Kalopanax septemlobus* extract followed first-order reaction kinetics with a rate constant of 0.1215 min^{-1} (Lu et al. 2018). The discoloration of the dye solution took place within 30 min under UV irradiation with 0.5 mg/mL catalyst dosage. The dye molecules get adsorbed onto the surface of the catalyst immediately after the introduction of ZnO nanoparticles in the solution, even in the absence of a light source. The photons emitted from the light source initiate the redox reaction for the

formation of hydroxyl radicals, which is responsible for the degradation of complex dye molecules (Khan et al. 2019).

Achouri et al. evaluated the catalytic efficiency of ZnO nanorods in two different ways for the degradation of orange II dye under sunlight irradiation. The ZnO nanorods in the powdered form exhibited better efficiency than the film immobilized on a glass plate. Authors claimed that immobilization might have restricted the available contact area and mass transfer, and the rate constants were estimated as 0.016 and 0.004 min^{-1} for powder and film, respectively (Achouri et al. 2018). The ZnO nanocomposites prepared using hydrazine hydrate as the reducing agent exhibited 98% decolorization efficiency against Coralene Red F3BS dye in acute alkaline conditions under solar irradiation (Yogendra et al. 2011). Similarly, a dumbbell-shaped ZnO nano photocatalyst prepared via microwave irradiation demonstrated remarkable decolorization efficiency (99.6%) in 75 min against methylene blue dye solution at pH 7–8 (Yang et al. 2010).

The noxious effect of chemical-reducing agents and the corresponding products are unavoidable. On the contrary, the biological methods not only exclude toxic chemicals in the synthesis protocol but also enhances the properties of the obtained nanoparticles. The extract of the *Suaeda japonica makino* plant revealed a natural tendency to degrade methylene blue dye under UV light irradiation (Shim et al. 2018). Nonetheless, the ZnO nanoparticles prepared using the same extract degraded the dye in a better manner with 54% efficiency in 1 h. The photocatalytic properties of the zinc oxide nanoparticles synthesized using biological sources were reinforced and strengthened by the bioactive molecules present in the plant extracts.

Prasad et al. assessed the selective degradation capacity of ZnO nanoparticles prepared using *Abelmoschus esculentus* mucilage towards four different dyes, methylene blue, rhodamine B, congo red, and methyl orange. It was noted that almost complete decolorization (95%) of methylene blue was achieved after 60 min, and 100% removal of rhodamine B was obtained after 50 min under UV irradiation (Prasad et al. 2019). Authors conferred the effect of catalyst loading (25–175 mg) on the photocatalytic degradation and recorded the maximum percentage efficiency at 175 mg. Further increase in the catalyst dosage resulted in the agglomeration of the particles thereby increasing the turbidity of the solution. Another interesting finding of the biologically synthesized ZnO nanoparticles using *Rubus coreanus* was that the nanoparticles were capable of generating reactive oxygen species even in darkness, causing the degradation of malachite green (90% in 4 h) under both light and dark conditions (Rupa et al. 2018).

The role of microorganisms in the fabrication of ZnO photocatalysts with varied morphology should not be overlooked. The ZnO nanoflowers synthesized using *Bacillus licheniformis* MTCC 9555 were evaluated for its photostability and efficacy in degrading methylene blue dye under UV irradiation (Tripathi et al. 2014). The photocatalytic activity of ZnO nanoflowers remained intact even after three consecutive cycles with 74% efficiency and the rate of recovery of 90%, representing the industrial applicability of ZnO nano photocatalysts. However, the nanoflowers tend to agglomerate after three cycles. The improved stability of ZnO nano photocatalysts prepared using *Plectranthus amboinicus* leaf extract exhibited extraordinary photo

degradative capacity (85%) even after four continuous cycles (Fu and Fu 2015). Table 4.2 represents the biological species, conditions, and the percent efficiency of the photocatalytic degradation of biogenic ZnO nanoparticles.

4.5.1 Factors Affecting the Photodegradation of Dye Contaminants

The dye degradation efficiency is influenced by the variation in the amount of zinc oxide nanoparticles, initial dye concentration, and pH of the solution.

4.5.1.1 Catalyst Dosage

The efficiency of photodegradation depends on the generation of hydroxyl radicals, the more the amount of catalyst loaded, the more the number of holes and hydroxyl radicals. However, there will be an optimum point beyond which the addition of catalyst may adversely affect the degradation efficacy (Suresh et al. 2015b; Mahdavi and Talesh 2017). This might be due to the tendency of particles to agglomerate, settle down, and hence decrease the active surface area. In some cases, a small increase in the catalyst dosage may not have any effect on the degradation efficiency.

The addition of ZnO nanoparticles in two different doses (0.05 mg/mL and 1 mg/mL) resulted in the same percent degradation of methylene blue solution, whereas the dye solution with plant extracts alone marked very less discoloration tendency (Chen et al. 2019). Furthermore, the solution became turbid and opaque due to the increase in catalyst dosage causing scattering of light. This phenomenon interrupts the passage of light irradiation through the suspension resulting in reduced degradation (Nethravathi et al. 2015). Taghavi et al. evaluated the effect of catalyst dosage (0.03, 0.04, 0.05, 0.06 g) in the degradation of DB 129 dye under visible light source at constant dye concentration (20 mg/L) for 105 min. The decolorization efficiency increased with catalyst dosage up to 0.05 g, and a further increase in the catalyst did not contribute towards the color removal (Taghavi et al. 2017).

4.5.1.2 Initial Dye Concentration

The initial dye concentration has a significant effect on the efficiency of photodegradation. The degradation efficiency was found to be lower at higher dye concentrations when all the other conditions were kept constant. With the increase in dye concentration, more and more dye molecules get adsorbed onto the catalyst surface thereby restricting the electron/hole pair to get adhered to the active catalyst sites (Chen et al. 2017). Because of the limited active sites on the surface of the photocatalyst, the generated reactive oxygen species would not be sufficient to degrade the organic contaminants.

Vidya et al. studied the effect of different dye concentrations (10, 20, 30, and 40 ppm) on the catalytic degradation of congo red dye under UV light irradiation. At higher concentrations, the hydrophobic interactions between the aromatic rings in the dye compound formed agglomerates and separated, accordingly, reducing the formation of hydroxyl radicals (Vidya et al. 2017). Furthermore, increased dye

Table 4.2 List of different dyes; degradation conditions and efficiency and the biological species involved in ZnO nanoparticles synthesis

Biological resource	Dye	Conditions	% removal	Reference
Abelmoschus esculentus	Methylene blue (32 mg/L) Rhodamine B (9.5 mg/L)	125 mg catalyst; 60 min; 100 mg catalyst; 50 min	100% 100%	Prasad et al. (2019)
Cassia fistula	Methylene blue (5 ppm)	200 mg catalyst pH -4; 120 min	98.71%	Suresh et al. (2015b)
Trianthema portulacastrum	Synozol navy blue-KBF dye (10 mg/L)	1 g/L ZnO nanoparticles; 159 min	91%	Khan et al. (2019)
Artocarpus heterophyllus	Congo red (20 ppm)	0.24 g/L ZnO nanoparticles; 60 min; pH -9	90%	Vidya et al. (2017)
Suaeda japonica makino	Methylene blue	1.8 mL; 60 min	54%	Shim et al. (2018)
Rubus coreanus	Malachite green (10 ppm)	50 ppm; 4 h	90%	Rupa et al. (2018)
Hippophae rhamnoides	Malachite green (10 ppm) Eosin Y (10 ppm)	500 ppm; 180 min	89% 95%	Hee et al. (2018)
Scutellaria baicalensis	Methylene blue (50μM)	0.05 mg/mL; 210 min	98.6%	Chen et al. (2019)
Allium sativum	Methylene blue (1×10^{-5} Mol/L)	10 mg;180 min	Complete decolorization	Stan et al. (2015)
Lagerstroemia speciosa	Methyl orange (10 mg/L)	0.01 mg/mL; 120 min	93.5%	Sai Saraswathi et al. (2017)
Kalopanax septemlobus	Methylene blue	0.5 mg/mL; 30 min	97%	Lu et al. (2018)
Tabernaemonta divaricate	Methylene blue (1×10^{-5} M)	1 mg/mL; 90 min	–	Raja et al. (2018)
Bacillus licheniformis MTCC 9555	Methylene blue (100μM/L)	0.25 g/L; 60 min	83%	Tripathi et al. (2014)
Arabic-gum biopolymer	DB 129 (20 mg/L)	0.05 g; 105 min	95%	Taghavi et al. (2017)
Nephelium lappaceum L.	Methyl orange (10 mg/L)	1 mg/mL; 120 min	83.99%	Karnan et al. (2016)
Ulva lactuca	Methylene blue (25 mg/L)	0.5 g/L; 120 min	90.4%	Ishwarya et al. (2018a, 2018b)
Azadirachta indica	Methylene blue	120 min	92%	Madan et al. (2015)

(continued)

Table 4.2 (continued)

Biological resource	Dye	Conditions	% removal	Reference
Phyllanthus niruri	Methylene blue (1×10^{-4} M)	20 mg; 30 min	Complete decolorization	Anbuvannan et al. (2015)
Lycopersicon esculentum	Methylene blue (15 mg/L)	1:1 (MB: ZnO); 60 min	73%	Castro-beltr (2017)
Vitex trifolia L.	Methylene blue (1×10^{-3} M)	30 mg; 90 min	92.13%	Elumalai et al. (2015)
Plectranthus amboinicus	Methyl red(1×10^{-4} M)	20 mg; 180 min	92.45%	Fu and Fu (2015)
Hydnocarpus alpina	Methylene blue (20 mg/L)	100 mg; 30 min	96%	Ganesh et al. (2019)

concentration hinders the intensity of photons reaching the surface of the catalyst and hence results in reduced degradation (Taghavi et al. 2017; Ani et al. 2018).

4.5.1.3 The pH of the Solution

The variation in the solution pH affects the surface properties of the ZnO nanoparticles and hence the photocatalytic degradation. The rate of formation of electron-hole pair due to the incident radiation and their adsorption/desorption mechanism, and transport of electron-hole pair through the catalyst-dye solution interface were altered by the changes in the pH of the solution. In acute acidic conditions, the hydrogen ions react with the azo linkage in the congo red dye, decreasing the formation of hydroxyl radicals (Vidya et al. 2017).

Ganesh et al. assessed the effect of pH on the photocatalytic degradation of biogenic ZnO nanoparticles using methylene blue as test pollutant. The results evidenced higher values of % color removal (96%) at higher pH values (9.8) than at lower values (4.8) (Ganesh et al. 2019). In the same way, the highest percent efficiency was noted under alkaline conditions during the degradation of malachite green in the presence of sunlight/UV irradiation and zinc oxide nanoparticles (Suresh et al. 2015a).

Netravathi et al. reported that lower pH values of the dye solution favored the photodegradation of methylene blue under sunlight with a maximum percent removal of 94.1 at pH 4 and a subsequent increase in pH reduced the degradation efficiency. The interaction between the positively charged ZnO nanoparticles and negatively charged dye molecules in acidic medium promoted the degradation process, and conversely, the surface charge of ZnO nanoparticles reversed with the rise in pH initiating repulsion between the dye molecules and nanoparticles. Additionally, higher values of pH may fasten the recombination rate of electron/hole pair, dropping the formation of hydroxyl radicals (Nethravathi et al. 2015).

4.5.2 Mechanism of Photocatalytic Degradation

When light energy equal to or greater than ($h\nu$) is incident on the catalyst surface, the electrons from the valence band (VB) get excited and shifted to the conduction band (CB) leaving a hole in the valence band (Eq. 4.1) (Suresh et al. 2015a). Thus, created electron-hole pair move to the surface of the ZnO nano photocatalyst and initiate the reactions generating the superoxide ions and hydroxide radicals (Eqs. 4.2, 4.3, and 4.4). Anbuvannan et al. explained the possible mechanism of degradation of methylene blue in the presence of biogenic ZnO nanoparticles and UV irradiation by the following set of equations.

$$ZnO\ \mathrm{NPs} + h\nu \rightarrow {h_{VB}}^{+} + {e_{CB}}^{-} \tag{4.1}$$

$$\mathrm{H_2}O + {h_{VB}}^{+} \rightarrow \mathrm{H}^{+} + O\mathrm{H} \tag{4.2}$$

$$O\mathrm{H}^{-} + {h_{VB}}^{+} \rightarrow O\mathrm{H} \tag{4.3}$$

$$O_2 + {e_{CB}}^{-} \rightarrow O_2 \tag{4.4}$$

$$O2 + {e_{CB}}^{-} + 2\mathrm{H}^{+} \rightarrow \mathrm{H2}O2 \tag{4.5}$$

$$2O2 + 2\mathrm{H}^{+} \rightarrow O_2 + \mathrm{H_2}O_2 \tag{4.6}$$

$$\mathrm{H_2}O_2 + {e_{CB}}^{-} \rightarrow O\mathrm{H} + O\mathrm{H}^{-} \tag{4.7}$$

$$\mathrm{D}ye\ molecules + O\mathrm{H} \rightarrow degraded\ \mathrm{p}roducts \tag{4.8}$$

The reactions of superoxide anions with electrons form hydrogen peroxide and subsequently hydroxyl ions (Eqs. 4.5, 4.6, and 4.7). The highly reactive hydroxyl radicals disintegrate the complex dye molecules into intermediate products and finally dissociate into carbon dioxide and water (Boon et al. 2018). Figure 4.4 illustrates the schematic representation of the mechanism of degradation of dye solution in the presence of sunlight or UV light source using zinc oxide nanoparticles.

4.5.3 Modification of ZnO Nano Photocatalysts

The green synthesized ZnO nanoparticles via *Caralluma fimbriata* extract were modified with Gd at different concentrations (1, 3, and 7 mole %) for the degradation of indigo carmine dye under UV and sunlight for 90 min (Mishra et al. 2016). It was noted that the dye degradation followed the same pattern under both UV and

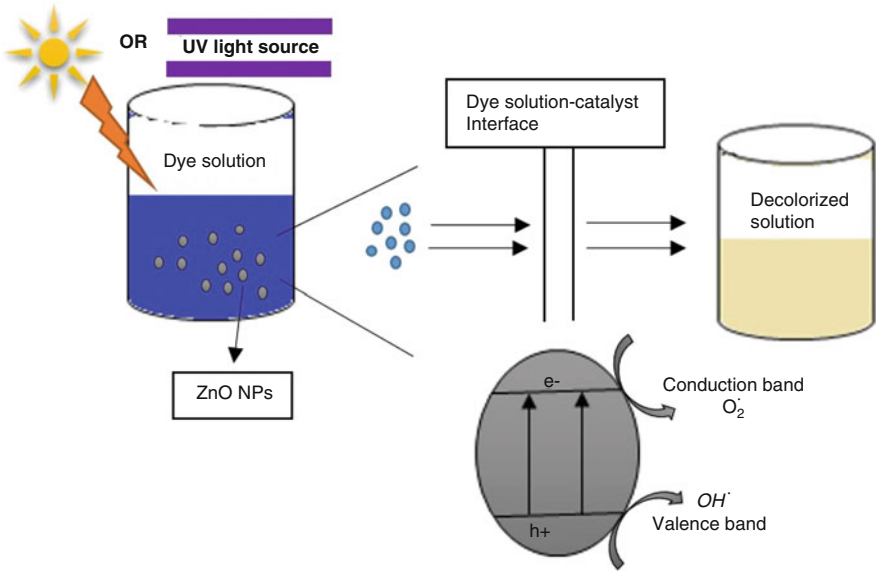

Fig. 4.4 Degradation of dye effluent in the presence of light source using zinc oxide nanoparticles. The incident light radiation excites the electrons to move from valence band to conduction band and the resulting electron hole pair facilitates the dye degradation on the catalyst surface

sunlight, and the highest photocatalytic activity was recorded by (1%) Gd-ZnO nanostructure. The addition of Gd^{3+} dopant to the ZnO nanoparticles initially enhanced the photocatalytic activity, and the subsequent increase in the Gd^{3+} content in the nanocomposite plummeted the efficiency. The findings emphasized the photocatalytic potential of biosynthesized zinc oxide nanoparticles for the degradation of toxic dye contaminants.

Similarly, the Al-doped ZnO nanoparticles exhibited better degradation capacity than the undoped nanoparticles. However, the increase in Al concentration above 5% reduced the degradation capacity (Mahdavi and Talesh 2017). The higher amount of doped metals on the catalytic surface might block the active sites hindering the generation of hydroxyl radicals required for the degradation of dye molecules. The surface integrity of the materials forming the interface amends the photocatalytic activity of a nanocomposite. The higher interfacial area facilitates faster transfer of photogenerated charges onto the catalyst surface and hence provides better activity (Jana et al. 2016). The composite prepared using zinc oxide and polymeric graphite-like C3N4 demonstrated superior degradation capacity in the treatment of rhodamine B dye solutions under solar irradiation (Osman et al. 2017). The interfacial interaction between the two semiconductor materials such as ZnO and polymeric graphite-like C3N4 improved the optical absorption and solar energy utilization of composite photocatalyst, resulting in the enhancement of photocatalytic degradation efficiency.

4.6 Conclusion

Textile mills are one of the oldest and most significant among all industries worldwide. The processes involved in garment manufacture and dyeing vary according to the type of the fabric, type of industry, location, and the demand of the finished products. The dyeing industry utilizes numerous chemicals inclusive of dyes, colorants, heavy metals, and other chemicals that are extremely toxic and nondegradable. The threats posed by the discharge of textile mill effluents would be a severe concern owing to the potential hazards to human health, aquatic life, and the environment. In the context of finding out the best method to treat dye effluents for safe disposal and probable reuse, nanotechnology has aroused the attention of the research community, considering the versatile properties of nanomaterials. This chapter analyzed the problems caused by dye wastewater, the conventional treatment methods for the treatment of dye effluents, and the implementation of nanotechnology in the treatment of industrial effluents.

Most importantly, this chapter emphasizes the role of zinc oxide nanoparticles in the active degradation of dyes, the green synthesis protocols and the various biological resources involved in the fabrication of ZnO photocatalyst with varied morphology, the photocatalytic degradation of different dyes and the impact of different factors in the photodegradation process. The mechanism of photodegradation and the performance of the modified ZnO nanocatalyst were also discussed. Textile mills are considered to be one of the largest consumers of water, and the generation of wastewater has become an inevitable part of the finishing process. However, there should be ways to optimize the utilization of chemicals and water to minimize wastewater production and discharge.

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5 Advancements on Biotechnological and Microbial Biodegradation of Textile Wastewater

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5.1 Introduction

The increase in industrialization has given rise to the pollutants like industrial effluents, other organic pollutants, pesticides, polycyclic aromatic hydrocarbons, etc., which are persistent in nature and they have greater potential to accumulate in biological systems (Shanker et al. 2017). Among all the organic pollutants, dyes have become a part of human lives and have been extensively used. Approximately 800,000 tonnes of dyes are manufactured worldwide, among which, 10–15% are lost during dyeing various processes and are discharged into the environment. China is the largest manufacturer as well as exporter of dyes, followed by India and several other countries. According to the annual report (2018–19) of the Government of India Ministry of Chemicals and Fertilizers Department of Chemicals and Petrochemicals, the total production of dyes and pigments was around 382 thousand MT. In India, Gujarat and Maharashtra are the highest producers of dyes and are responsible for about 90% of the total dye produced annually. Textile remains as the largest consumer of dyes and in return, a lot of dyestuff-producing industries are dependent on it. Textile sector mainly requires azo dyes, vat dyes, and reactive dyes

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_5

for dyeing cotton fibers. It is also very evident that high amount of colored effluent is released every now and then. Almost 10–25% of these dyes are lost in the form of effluents and 2–20% are present as aqueous effluents, directly discarded in the natural environment.

Textile industries use large quantities of water in their operation and the effluent may contain high amounts of heavy metals and suspended solids. The colorants used are prone to biological assimilations, which can result in eutrophication, ultimately leading to genotoxic and microtoxic effects (Verma et al. 2012). If the effluents are not treated properly, it may even lead to contamination of groundwater, other water reservoirs, and agricultural lands. Because of high variation in their physiochemical properties in water, it is difficult to treat these effluents (Crini and Lichtfouse 2019).

5.2 Dyes and Their Variants

Dyes are soluble, organic substances that can impart color to the substrates, temporarily or permanently, either by destructing their crystal structures or by adhering to the surface. Natural dyes and colorants are undoubtedly environment friendly but have limited colors, the shades obtained may or may not be reproducible, and are sometimes light sensitive and sensitive to detergents (Khatri and White 2015). The modification of natural dye started in 1771 when Woulfe created yellow-colored picric acid from indigo and nitric acid. But a major revolution began with the production of a cationic dye called mauve and mauveine salts by William Perkin in 1856. Since then, the use of synthetic dyes has increased drastically (Singh and Arora 2011).

Synthetic dyes are the derivatives of basic organic compounds like benzene, anthracene, etc. They are classified according to their chemical structure, color index, or their usage in a specific kind of industry. Another way of classifying these dyes is according to their ionic charge when dissolved in an aqueous solution. The cationic dyes are positively charged and alkaline in nature. Anionic dyes are negatively charged and further subdivided into acid dyes, reactive dyes, or direct dyes. Most convenient classification of dyes is based on the chemical structure of the chromophores present. Textile dyes majorly include Azo dyes, nitro dyes, indigo dyes, anthraquinone dyes, phthalein dyes, triphenyl methyl dyes, nitrate dyes, nitroso dyes, etc. Two of the most important textile fabrics are cotton and polyester and the dyes are selected according to the fabric. Among all the dyes mentioned above, azo dyes are studied extensively and they are the largest class of synthetic dyes and are used in all possible industries. The degradation of azo dyes yields aromatic amines, which can undergo biomagnification or bioaccumulation. The dye effluents from the industry that use azo dyes have emerged as a major pollutant.

Thiazine dyes are basic in nature and it contains nitrogen and sulfur atoms, which form a ring with the benzene carbons. They mainly include methylene blue, thionine, methylene violet, red methylene blue, Azure B, Azure C, etc. (Mills et al. 2011). Anthraquinones are the second most widely used textile dyes and are a little resistant to decolorisation. This is because the chromogenic groups of these dyes are highly

stable and can retain their characteristics for a longer period. Therefore, if released in water without any kind of treatment, they can cause acute mutations in aquatic animals and can thus disturb the aqueous ecosystem (Li et al. 2019).

5.3 Dyeing Effluent: a Dreadful Pollutant

The environment has become one of the leading topics of discussion because of the increasing pollution by textile industrial effluents. Dying effluents released from industries are recognized easily because of their pigments and sometimes due to the pungent odor. This prevents the exchange of gases and affects gas solubility in the water reservoirs. The penetration of light is severely affected which results in the loss of aquatic life due to decreased photosynthesis and affects the biochemical and chemical oxygen demand of water bodies (Ogugbue and Sawidis 2011). Dyes usually have aromatic structure, which makes them highly stable and sometimes recalcitrant. Therefore, once incorporated into any level of food chain, they can undergo biomagnification and bioaccumulation. Dyes can behave as an allergen, mutagen, and potential carcinogen as well as exert immunological effects causing respiratory diseases, skin irritations, and can even cause chromosomal aberration in mammals (Sharavanan et al. 2020). Discharging dye effluents in agricultural soil can result in loss of soil fertility, reduces the soil porosity, and can harden the soil so that the roots can no longer penetrate. Owing to the above threats, there is a need to treat the effluents before releasing them into the environment.

5.4 Methods for Treating the Dyeing Effluents

5.4.1 Physical Methods

The physical methods include precipitation, flocculation, filtration, adsorption, and a very simple principle of adsorption used to remove dyes in which natural components as adsorbents to decrease the overall cost of the process. These adsorbents are readily available and accepted by technologists. The materials like perlite, clay, activated charcoal, and zeolite are extensively used to remove dyes (Rafatullah et al. 2010; Varghese et al. 2019). Graphene oxide has also been proven a very good absorbent with an efficiency of almost 99% and showed a successful decolorization of the solution (Yang et al. 2011). But with the leading advantages, there are problems associated with the regeneration of the same results every time. Moreover, the synthetic adsorbents ultimately add up to the overall cost of the treatment.

Coagulation-flocculation also gives efficient results for the decolorization of dyes by completely removing the molecules from wastewater. To make the process better, synthetic coagulants or flocculants are added to the system, which again adds up to the total cost. Other conventional methods include membrane filtration, irradiation, ion exchange, photochemical sonolysis, and oxidation with the help of

heterogeneous catalysts. However, with each method, there comes a limitation of either the cost or the production of high amounts of sludge waste (Verma et al. 2012).

Filtration is mainly employed to recycle water used in the textile industry. With the help of ultrafiltration and nano-filtration, the color, and oxygen demands of wastewater can be reduced. Filtration allows us to achieve a continuous separation of dye from effluent. Again, the problem of sludge disposal remains as large quantities are separated from the effluent. Ion exchangers can be used to remove dyes and dyestuff from the effluent. Cationic or anionic exchange resins can be used to remove the ions present. This is used to remove ions like Ca^{2+} and Mg^{2+}, but this method cannot be used to remove heavy metals from effluents (Li et al. 2007).

5.4.2 Chemical Methods

The chemical methods include ozonation, electrolysis, and other advanced oxidation processes. Ozone has the capacity to oxidize the organic components present in the dye effluent by directly oxidizing the compound or can form free radicals in water. Studies suggest that ozonation can decolorize almost all types of dyes except some disperse dyes and vat dyes. Ozonation is also capable of altering the pH of the solution and may or may not lead to complete oxidation of organic compound, instead can yield intermediates that are more toxic. To avoid such outcomes, the process is coupled with UV radiation and this process is termed as photochemical oxidation (Liu et al. 2011; Tarkwa et al. 2019).

Another method for the treatment of wastewater is by advanced oxidation processes; based on the formation of highly reactive hydroxyl free radical in order to remove the pollutants (Verma and Samanta 2018). Fenton's reagent is prepared using hydrogen peroxide and ferrous ion and is widely used for decolorization of effluents having high chemical oxygen demands. pH of the system also plays a major role in this method. The treated wastewater may have high amounts of anions and large quantities of ferrous ion sludge are generated which can lead to another problem (Hansson et al. 2012).

Electrocoagulation is a modification of electro-floatation where iron and aluminum are used as electrodes. The organic component is precipitated and adsorbed on the electrodes. Change in chemical oxygen demand levels and decrease in the color depends on the density of the current applied at the electrodes. Electrocoagulation provides removal of dye components that cannot be removed by normal chemical methods. Sludge generated is less and can be used as a fertilizer within a lesser induction time. However, the electrodes used needs replacement and the overall cost increases due to continuous requirement of electricity (Khandegar and Saroha 2013). Other most common advanced oxidation processes include electrochemical oxidation, photolysis using hydrogen peroxide and ozone process, titanium dioxide photolysis, wet oxidation, and the use of electronic beams (Guivarch et al. 2003; Lahkimi et al. 2007).

5.4.3 Biological Methods

Physiochemical methods cannot always mineralize recalcitrant dyes or their organic intermediates thus the biological systems are employed for treating the dye effluents. Bioremediation is a slow process but can give maximum result at a very low cost and is also environment friendly (Kasiri and Safapour 2014). It includes the use of any kind of biological systems like plants or microbial cells, for the removal of pollutants. Three types of bioremedial strategies are used to treat the dyeing effluents such as biosorption, bioaccumulation, and bioaugmentation. Biosorption involves the use of viable or non-viable biological material for the elimination of pollutants from the solution (Crini et al. 2019). The accumulation of pollutants in actively dividing cells is termed as bioaccumulation. It can be achieved when simpler carbon sources are provided in the media. Microbes will utilize these substrates as a carbon source instead of the pollutant present. Bioaugmentation can be done either by directly introducing the microbes either in situ or ex situ (Khalid et al. 2010).

5.4.3.1 Phytoremediation

Phytoremediation is the use of plants for the restoration of polluted sites. The cost-effectiveness is the main advantage of using plant-based techniques. Phytoremediation involves various strategies like phytoaccumulation, phytovolatilization, phytoextraction, phytostabilization, phytoimmobilization, rhizofiltration, and phytodegradation (Muthusaravanan et al. 2018). Reports suggest that phytoremediation has been used for the removal of dyes, toxic compounds like arsenic, and various other heavy metals (Vithanage et al. 2012). The use of field crops that are edible or used as animal fodder is not advisable in phytoremediation. Various strategies including recombinant DNA technology, genetic engineering, and biofortification are employed in order to improve the phytoremediation efficiency of plants (Table 5.1) (Vamerali et al. 2010).

5.4.3.2 Microbial Remediation

Microbes can adapt themselves to the toxic environment and can convert the toxic compounds into less harmful components or can help in the complete mineralization of complex organic compounds. Many species of bacteria, algae, fungi, and yeast

Table 5.1 Decolorization of dyes using phytoremediation

Dye	Plant	References
Basic Red 46	*Azolla filiculoide*	Vafaei et al. (2012)
Acid Blue	*Nasturtium officinale*	Torbati et al. (2014)
Scarlet RR	*Ipomoea herderifolia*	Rane et al. (2014)
Reactive Blue 19	*Typhaangustifolia*	Mahmood et al. (2014)
Remazol Red	*Alternanthera philoxeroides*	Rane et al. (2015)
Acid Red 114	*Ipomoea carnea* J	Jha et al. (2016)
Textile Wastewater	*Eichhornia crassipes*	Priya and Selvan (2017)
Methylene Blue	*Azolla pinnata*	Al-Baldawi et al. (2018)

have the ability to degrade or decolorize the dyeing effluents either by producing certain enzymes or by consuming the organic material as a carbon source (Saratale et al. 2011).

Fungi are found in a variety of ecological niches and the main requirements for carrying out our proper metabolic cycles are carbon and nitrogen. They even have the ability to produce many intracellular as well as extracellular enzymes required for their metabolism. Due to this ability, fungi can utilize a variety of organic compounds like lignocellulose, hemicellulose, and other polyaromatic hydrocarbons including dye. *Phanerochaete chrysosporium* has been studied extensively because of its ability to produce enzymes like ligninases and peroxidases, which are required for degradation. Unlike bacteria, fungi do not require an adaptation period. However, it still has long growth periods, uncontrolled growth rate, and is completely dependent on the substrates. Due to such reasons, enzymes can be directly isolated and used. Nevertheless, the production and purification of the enzymes add up to the total cost. Yeasts can grow rapidly and are resistant to extreme conditions up to a certain extent. Viable as well as dry yeast can behave as a very efficient biosorbent, cost-effective, and does not require any expensive culture media. Many species of *Candida* have shown effective biosorption of distinct dyes (Aksu and Dönmez 2003).

Algae are unicellular, photosynthetic organisms, which can be found in many habitats. They can utilize organic components like dyes in order to increase the biomass and can convert them to carbon dioxide and water (Khataee et al. 2011). Biosorption of reactive dyes like Remazol Black B, Remazol Red RR, and Remazol Golden Yellow RNL was done using a green alga, *Chorella vulgaris* (Aksu and Tezer 2005). Some species of actinomycetes are known to produce certain extracellular enzymes that can act on organic pollutants. They were able to decolorize and degrade some azo dyes in aerobic condition. *Streptomycetes* sp. is capable of producing extracellular peroxidases that help in decolorization (McMullan et al. 2001; Dholakiya et al. 2018).

5.5 Bacterial-Aided Dye Degradation

5.5.1 Pure Bacterial Strains for Dye Decolorization

Bacterial degradation is the most studied method and it has its own advantages. It is inexpensive, environment friendly, and produces less sludge when compared with other techniques. Bacteria can utilize a wide variety of organic materials based on their metabolic needs. Bacterial degradation can be aerobic as well as anaerobic. Bacteria are used either as a pure culture or as a consortium. The advantage of using bacterial consortia is that it can result in complete mineralization of organic pollutant due to the synergistic effect between different cultures. Isolating a pure bacterial strain can become a tedious job and it may even require a longer adaptation period (Cerqueira et al. 2011). The single-pure bacterial strains can easily degrade dyes and the main advantage that they offer is the ability to give reproducible results that are

Table 5.2 Different bacterial strains used in dye degradation

Bacterial strain	Dye	References
Pseudomonas desmolyticum NCIM 2112	Direct blue 6	Kalme et al. (2007)
Brevibacillus laterosporus MTCC 2298	Methyl red	Gomare and Govindwar (2009)
	Golden yellow HER	Gomare et al. (2009)
Acetobacter calcoaceticus NCIM 2890	Direct brown MR	Ghodake et al. (2009)
Kocuria rosea MTCC 1532	Methyl orange	Parshetti et al. (2010)
Proteus vulgaris NCIM 2027	Green HE4BD	Saratale et al. (2010)
	Scarlet RR	
	Navy blue HE2R	
Bacillus algicola	Blue azo dye	Chukowry et al. (2017)
	Red azo dye	
Klebsiella sp. C NCIM 5546	Reactive Blue 19	Holkar et al. (2018)

easily interpreted. Pure strains can degrade efficiently in aerobic, anaerobic, and anoxic conditions. Table 5.2 gives a precise review of the different pure bacterial strains involved in dye degradation.

5.5.2 Acclimated Bacterial Strains for Dye Decolorization

Polluted sites usually have a diverse category of microorganisms which are not only adapted to the pollutants present but can even consume them for their survival. These indigenous strains are more effective as they behave as extremophiles and can easily get adapted to the biotic and abiotic stresses for their survival. These microorganisms adapt to the environment by following an alternative form of metabolism. This phenomenon is known as acclimation. At lower dye concentrations, the acclimated microbes exhibit negative inhibition while at higher dye concentrations, the microbial strains get acclimated to a greater extent (Santhiya and Ting 2006). This method is very well studied due to the advantages that the strains offer like easy screening, eco-friendly and economically viable nature, and less sludge production with metabolites of mineralized nature. Table 5.3 exhibits the dye decolorization potential of different bacterial isolates.

5.5.3 Mixed Bacterial Cultures for Dye Degradation

The bacterial consortia show a synergistic mechanism among the coexisting strains wherein, the individual strains attack the dyes at different positions to form intermediates, which are further decomposed, by other synergistic strains (Lade et al. 2015; Kumar et al. 2017a, b). Table 5.4 gives a snapshot of the reports of

Table 5.3 Application of identified bacterial strains for dye degradation

Bacterial strain	Dye	References
Geobacillus stearothermophilus UCP 986	Orange II	Evangelista-Barreto et al. (2009)
Pseudomonas aeruginosa	Remazol Orange	Sarayu and Sandhya (2010)
Bacillus sp. strain AK1 and *Lysinibacillus* sp. strain AK2	Metanil yellow	Anjaneya et al. (2011)
Brevibacterium sp. strain VN-15	Reactive yellow 107	Franciscon et al. (2012)
	Reactive Black 5	
	Reactive Red 198	
	Direct Blue	
Sphingomonas paucimobilis	Methylene Blue	Noraini et al. (2012)
Bacillus cereus RMLAU1	Acid Orange 7	Garg and Tripathi (2013)
Staphylococcus hominis RMLRT03	Acid Orange	Singh et al. (2014)
Pseudomonas entomophila BS1	Reactive Black 5	Khan and Malik (2016)
Stenotrophomonas maltophilia	Methylene Blue	Kilany (2017)
Serratia liquefaciens	Azure B	Haq and Raj (2018)
Staphylococcus sp. strain K2204	Remazol Brilliant Blue R	Velayutham et al. (2018)
Lysinibacillus boronitolerans CMGS-2	Reactive Red 11	Basutkar and Shivannavar (2019)

dye degradation by mixed bacterial population and bacterial consortium for the degradation of dyes.

5.6 Instrumental Methods of Biodegradation Analyses

Biodegradation of dyes either takes place through several mechanisms that produce different intermediates simple organic molecules or gets completely mineralized. The underlying mechanisms of biodegradation are characterized by analytical techniques based on samples tested, and are classified into spectrophotometric, spectroscopic, chromatographic, and mass spectral analyses (Kilany 2017).

Ultraviolet-visible (UV-Vis) spectrophotometry is a primary analytical technique to measure qualitative as well as quantitative extent of decolorization by comparing the absorbance values of the dyes at their respective wavelengths before and after microbial treatment. The spectra are interpreted by visualizing the disappearance of the sharp peak after the dye decolorization (Kumar et al. 2012). To observe the changes in the functional constituents and moieties of the dyes and their metabolites, Fourier transform infrared (FT-IR) spectroscopic studies are employed. FT-IR

Table 5.4 Application of mixed bacterial cultures for dye degradation

Mixed bacterial culture	Dye	References
Seed sludge from a municipal wastewater treatment plant	Acid Red 42	Gonçalves et al. (2001)
	Acid Red 73	
	Direct Red 80	
	Disperse Blue 56	
Mixed and methanogenic cultures	Acid Orange 7	Brás et al. (2001)
An uncharacterized aerobic biofilm	Acid Orange 7	Coughlin et al. (2002)
Facultative consortium-PDW	Reactive Orange 16	Kapdan and Oztekin (2003)
Granulated anaerobic mixed culture	Reactive Black 5	Işık and Sponza (2004)
	Direct Brown 2	
Dried activated sludge	Reactive Black 5	Gulnaz et al. (2006)
Mixed bacterial strains	Mixed dyes	Rajeswari et al. (2011)
Bacterial consortium-AVS	Acid Blue 15	Kumar et al. (2012)
	Methylene Blue	
Mixed culture SB4	Reactive Violet 5R	Jain et al. (2012)
Microbial community	Actual textile wastewater	Forss et al. (2017)
B. firmus, B. macerans, S. aureus, and *K. oxytoca*	Vat dyes	Adebajo et al. (2017)
Bacterial concoction	Textile mill effluent	Kumar et al. (2017a, b)

spectrum allows us to determine the type and strength of interactions that occur within the dyes and the microbial cells during biodegradation (Cui et al. 2016). Nuclear magnetic resonance (NMR) spectroscopy is a tool to analyze the structural information concerning molecular compounds in either solution or solid state. The resonance of the protons attached to the structural moieties is described by these spectra and the chemical spins with shifts are explained with a comparative analysis of the initial dye molecules and the biotransformed products (Kumar et al. 2017a, b).

High-performance liquid chromatography (HPLC) utilizes very small packing particles with a relatively high pressure for the separation of the analytes and further recording the chromatogram. The analytes are eluted from the column at different retention times which is the time required for a band to travel the column length and is generally represented in seconds or minutes. For dye degradation studies, the breakdown is confirmed by the occurrence of new peaks with different retention time in comparison to that of the original dye, indicating the formation of new structure analogs (Bandary et al. 2016). Thin layer chromatography (TLC) relies on the separation power to resolve dyes and metabolites from the mobile phase mixture containing the analytes (Mohana et al. 2008). The separation of the mixtures is observed as bands or spots on the sheet and eventually, the retardation factor corresponding to each of such bands/spots is calculated and compared to the initial and final compounds for explaining the degradation (Komal et al. 2017; Kumar et al. 2017a, b).

The mass spectral analysis is used to identify the products of biotransformation catalyzed by the bacterial cells. Compounds can be identified by comparing their retention times with those of standards and confirmed by their mass spectra. Mass spectra provide a detailed qualitative analysis of the products, information on molecular weights, and structural information of the metabolites and also help to propose dye degradation pathways (Dettmer et al. 2007).

5.7 Induction of Oxido-Reductases during Dye Degradation

Enzymes are a better option for bioremediating environmental contaminants, and the oxido-reductive enzyme systems comprising oxidases and reductases that are involved in the degradation of aromatic pollutants (de Gonzalo et al. 2016). Enzymes like lignin peroxidase, manganese peroxidase, azoreductase, dichlorophenol indophenol reductase, tyrosinase, laccase, veratryl alcohol oxidase, triphenylmethane reductase, horseradish peroxidase, and aminopyrine N-demethylase can catalyze biotransformations of dyes (Kabra et al. 2011). But the major limitation of enzyme-based biodegradation is that these enzymes easily get denatured at high temperatures which results in a decrease in their activity when exposed to higher concentrations of pollutants. The production and purification of these enzymes are relatively expensive and require controlled environmental conditions (Rao et al. 2010). Generally, dye degradation by bacterial cells involves the oxidation and reduction of the chemical bonds by the enzymes and these enzymes may be extracellular or intracellular (Mahmood et al. 2014; Yadav and Yadav 2015). Lignin-degrading enzymes constitute two types of peroxidases such as lignin peroxidase and manganese peroxidase, which bioremediates diverse pollutants. Lignin peroxidase utilizes hydrogen peroxide as a co-factor for oxidizing the phenolic group (de Gonzalo et al. 2016). Manganese peroxidase catalyzes the oxidation of phenolic contaminants in the presence of divalent manganese ions (Haq and Raj 2018).

Laccase consists of histidine-copper binding domains used for the biodegradation of textile dyes and polycyclic aromatic hydrocarbons (Yang et al. 2015). It has the ability to degrade the substrates having high redox potential and catalyzes the removal of hydrogen atom from the hydroxyl group of the substituted mono and polyphenol compounds (Akkaya et al. 2016). Tyrosinase is a monophenol monooxygenase, which catalyzes the phenol oxidation using molecular oxygen rather than hydrogen peroxide (Ramsden and Riley 2014). Hydroxylation and oxidation are the two main steps involved in catalysis (Durán and Esposito 2000; Mahmood et al. 2014).

Azoreductase catalyzes the reductive cleavage of azo bonds to produce colorless aromatic amines (Misal et al. 2014). They are categorized into flavin-dependant and flavin-independent azoreductases based on their function (Parmar and Shukla 2018). Triphenylmethane reductase is a dinucleotide-binding motif-containing enzyme (Kim et al. 2006). The veratryl alcohol oxidase is another oxidative enzyme induced by the microbial system during dye degradation (Bourbonnais and Paice 1988).

5.8 Assessment of Detoxification

The produced intermediates should be analyzed for their potential toxicity as they can prove to be even more hazardous to the environment as compared to the dyestuff itself. The lack of proper toxicity assessments of these metabolic intermediates makes environmental technologists think of the biodegradation technique as an unreliable option. The toxicity of dyes and degraded products are assessed and compared using toxicity tests like phytotoxicity, microbial toxicity, and cyto- or genotoxicity. The phytotoxicity of such dyes and their intermediates can be checked by growing model plants in the presence of treated and untreated compounds. The commonly used plant seeds for assessing the toxicity of dyes and their degraded products include *Triticum aestivum, Oryza sativa, Sorghum vulgare, Phaseolus mungo, Sorghum bicolor,* and *Zea mays* (Phugare et al. 2011). The radicle and plumule height and the rate of seed germination are measured to assess the plant's growth (Saratale et al. 2010; Roat et al. 2016).

To check the toxicity of treated as well as untreated dyestuff on animal, Artemia model is taken into consideration. *Artemia nauplii* larvae are allowed to grow in the presence of treated as well as untreated dyestuff and then after that acute toxicity is measured in terms of the mortality of the organism. Various dilutions of the test compound can be used with respect to different incubation times and the results are compared with controls (Prasad and Rao 2013).

In addition to phytotoxicity, microbial toxicity assessments are done using bacteria belonging to nitrogen-fixing classes *A. vinelandii, S. paucimobilis, Cellulomonas biazotea, E. coli* DH5α, *B. laterosporus, P. aeruginosa, R. radiobacter,* and *Acinetobacter* sp. (Roat et al. 2016). The toxicity assessments of dyes and their products have also been made using Comet assays and cyto/genotoxicity assays (Rajaguru et al. 2001). Studies related to oxidative stress, antioxidant enzymatic status, protein oxidation, and lipid peroxidation analyses are done to monitor toxicity. Sensitivity tests using neonates and acute toxicity tests using *Daphnia magna* are also explored for assessing the toxicity of dyes and their products (Elisangela et al. 2009).

5.9 Conclusion

The dyes and their effluents have caused a major environmental change and indirectly decrease the overall esthetic value. Thus, the demand for developing new and environment-friendly methods have increased. Using microorganisms to remediate these organic compounds have gained huge attention because of the benefits they offer. Biological treatments are usually less expensive and feasible, and the sludge production is very less. Microbial treatment or enzymatic treatments can also lead to complete mineralization of dyes and dyestuff. However, there is a need to scale up such treatments with an aim of treating industrial effluents. Conclusively, the appropriate mechanism of degradation should be cautiously studied to understand

bioaccumulation and biomagnification of the toxic by-products as well as to assess the fate of the intermediate metabolites.

Acknowledgment The authors are thankful to Director, CSIR-CSMCRI for the support and the manuscript has been assigned CSIR-CSMCRI-168/2019 registration.

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6 Emergence of Antimicrobial Resistance among Microbiome in Wastewater Treatment Plant and Strategies to Tackle their Effects in Environment

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6.1 Introduction

Antimicrobial resistance (AMR) embodies a key source of health threat around the world as well as acts as a chief risk to maintainable financial progress and social safety. Therefore, it is important that present research line up with policy growth and execution to lessen a latent catastrophe. Regrettably, current industrial scenario and its pollution influence on the incidence of environmental AMR have largely been overlooked. Other than the natural environmental stresses, anthropogenic activities that result in polluting inputs can adequately form taxing circumstances to the microbiome thereby encouraging selective pressures to alter the resistome (Laxminarayan et al. 2013). This would in turn cause a change in the survival mechanisms of the bacteria through co-selective gravities which would ultimately affect their vulnerability to antibiotics.

The progress of antimicrobial resistance (AMR) presents a vital contributor to the environmental imbalance and habitat-based health effects. But these pathogens do not just advance from exposure to antibiotics, they are also exposed to natural and anthropogenic settings in their surroundings. As an endurance mechanism, these pathogens attain genes as a way to counterattack the stressors (Liao et al. 2020). Any acquired or established resistance traits that have been effective in their survival becomes established in coming generations and intensifies the spread of ARGs in a

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_6

solitary populace. There are also instances where ARGs can be transferred to other bacteria through horizontal gene transfer (HGT). The danger here is that the inheritor bacteria could be pathogenic. This highlights the likelihood that strained bacteria could activate genetic exchanges, which may then lead to increased antibiotic resistance.

Thus, exposure to antibiotics in the natural habitat and surrounding environment can lead to bacteria developing resistance towards antibiotics as a natural survival mechanism (Ribeiro da Silva et al. 2020). It has been observed that antimicrobial resistance has also been reported in other microorganisms such as fungi, viruses, and some parasites, which as a whole would be identified as resistant organisms (WHO 2012). All these organisms together are responsible for antimicrobial resistance in the environment.

6.2 Prevalence of Antimicrobial Resistance (AMR) in Environment

Antimicrobial resistance (AMR) is a grave worldwide civic health challenge. There are a variety of problems that can arise from antibiotic resistance in human pathogens, such as treatment and diagnostic failure, extend the period of disease and hospital stay, and aggravate mortality rates, incurring great human and financial costs to society (Redfern et al. 2020). The prevalent and increasing use of antibiotics and antimicrobial agents in human health care, veterinary science, animal husbandry, horticulture, and even in the household environment have heightened the competitive selection and spread of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Davenport et al. 2020). The imminent part the environment plays, specifically terrestrial and aquatic environment in the transmission trails of ARB and associated ARG, has been the subject of much recent debate and attention (Aarestrup and Woolhouse 2020). Almost all of the general populace's actions, including those of everyday life, health care, and agriculture, generate waste that includes various levels of antibiotics (and other pollutants), ARB, and ARG. These wastes are eventually discharged into the environment. Point sources, defined as "any single identifiable source of pollution from which pollutants are discharged" (Bueno et al. 2017), embody a significant contribution to this discharged effluent. When exposed to the environment, these ARBs and ARGs stance great health hazards to humans as well as animals. They can remain in the environment or spread over a vast area of land and water (Swift et al. 2019). In environmental fortes, ARGs can increase clonally when a bacterial cell containing an ARG divides or can be transferred between bacterial cells through horizontal gene transfer (HGT) (Deng et al. 2020). Even though there is an increase in the incidence of reports and research indicating AMR in multiple natural habitat zones, including water, soil, sediment, and wildlife, the comparative input of particular anthropogenic activities and sources that contribute towards the presence of ARB and ARGs in the environment is an area of debate and dissent (Lübbert et al. 2017).

6.2.1 Occurrence of AMR in Indian Environment

Antimicrobial resistant pathogens and their genes have been reported from various water sources of India. The foremost source of this contamination is pharmaceutical waste waters and hospital effluents that are discharged into the water bodies without appropriate treatment. The two largest rivers of India, Ganges and Yamuna and its tributaries and distributaries, extend across a huge area of land and collects several sewage and effluent inputs having varying and lethal concentration of drug-resistant bacteria. The rate of extended spectrum beta-lactamase (ESBL) producers was reported to be around 17.4% among Gram-negative bacteria isolated from these north Indian rivers.

Resistance genes like blaNDM-1 and blaOXA4823 were also detected (Marinescu et al. 2015). Of the three E. coli isolates from the south Indian river Cauvery in Karnataka, 100% were resistant to third-generation cephalosporin. The groundwater and surface water that are used for drinking and other household and recreational purposes have been reported with 17% rate of E. coli, resistant to third-generation cephalosporin, in central India, 7% in north India (Kashmir), 50% in east India (Sikkim), and 100% in south India (Hyderabad) (Taneja and Sharma 2019). The samples that were collected for carrying out these studies were procured from various water sources like rivers, lakes, hand pumps, open wells, and tube wells.

6.3 Point Source of Antimicrobial Resistant Microbes

The various point sources for the emergence of antimicrobial resistance in the environment are represented in Fig. 6.1.

6.3.1 Antibiotic Manufacturing Industrial Effluent

Reports have shown that even after guidelines and various other measures issued by the responsible authorities, a number of pharmaceutical companies continue to release antibiotics into the environment on a massive scale through the wastewater that is being generated from their production plants. In spite of this, the past few years have witnessed pharmaceutical industries trying to make amendments and policies for reducing and monitoring antibiotic waste. This commitment and improvement have been taken up as a matter of prime urgency in the Industry Roadmap presented at the Davos Forum in 2016 (Tell et al. 2019).

Hyderabad is a city in India with a lot of pharmaceutical industry. When researchers analyzed water in villages surrounding Hyderabad's industrial suburb Pattancheru, antibiotics were found in every village where samples were taken (Lübbert et al. 2017). More than 10 years ago it was reported that effluent from drug manufacturers that are released into the water contains extremely high levels of antibiotics. In some places concentrations of antibiotics were found to reach up into

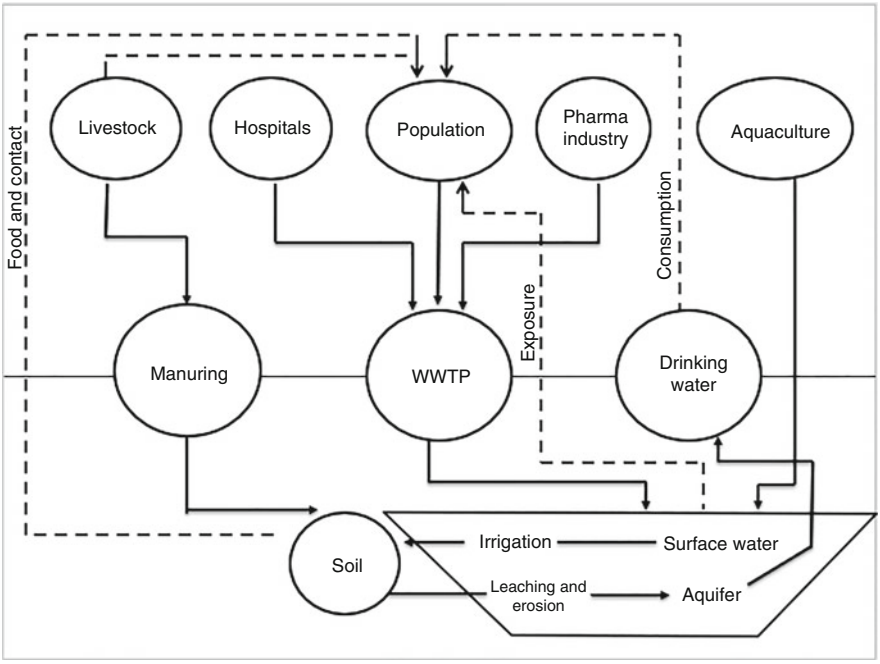

Fig. 6.1 Various point sources for the emergence of antimicrobial resistance in the environment

the mg/L range, higher than the concentration you find in the blood of patients taking antibiotics.

Researchers found that the industrially polluted sites stand out as extreme when it comes to housing antibiotic resistance genes (ARG). It has also been shown that resistant environmental bacteria are efficient in sharing their resistance genes, for instance, with *E. coli* through HGT. The chance that resistance genes present in bacteria found in the natural surroundings would be transferred to pathogenic bacteria may be limited, but as the variety and quantity of antibiotic resistance genes in the environment increases, so does the risk that it will occur (Pal et al. 2015).

One of the major obligations made by pharmaceutical companies is the Antibiotic Manufacturing Framework that was formed by the AMR Industry Alliance. The AMR Industry Alliance is introduced by the International Federation of Pharmaceutical Manufacturers & Associations (IFPMA), and it brings together various pharmaceutical companies, diagnostics, and biotech companies to jointly work towards an antimicrobial resistance-free environment (Jenner and Kowalski 2015). It is formulated along the lines of the Industry Declaration on AMR at the World Economic Forum in Davos in 2016, and a roadmap adopted by a number of pharmaceutical companies at the United Nations High-Level Meeting on AMR, also in 2016 (Sciarretta et al. 2016).

In the Roadmap, the reduction of the environmental impact from production of antibiotics by pharmaceutical companies was set as a top priority by the industry itself. The Antibiotic Manufacturing Framework is the outcome after 2 years of work. While the proposed framework and guidelines recognizes the specific problems of antibiotics in comparison with other pharmaceuticals in the environment, its major focus is on the production process and pharmaceuticals as an entire entity, and because of that there is a lack of specific focus on limiting antibiotic resistance. Restrictions and limitations on the release of antibiotics can effectively be implemented by local responsible bodies, but to date specific regulations on such emissions have rarely been executed (Daughton 2016).

Despite the described limitations and concerns, the Common Antibiotic Manufacturing Framework should be considered as a step in the right direction. But the willingness among pharmaceutical and other stakeholder companies to agree, sign up, and work for it has so far been minimal (Manuscript et al. 2016).

A substitute way to progress and uplift antibiotic production sustainably is by inculcating criteria in procurement (WHO 2012). In a few developed countries companies are required to report to the responsible government authority when registering a new pharmaceutical product on the market and to specify where and by whom the active pharmaceutical ingredient (API) is being produced. This way countries would be aware of where the medicines and the APIs are produced and procured from, even though they may not be fully aware of the environmental impact of the release of these active pharmaceutical ingredients (Fortunak et al. 2014).

6.3.2 Municipal Wastewater Treatment Plant

Discharged water from municipal wastewater treatment plants and the sludge that it contains provide favorable habitat for the housing and spreading of ARGs. It consists of a variety of consortia of bacteria, minerals, and antimicrobial resistance-causing agents, for existence, gene transmission (Santajit and Indrawattana 2016) and plays a role in dispersing resistant pathogens in both water and land environment. These ecosystems provide favorable surroundings for the antibiotic resistant bacteria to find a way of reentering the food chain of humans through drinking water, domestic water uses or recreational contact, or indirectly, by consuming dairy, poultry, and vegetables that have been reared or bred using such contaminated water (Lewis 2020).

The sewage treatment plants that are traditionally constructed function primarily to retain solid matter. This allows immense amounts of pathogens to be discharged into the water bodies. The least efficient performances in controlling and managing pathogenic cells were identified among the old sewage treatment plants that have low treatment efficiency and those collected in drizzly weather as the runoff water can play a major role in the doubling or even tripling of the day-to-day effluent quantity that is being generated for treatment thereby drastically bringing down the sedimentation, retention, and purification time (Zahmatkesh and Pirouzi 2020). This would invariably lead to large quantities of biosolids being discharged into the water

bodies. The secondary usage of sludge on fields, fallow lands, and pastures as fertilizers and growth promoters is another important gateway of AMR into the environment (Antonkiewicz et al. 2020).

Lethal and inhibitory quantities of antimicrobials and disinfectants which are at times found in antibacterial hygiene products are at times found in the sewage bulk and this might in turn encourage the development of antimicrobial-resistant bacteria or even horizontal gene transfer between pathogens present in the WWTP tanks (Meza et al. 2020).

The presence of such large quantities of multidrug-resistant bacteria in urban and suburban sewage and sludge signifies that these pathogens are not being prohibited from entering the natural environment by conventional treatment mechanisms established in WWTPs. But the ultra-violet light irradiation in the ultimate stage of tertiary treatment of the effluent has the potential to cause a momentous decrease in the number of resistant bacteria that is being discharged into the aquatic environment (Sharma et al. 2016).

6.3.3 Hospital Effluent

The emergence of AMR in clinical, health, and diagnostic settings is due to the contribution of human medicine, its impact on medical-environmental interaction, and unregulated distribution of antibiotics to outpatients (Ferri et al. 2017). This leads to exposure to sublethal and sometimes lethal concentrations of antibiotics in patients as well as general public. Various factors other than environmental-based exposures have also been reported to be the causative agent for AMR in clinical settings. The consequence of the development of AMR in the general populace includes therapeutic and diagnostic fiasco, extended hospital admission, increased cost of alternative treatment option, and admittedly higher mortality rate. The control on antimicrobial drugs (AMD) administration is not very stringent in some countries and these antibiotics are being sold as over-the-counter (OTC) drugs, individuals with even mild infectious symptoms engage in self-medication. Another major cause for the prevalence and development of antimicrobial resistance is self-medication. Antibiotics that can be orally administered are more used in self-medication than parenteral leading to several complications along with the rise of AMR (Mehmood et al. 2016).

Thus, the emergence of resistance induced by introduction of ARGs in the soil, sewage water discharges, and in the hospital environment is contributing to the upsurge in the level of threat of AMR to public wellbeing. The centers for Disease Control and Prevention has identified the following noted organisms as posing grave concerns: pan-drug-resistant (PDR) or extended spectrum drug-resistant (XDR) *Acinetobacter* spp., drug-resistant *Campylobacter* spp., fluconazole-resistant *Candida* spp., extended spectrum β-lactamase- producing *Enterobacteriaceae* (ESBLs), vancomycin-resistant *Enterococci* (VRE), multidrug-resistant *Pseudomonas aeruginosa*, drug-resistant Non-typhoidal *Salmonella* spp., drug-resistant *Salmonella*, methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant

Streptococcus pneumoniae, total drug-resistant *Mycobacterium tuberculosis*, etc. (Aparecida et al. 2020).

6.3.4 Other Types of Sources

In addition to the abovementioned point sources some other less prevalent but equally grave sources of AMR in the environment are as follows:

6.3.4.1 Wastes from Animal Farms

Antimicrobial-resistant (AMR) bacteria, which also include multidrug-resistant pathogens, have been reported to be present in animal waste-based fertilizers that are produced at food-producing animal farms (Coeffic et al. 2020). Emerging resistances are due to the selective pressure that the antimicrobials exert that are frequently found in food animal production systems. Antimicrobial-resistant bacteria can also be exposed into the environment through biosolids that might be utilized to fertilize agricultural land (Ferri et al. 2017).

6.3.4.2 Aquaculture

Antimicrobials are used across the globe in aquaculture and fisheries field, particularly in intensive breeding organizations, to control rampant and potentially lethal diseases. These are generally administered in feed along with nutritional supplements. Deliberations are in place questioning if the combined use of antimicrobial agents in aquaculture presents a significant portion of use in all food animals or not. But there is enough gravity to the concern that its use, if not followed sustainably, could taint the environment and cause resistance development in pathogenic organisms (Defoirdt et al. 2011).

6.3.4.3 Crop Pesticides

Antimicrobials are frequently used around the world as pesticides to manage blithe and crop diseases (Shitole 2020). If left without treatment these diseases can prove to be very hard to control and extremely destructive which would seriously impact the income from farms. Even though added research is required to fix the problems that are faced by the society at large due to usage of antimicrobial-based pesticides, explicit apprehensions are present for human health where antimicrobial pesticides are either similar to or are the same as that of antimicrobials used in human health care (Sievert et al. 2013).

6.4 Analytical Techniques for the Detection of AMR

6.4.1 Phenotyping Methods

Globally, Public health is under threat due to the emergence of antimicrobial resistance in pathogens. Each year, over 2 million people were infected with antimicrobial-resistant microbes, out of which nearly 23,000 people die (Sievert et al. 2013). Antimicrobial resistance in bacteria from wastewaters arise due to various factors. In the external environment, misuse of antibiotics as feed additives to increase meat and milk production, the use of chemical fertilizers in agriculture and aquaculture, pollution from pharmaceutical industrial plants, residual antibiotics excretion from humans and animals ending up in rivers, lakes, soils, and even food products such as milk and meat can all lead to sublethal concentrations of antibiotics. Due to the foretold factors, a small subpopulation of AMR microbes emerges, which is challenging to detect and treat thereby causing untreatable AMR infections (Lyu et al. 2018). Disc and well diffusion test methods are primitive phenotyping methods for the detection of AMR microbes. Eventually, they lead to the rise of advanced tests like Double Disc Synergy Tests, Imipenem-EDTA Synergy Test, D-Test, and CCCP Test.

6.4.2 Genotyping Methods

The increased demand and usage of expensive and alternative antimicrobial agents leads to significant widespread antimicrobial-resistant (AMR) strains. It is essential to control the evolution and widespread epidemic and endemic Antimicrobial-resistant strains. Identification of these infectious pathogenic strains and their genes facilitate the detection and control of these AMR strains. Genotyping methods have been under development for the past four decades, but there is a significant rapid development from the past decade, which facilitates us to detect AMR strains and their genes accurately. Primitive technologies like hybridization are based on the binding of rendered single-strand DNA with the single-stranded probe. Even though it was a primitive technology, it paved the way for the development of other advanced applications like molecular labeling, molecular probing, DNA array, and DNA chips (Fluit et al. 2001). Despite its enormous potential and application, it is still limited by its high cost (Head et al. 1999). Current technologies such as DNA microarrays facilitate the detection of several thousand genes simultaneously. Despite this advantage, this technology is limited by time, the requirement of template for each gene, design, and synthesis of specific gene primers, synthesis, and purification of PCR products (Frye et al. 2006). In the current decade, the cost of sequencing and identification of AMR genes has lowered due to the development of advanced online gene database tools such as NCBI nucleotide database, which provides sequence data to build BLAST integrated ResFinder database. ResFinder is an emerging technology, and it must be continuously updated to include emerging novel AMR genes allowing it to identify virulence genes and species, pangenome

analysis, and phylogenetic analysis based on single-nucleotide polymorphism. Despite its multiple advantages, the original ResFinder does not detect resistant genes in multiple copies. To detect the broad spectrum of AMR genes, a new bioinformatic tool "ARG-ANNOT" has been invented which is highly desirable for the detection of AMR genes through point mutations in chromosomic target genes thus supporting outbreak inspection, source tracing, therapeutic medical investigations, epidemiological monitoring, and far more research (Zankari et al. 2012). The utility of online and bioinformatic tools provides us vital information about various AMR genes. Regardless of its crucial role, they are still under development, and it needs to be easily accessible to all researchers. International and national level databases developed in the future for the efficient detection and control of widespread AMR strains.

6.5 The Fate of AMR during Wastewater Treatment

In general, wastewater from different sources is treated in a wastewater treatment plant (WWTP) through three different stages: primary, secondary, and tertiary treatment. The primary unit operations remove the solids in the wastewater. Most of the organic components of the wastewater are removed through secondary treatment, where biological or chemical-based treatment methods are used. The advanced treatment processes like UV, Ozone, Chlorination a Fenton's collectively called tertiary treatment process are used to remove leftover organics from secondary treatment (Wagner and Loy 2002). At each stage of the treatment process, the bacterial population changes significantly, including antimicrobial-resistant microbes (Guardabassi and Dalsgaard 2002; Huang et al. 2012).

Even though there is not enough literature to state that antimicrobials in wastewater lead to the emergence of AMR bacteria, their presence in wastewater is much more significant than the surface domestic water (Huang et al. 2012; Schwartz 2003). Certain literature have reported that the wastewater treatment plant's environmental conditions are much more favorable for the proliferation of AMR bacteria thereby increasing the risk of AMR gene transfer to non-AMR microbes (Poté et al. 2003). The schematic representation of the occurrence of AMR microbes in the wastewater treatment plant is provided in Fig. 6.2.

6.5.1 AMR in Primary Treatment Methods

The primary treatment of WWTP is a type of physical unit operation method where its objective is to remove insoluble solids by physical unit operations like sedimentation and floatation. Roughly, 25–50% of Biological Oxygen Demand (BOD), 50–70% of the total suspended solids (TSS), and 65% of the oil/lipid content are removed during primary treatment (Sonune and Ghate 2004). The effluent released from the primary clarifiers mainly contains colloidal solids, dissolved salts, organic, and inorganic components. Even though most of the solids are removed from the

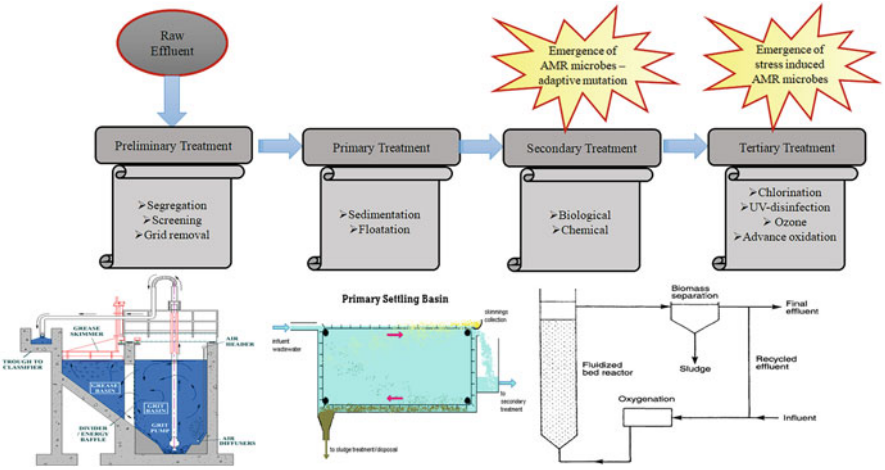

Fig. 6.2 Schematic representation of the occurrence of AMR microbes in the wastewater treatment plant

effluent, the active pharmaceutical ingredients and residual pharmaceutical products from the pharma industries; the pharmaceutical residues from human excreta; unused, expired, or discarded drugs; Various chemicals from various industrial processes and laboratory wastewater increases the chances of arousal of AMR microbes. The physical sedimentation processes technically remove these active compounds from the wastewater but not from the environment. Eventually, these active compounds one or another way leads to the arousal of AMR microbes. Therefore, there is a need for understanding regarding primary physical treatment and its effect on the environment. New novel primary treatment methods should be applied in WWTP to destruct but not remove these active compounds from the environment.

6.5.2 AMR in Secondary/Biological Treatment

After removal of the major load of pollutants, the effluent is released from the primary clarifiers to the secondary treatment unit. Depending on the type of effluent, the colloidal, dissolved organic, and inorganic solids containing effluent is treated chemically or biologically. Domestic wastewater usually does not contain complex pollutant loads. Therefore, indigenous microbes are efficiently used for the effective removal of pollutants. In the case of effluents from the industries, they cannot be treated as same as domestic wastewater. The complex toxic pollutant load containing industrial wastewater thus requires microbes from different environments that can tolerate, adapt, and degrade the complex toxic pollutants to simpler nontoxic forms. Even though there is not enough literature to state that antimicrobials in wastewater lead to the emergence of AMR microbes, their presence in wastewater is much larger

than the surface domestic water (Huang et al. 2012; Schwartz 2003). Marinescu et al. (2017) stated that the presence of tetracycline-resistant microbes in all the units of WWTP thereby denoting they may be a key source for the origin of AMR microbes. Similarly, Munir et al. (2010) reported that five WWTP in Michigan contains a huge level of tetracycline- and sulphonamide-resistant microbes. Generally, the concentration and activity spectrum of toxic pollutants do not correspond to the level of presence of AMR microbes and their genes. Studies in European WWTPs reported the presence of vancomycin-resistant microbes even though vancomycin usage is limited in that area. Nevertheless, many works of literature hypothesized that these AMR microbes might arouse due to the mutations caused by the high concentration of active pollutants. Unfortunately, there is still a lack of insightment regarding the emergence of AMR microbes and their genes. *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter* spp., and *Enterobacteriaceae* are some of the resistant and multidrug-resistant organisms which may originate from various units of WWTPs, but their origin does not correspond to the antibiotic production and their usage. Furthermore, acquiring dependable data on antibiotic production and its usage is much more complicated, and their statistics fluctuate in each country (Alda et al. 2003; Kümmerer 2009).

6.5.3 AMR in Tertiary Treatment Methods

The objective of tertiary or advanced treatment is to achieve additional removal of organic and inorganic undissolved colloidal solids, nitrogenous oxygen demand, excess nutriment, and recalcitrant toxic compounds after the biological/conventional treatment. Generally, for domestic wastewater, after the biological treatment, the biomass is separated from the effluent as activated sludge, which is further utilized as natural fertilizers for gardens. However, this type of sludge disposal method cannot be followed in the industrial effluent treatment process due to the presence of AMR microbes. Therefore, certain pharmaceutical industries follow effluent disinfection methods for the inactivation of AMR microbes. To control human health risk, the disinfection of treated wastewater and recovered water is essential. Chlorination, UV, and ozone are some of the commonly used disinfection methods for industrial effluents. Among various disinfection methods, chlorination is a widely opted method in industries due to its affordable cost despite its harmful by-product production (Wang et al. 2007; Wu and Hu 2009). Typically, chemical treatment alone is not effective in the removal of AMR microbes (Adams et al. 2002), but the efficacy can be enhanced by the integration of biological and chemical treatment processes (Rico et al. 1995). Anh et al. (2008) published that the number of identified AMR microbes reduced by up to 99% after the disinfection process in WWTPs. A study by Guardabassi and Dalsgaard (2002) reported that the municipal wastewater had E. *coli, Pseudomonas* sp., and *Enterococcus* sp. showed a 95–99% reduction of bacteria, and the hospital wastewater containing resistant strains showed a 93.5–100% reduction of bacteria. Besides this elimination rate, Guo et al. (2020) reported that there was no reduction of tetracycline and sulphonamide-resistant

bacteria in the treated effluent. Even though chlorine is an effective disinfectant, certain literature reported that chlorine-induced antibiotic-resistant bacteria might be more resistant, and their mechanism is still unknown. Since the chemical-based disinfection process can induce resistance in microbes, many industrial wastewater treatment units have opted for UV light as a disinfecting agent rather than chemical agents. Despite the advantage of low cost and maintenance, U.V. disinfection efficacy is affected by turbidity, color, and quantity. Due to the foretold disadvantages of the UV, advance oxidation processes like ozone have also been used as a disinfecting agent, but their usage is limited due to the high cost. Moreover, the effluent release standards of EPA compel industries to adopt these advance disinfection processes for the wellness of our environment. Therefore, a clear understanding of disinfection processes is required, and new biological, chemical, and physical disinfection processes should be developed to reduce the emergence of AMR microbes and their gene distribution in our environment.

6.6 Strategies for the Tackling of AMR in Environment

There are a number of remediation strategies that can be adopted for preventing and bringing down the number of antimicrobial-resistant microbes including pathogenic organisms in the natural surroundings.

While introducing mitigation measures, it becomes vital to consider the applicable target, e.g., antimicrobial-resistant organisms that may be pathogenic to humans. The chief aim is to lessen human exposure to antimicrobial-resistant pathogens.

Some of the easily practicable and implementable mitigation measures for tackling AMR are as follows:

(a) Preventing water bodies from contamination with residues that may contain antibacterial agents is the foremost step in reducing the impact of AMR on the environment. This may be achieved by regulating and enforcing the number of antimicrobial residues discharged into the environment (Andleeb et al. 2020).
(b) Reducing the usage of antimicrobial-based medicines through improved animal health and hygiene practices would be very effective in reducing the contamination of animal wastes with antimicrobial residues and AMR bacteria (Coeffic et al. 2020).
(c) Efficient treatment of municipal and hospital wastes to reduce and eliminate residual antimicrobials would go a long way in mitigating environmental contamination. Since most waste treatment strategies have not been designed specifically to address antimicrobial residues, their effectiveness to manage these residues is highly inconsistent. A more effective tactic would be required to overcome the challenges of limited or absent waste treatment facilities and standard operating procedures (Acharya et al. 2019).
(d) Another field of improvement would be to increase the present limited awareness. Building new and improved infrastructure and to strengthen weak or poorly enforced regulations and guidelines. These challenges are especially

marked in low- and middle-income countries (LMICs) and require pressing consideration (Al-haboubi et al. 2020).

(e) Providing better and efficient municipal wastewater collection possibilities that would interrupt fresh fecal matter from inflowing into the whole collected wastewater water (e.g., portable toilets, septic tanks, soakaways).

(f) Establishing sewer assortment systems that would convey the wastewater collected from the community as well as other sources to a centralized treatment facility which comprises of different stages of treatment and management namely primary, secondary (biological), and tertiary treatment (Terada 2019).

(g) Incineration is a decisive way of eradicating all antimicrobial activity thereby reducing the cases of the upheaval of antimicrobial-resistant pathogen. In spite of it being an effective strategy, it has one major disadvantage that it is an energy-consuming process and hence not always economically feasible (Aparecida et al. 2020).

(h) A better alternative would be to use an enzymatic method that utilizes particular enzymes that degenerate chemicals and other harmful substances in the wastewater like high-strength antimicrobial agents. This way of treatment does not use live microorganisms hence the problems that arise due to toxicity can be avoided. Since the enzymes get degraded naturally it also does not pose the risk of downstream contamination (Suzuki et al. 2020).

(i) Another method that can be adopted is targeted and specific treatment at particular and most polluting point sources like wastewater from hospitals. This would prove as an important step towards minimizing the load on existing wastewater treatment plants. This approach would help in reducing the volume of wastes that have to be managed at a time in WWTPs which would in turn allow implementation of efficient and sometimes costly technologies at these point sources that are targeted in treating a particular kind of waste composition.

(j) A variety of legal, financial, and public incentives can minimize environmental pollution caused by the industrial discharges from pharma industries. These subsidies can be executed through the participation of various stakeholders, including responsible authorities, the general populace, media and journalists (by creating awareness), international organizations (e.g., WHO), etc.

(k) It has been estimated that around 20–30% of antimicrobials that are dispensed from medical care facilities are being put to inappropriate use. Strict antimicrobial stewardship and preventing and withholding self-medication can reduce antimicrobial misuse which in turn would cause a decline in production thereby reducing the ill effects they have on the environment (Davenport et al. 2020).

(l) Even though quite a few developed countries are mindful of the ill effects of AMR and practice some primary level of environmental protection, more extensive and severe execution of these practices particularly aimed at plummeting antimicrobial residue pollution in the environment would go a long way towards decelerating the development of AMR—which is an urgency for all countries in the context of the Global Action Plan on AMR.

6.7 Future Perspectives and Research Recommendations

CRISPRs (clustered regularly interspaced short palindromic repeats) are the innate immune defense system of primitive and modern bacteria. Due to its cytotoxic antimicrobial activity against resistant plasmids, they can be a crucial application in WWTPs (Bikard et al. 2014; Hsu et al. 2014; Sorek et al. 2013). The schematic sketch of disinfection technologies for AMR microbes in WWTPs—as Future directions is represented in Fig. 6.3. In detail, merging with nanotechnological delivery techniques, such as nanocapsules, the CRISPR-Cas system can be effectively used to selectively target and eliminate various groups of medical pathogens that pose a severe threat to human and animal health. Metallic nanoparticles, such as silver nanoparticles associated with disinfection processes, could be a promising technology. Silver nanoparticles irreversibly bind to the cell wall of the bacteria thereby changing electrostatic potential. Moreover, they may even penetrate the protoplasm, interact and deactivate the enzymes and genetic matter thereby bypassing the resistant mechanism of bacteria. However, despite the high production cost, the recoverability and reusability efficiencies are very low thus limiting the usability in industries. Instead of high-cost metals like silver, various combinations of other metals should be developed and tested for antimicrobial activity so that industries can effectively adapt them in their effluent treatment processes. River Ganga is one of the sacred rivers of India, but the river is highly contaminated due to excessive human usage and disposal. Even though it is highly contaminated, the bacterial population is naturally controlled by bacteriophages. Bacteriophages are viral particles that selectively infect and eliminate various groups of bacteria. Since most of the bacteriophages are bacterial strain-specific, they cannot be directly employed in wastewater treatment units. Instead, recombinant bacteriophages, coupled with CRISPR technology, should be developed specifically to eliminate

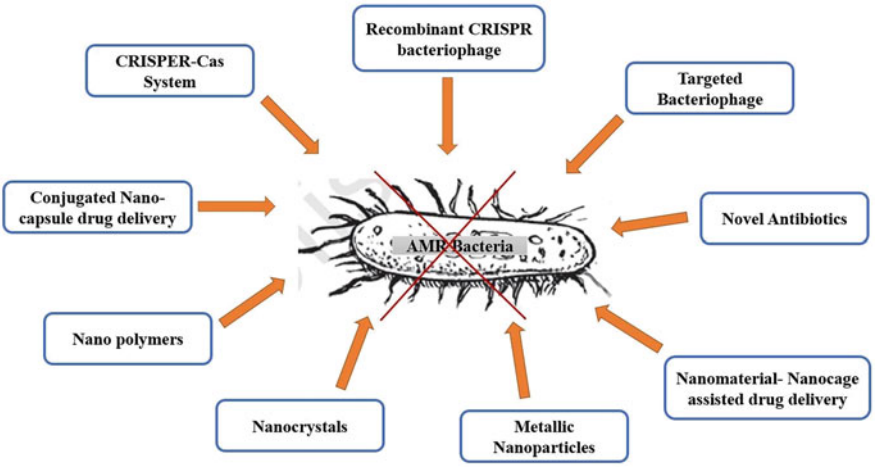

Fig. 6.3 Disinfection technologies for AMR microbes in WWTPs—Future directions

resistant bacterial strains. New novel MDROs disinfection technologies should be structured, developed, and made as readily available so that conventional disinfection methods can be replaced; thereby, the emergence and escape of MDROs associated pathogens can be eliminated/restricted from the environment.

6.8 Conclusion

The research resources which we analyzed above showed that AMR microbes and their genes originate from treatment units of wastewater treatment plants (WWTP), but those data do not prove that AMR microbes and their genes arose only due to excessive antibiotic usage. Moreover, the data varies from country to country each year. A deep understanding of AMR genes and their fate in WWTP is required. The mechanisms and health effects of AMR genes transfer from WWTP to the environment should be evaluated and studied. Even though industries use various technologies for effluent treatment, their operation efficiencies are very low. New innovative technologies with high efficiencies should be implemented to remove AMR microbes. Eventually, new antibiotics will emerge in therapeutic medical treatment, but they will not cease the emergence, spread, and pathogenesis of AMR microbes. Thus, extensive research on disinfection technologies, optimal medicine production, usage, and disposal in the future will help us to protect ourselves and the environment from AMR microbial hazards.

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The Role of Wastewater Treatment Technologies in Municipal Landfill Leachate Treatment

7

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7.1 Introduction

The increase in municipal solid waste (MSW) generation has resulted in a significant problem to the society due to the increased environmental risk during landfilling (Bai and Sutanto 2002; Chen et al. 2010; Luo et al. 2019a). Municipal landfilling is a widely used, inexpensive method for the management of MSW when compared to previously adopted technologies such as composting and incineration (Renou et al. 2008; Luo et al. 2017). It is claimed that around 95% of the total global MSW generated is disposed off in landfills (Gao et al. 2014). Disposal of MSW over the land usually originates risk, due to the disposing of hazardous wastes which pose a significant risk to the environment. Although currently MSW disposal is carried out in highly engineered modern landfill facilities, the generation of landfill leachate (LL) is still a major problem for modern landfills, due to the significant risk of contaminating soil, surface, and groundwater (Kjeldsen et al. 2002; Yan et al. 2015; Luo et al. 2019b). The composition of leachate is highly dependent on the age of the landfill, as the leachate parameters (Ammoniacal nitrogen, BOD, BOD/COD ratio) significantly changes as the landfill tends to stabilize (Kjeldsen et al. 2002; Kulikowska and Klimiuk 2008). Various physicochemical and biological methods, as well as combination of both, have been adopted in order to fulfill the stringent discharge standards in different parts of the world (Wiszniowski et al. 2006; Silva

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et al. 2017; Torretta et al. 2017). Physicochemical methods have been used for the removal of refractory organics as well as a refining step for the biologically treated leachate (Kurniawan et al. 2006; Chys et al. 2015).

Biological aerobic and anaerobic methods are considered to be simple, reliable, and highly cost-effective and are used for the treatment of bulk leachate containing a high concentration of organics (Miao et al. 2019). In the aerobic biological treatment, microorganisms result in the breakdown of organic compounds to carbon dioxide and sludge and to biogas (carbon dioxide and methane) under anaerobic conditions. Furthermore, combination of physicochemical and biological treatments can accomplish acceptable treatment efficiencies of contaminants such as ammoniacal nitrogen, heavy metals, refractory organics, BOD, and COD (Hassan et al. 2017; Pastore et al. 2018; Gomes et al. 2019). Several studies, exercising the use of physicochemical, biological, and combined treatments for the treatment of landfill leachate have been studied worldwide in the last decades. However, not many endeavors have been made to acquire a detailed overview of all the treatment methods in terms of optimum conditions for the maximum removal of ammoniacal nitrogen and COD for the landfill leachate. This chapter covers the state-of-art treatment technologies, technical applicability, and efficiency of all available physicochemical, biological, and combined treatments for the LL treatment. This chapter shall significantly contribute to the future sustainable treatment of landfill leachate.

7.2 Landfill Leachate Treatment

Treatment of LL in past has been carried out using three major groups: (1) physicochemical processes; (2) biological processes (aerobic and anaerobic), and (3) a combination of physicochemical and biological processes (Wiszniowski et al. 2006; Torretta et al. 2017; Renou et al. 2008).

7.2.1 Physicochemical Processes for LL Treatment

Physicochemical treatments are considered to be the most appropriate for the removal of refractory compounds from the leachate. Various physicochemical processes are employed in the treatment of LL for the removal of COD, BOD, NH3-N, and/or heavy metals. In recent years, various studies have been carried out worldwide on the performance of different physicochemical methods for the treatment of SL. Various physicochemical methods and their technical applicability, and performance are discussed below. The combined effect of different processes and their advantages and limitations are also discussed.

7.2.1.1 Coagulation-Flocculation

Coagulation-Flocculation is a two-step process employed for the removal of organic compounds present in LL that are usually nonbiodegradable (Amokrane et al. 1997; Diamadopoulos 1994; Urase et al. 1997). It is an ion-dependent process where the

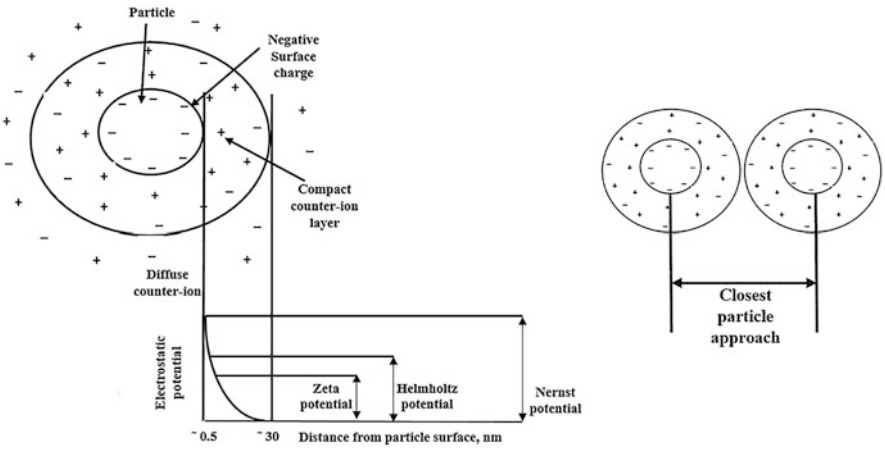

Fig 7.1 Schematic mechanism of coagulation process in landfill leachate treatment

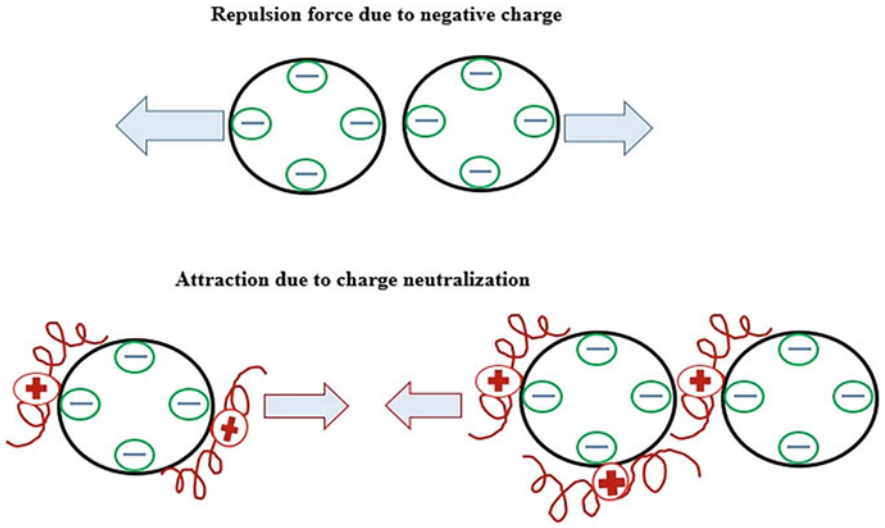

Fig 7.2 Schematic mechanism of flocculation process in landfill leachate treatment

colloidal particles are destabilized by the addition of a coagulant. The colloidal particles present in the leachate are negatively charged and thus remain suspended. Upon the addition of a positively charged coagulant, these suspended particles get attracted to the coagulant, forming clusters and this process is called coagulation (Fig. 7.1). In order to enlarge the size of those particles, coagulation is usually followed by flocculation where there is the formation of bulky floccules thus settling occurs more rapidly as represented in Fig. 7.2 (Cheng et al. 1994). This process can

be employed for heavy metal removal from LL and usually involves pH adjustment as the first step which is followed by the addition of a coagulant such as alum or ferric salts and then flocculating agent (Tatsi et al. 2003). Examples of coagulation-flocculation process include the removal of heavy metals using FeCl3 and the removal rate was found to be more at pH 9.0 than pH 4.0 which was the actual pH of that leachate. Thus, it demonstrates that the removal of heavy metals by precipitation is more effective in basic conditions than the actual pH condition (Urase et al. 1997). A comparative study between the effectiveness of alum and FeCl3 was carried out and the results showed that ferric chloride gave a higher removal of organic compounds (55%) than alum (43%) thus proving that ferric chloride is more efficient than alum in the coagulation process (Amokrane et al. 1997).

Overall, the coagulation-flocculation process is effective for the removal of heavy metals and organic compounds from the LL. Lime can also be used as a coagulant for better COD removal. High cost due to the consumption of chemicals, generation of sludge as a result of the settlement of suspended particles, and sensitivity to pH are the drawbacks associated with this technique. pH, velocity gradient, and settling time have to be noted carefully since they play key roles in the settlement of colloidal particles (Kurniawan et al. 2006).

7.2.1.2 Ammonium Stripping

It is the most extensively used technique for the removal of ammoniacal nitrogen (NH3-N) from LL (Calli et al. 2005; Cheung et al. 1997; Diamadopoulos 1994; Marttinen et al. 2002; Ozturk et al. 2003). Here, the leachate containing NH3-N is allowed to interact with the air phase in a counter-current flow in a stripping tower where the NH3-N from the leachate is transported into the air and is then adsorbed into a strong acid like H2SO4. It can also be directly fluxed into the ambient air (Bonmatí and Flotats 2003). Ammonium stripping is considered as the most effective technique for NH3-N removal.

In a case study, nanofiltration and ammonium stripping were applied for the treatment of a YL from Finland (Bonmatí and Flotats 2003). When nanofiltration alone was applied, only 50% of NH3-N and 66% of COD were removed whose initial concentrations were 220 mg/L and 920 mg/L, respectively. But at pH 11, 89% of NH3-N and 21% of COD were removed by the application of ammonium stripping with the same initial concentrations and these results obtained agree with those obtained in another study where about 85% of NH3-N was removed by ammonium stripping from anaerobically pretreated leachate from Turkey (Oyaderi landfill) whose initial concentration was 1025 mg/L (Calli et al. 2005).

Thus, ammonium stripping is an efficient technique for the removal of ammoniacal nitrogen from LL and this can be followed by biological treatment for better COD removal. To enhance the removal of NH3-N, the pH of the leachate can be adjusted to basic conditions before the treatment. Being able to meet the ammoniacal nitrogen discharge standard using ammonium stripping alone is another advantage of this process (Bae et al. 1997). It is also more economical when compared to other techniques like reverse osmosis and nanofiltration (Kurniawan et al. 2006). Despite all the advantages, this technique has a few drawbacks. The major problem

associated with this technique is the release of ammonia gas into the air and its impact on the environment. Thus, there is a requirement for additional treatment of the gas with acids like hydrochloric acid or sulphuric acid which in turn increases the operational cost of the treatment process. Other limitations include the requirement for pH adjustment of treated effluent before discharge since the operation occurs in basic conditions, CaCO3 scaling of stripping tower during pH adjustment using lime and the difficulty in removal of lesser concentration of NH3-N (<100 mg/L) (Li and Zhao 1999; Tanaka and Matsumura 2002).

7.2.1.3 Chemical Precipitation

Chemical precipitation has been used for the exclusion of heavy metals, NH3-N, and nonbiodegradable organics from LL because of its simplicity and cost-effectiveness (Calli et al. 2005; Cecen and Gursoy 2000; Li et al. 1999; Ozturk et al. 2003). During the process of chemical precipitation, the ions dissolved in the mixture get converted into insoluble ions by chemical reactions. Mostly, metal precipitates in the form of hydroxide from the solution. Depending on the target removal magnesium ammonium phosphate (MAP), known as Struvite (for NH3-N) or lime (heavy metals) is used as the precipitant (Kurniawan et al. 2006).

Chemical precipitation using struvite was applied for NH3-N removal from anaerobically pretreated leachate from the Oyaderi landfill (Turkey) (Ozturk et al. 2003). Here, ammonia that was present in the leachate got converted into a nitrogen fertilizer such as urea (Eq. 7.1). Around 50% and 90% of COD and NH3-N have been removed whose initial concentrations were 4024 mg/L and 2240 mg/L, respectively. These results indicate that struvite can efficiently remove NH3-N from leachate than organic compounds. This is in agreement with the results of another study where struvite was used to decrease the ammoniacal nitrogen concentration in the leachate (Calli et al. 2005). Around 98% of NH3-N was precipitated at pH 7.5 along with 20% of COD removal.

$$\begin{array}{r} MgCl_2{\cdot}6H_2O + Na_2HPO_4 + NH_4{}^+ \leftrightarrow MgNH_4PO_4{\cdot}6H_2O \\ \downarrow +2NaCl + H^+ \end{array} \qquad (7.1)$$

7.pKs = 12.6 (25 °C)

The main advantage of using struvite as the precipitant is that if there is no presence of heavy metals in the leachate, the sludge produced from this process can be used as a fertilizer. However, biological treatment has to be carried out to lower the level of COD of the leachate (Li and Zhao 2001). In the case of the uptake of heavy metals like copper, nickel, manganese, lead, and iron, lime was found to be an effective precipitant (Cecen and Gursoy 2000). Besides the use of lime, adjusting the pH to basic conditions enhanced the level of metal precipitation. The limitations of this method include the requirement of high dose precipitant, sensitivity to pH and sludge generation, and further disposal of it (Kurniawan et al. 2006).

7.2.1.4 Membrane Filtration Tecnologies

Microfiltration (MF)

It is employed in the removal of colloidal particles whose size ranges from 0.05 to 1.0μm by a cross-flow at low pressure. Since the rate of retention was not significant and only 25–35% of the COD reduction was achieved, it is usually employed as a pretreatment for other membrane processes or in combination with chemical treatments (Fig. 7.3) (Abbas et al. 2009).

Ultrafiltration (UF)

In this process of selective fractionation using pressure of up to 10 bar, suspended solids and solutes weighing more than 1000 Da are concentrated. The infiltrate has salts and solutes of low molecular weight. Depending on the type of material used as a membrane, UF can be beneficial in the removal of molecules of macro size as represented in Fig. 7.3. It can be used employed to fractionate organic compounds and so can assess the molecular weight of those compounds present in the leachate. In addition, by analyzing the permeates of the membrane, knowledge about the nature and toxicity of the compound can be gained. It has also been stated that UF can be an effective pretreatment process for reverse osmosis. It can remove larger molecules that result to foul the membranes used in reverse osmosis. In combination

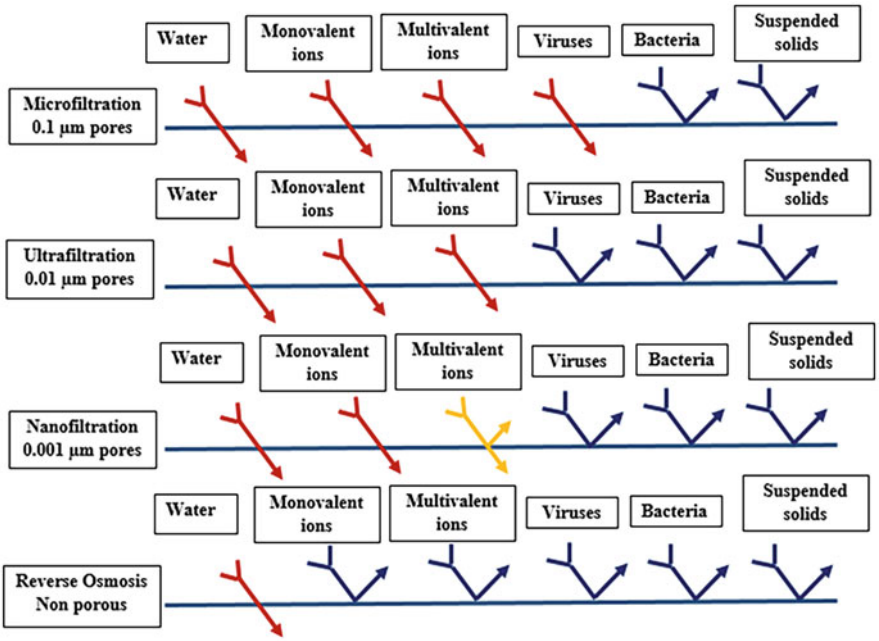

Fig 7.3 Schematic representation of membrane filtration process in landfill leachate treatment

with biological treatment, UF has been employed and successful treatment of leachate has been achieved (Abbas et al. 2009).

Nanofiltration (NF)

Nanofiltration has exceptional properties that ultrafiltration and reverse osmosis membranes do not have and thus have been employed in the treatment of LL (Linde and Jönsson 1995; Ozturk et al. 2003; Urase et al. 1997). Through the electrostatic interactions between the ions and the membranes, particles of molecular mass greater than 300 Da along with inorganic substances are removed in this process. The surface charges on the membrane reject the charged solutes smaller than the membrane pores along with bigger neutral solutes and salts which makes the process significant (Fig. 7.3) (Kurniawan et al. 2006).

The use of NTR-7250 for the heavy metal removal achieved 99% of removal whose original metal concentrations were 0.69 mg/L and 0.23 mg/L of Cr^{3+} and Cu^{2+}, respectively (Urase et al. 1997).

Nanofiltration was used for the treatment of anaerobically pretreated leachate (Oyaderi landfill) (Ozturk et al. 2003). Around 90% and 70% of COD and NH3-N have been removed whose initial COD concentrations were 3000 mg/L and 920 mg/L, respectively. The total operational cost was US$ 0.8/m^3 which made the process more efficient.

Thus, NF has established a reasonable treatment performance in the reduction of organic load with COD concentrations ranging from 900 to 3000 mg/L. Due to the presence of negatively charged groups in its membrane, NF can also effectively remove heavy metals. Monovalent and divalent ions dissolved in the solution can also be separated by the application of NF, the materials dissolved in the solution can be separated into monovalent and divalent ions as represented in Fig. 7.3. Unlike RO, NF has a looser structure that enables higher fluxes and lowers operating pressure during the treatment process.

Reverse Osmosis (RO)

Reverse osmosis is another physicochemical method applied for the treatment of SL that has higher flexes and can operate over a wide temperature and pH range. In RO application, the metal concentration is reduced by controlling the flow of metal cation-containing solvent into the membrane (Jenkins et al. 2003). Organic compounds, dissolved solids, and suspended/colloidal particles can be removed by the application of RO with a 98–99% rejection rate of organic and inorganic contaminants (Fig. 7.3) (Kurniawan et al. 2006).

Removal of dioxins like polychlorinated biphenyls (PCB), polychlorinated dibenzofurans (PCDF), and polychlorinated dibenzo-*p*-dioxins (PDPD) by the application of RO was studied and results showed complete removal of dioxins whose initial concentration was 2.35 mg/L. COD was completely removed and 98% removal of NH3-N was also removed whose initial concentrations were 97.4 mg/L and 33.7 mg/L, respectively (Ushikoshi et al. 2002).

The application of RO membrane during the treatment of YL from a landfill in South Korea, resulted in 96–97% COD and NH3-N removal whose initial

concentrations were 1500 mg/L and 1400 mg/L (Ahn et al. 2002). The results prove that RO can be used to enhance the treatment efficiency by the removal of nonbiodegradable organic compounds from LL.

A comparative study between RO and NF for the treatment of SL revealed that RO (99%) was more efficient than UF (52%) in the removal of COD with an initial concentration of 1780 mg/L (Peters 1998a, b). However, due to varying compositions, a combination of biological treatment and RO has to be carried out for the effective treatment of leachate.

There are various factors that govern the efficiency of the RO. The characteristic of the membrane used greatly affects the treatment performance on the basis of the removal of organic compounds and ammoniacal nitrogen. Charge, porosity, hydrophilicity, thickness, roughness, and the material used are the factors that affect the water passage through the membrane. Higher removal of organic compounds and ammoniacal nitrogen can be obtained by the use of membranes made of cellulose acetate or polyamide which can also work in a wide range of temperatures (5–35 °C) when compared to membranes composed of PVC.

It may be because of the high permeability and hydrophilicity of polyamide when compared to other materials like polyethylene-terephthalate and polysulphone. Other factors that have to be considered while choosing a membrane are the characteristics, pH, temperature of the leachate, nature, and concentration of components present in it (Alvarez-Vazquez et al. 2004).

Overall, RO is an efficient technique for the removal of both COD and ammoniacal nitrogen from LL. However, the main disadvantage of RO is membrane fouling, where, suspended or dissolved substances get undesirably deposited on the outer surface of the membrane (Choo and Lee 1996). Another limitation of RO is those small molecules that pass through the membrane have low retention time and also there is high energy consumption. It is also reported that about 60–80% cost of RO treatment accounts for energy consumption (Peters 1998b). Therefore, during the selection of treatment, affordability has to be considered to justify it as the solution.

7.2.1.5 Activated Carbon Adsorption (ACA)

Of all the treatment technologies discussed, adsorption is the most broadly employed method for the removal of toxic contaminants from LL (Abdul Aziz et al. 2004; Babel and Kurniawan 2004, 2003; Fettig 1999; Heavey 2003; Imai et al. 1998; Morawe et al. 1995; Wasay et al. 1999). In general, during adsorption, by means of mass transfer, a substance is transferred from the liquid phase to the surface of the solid and becomes bound by physical and/or chemical interactions. Significant attention has been given in the past few years to adsorption using granular activated carbon (GAC) and/or powdered activated carbon (PAC) in the removal of contaminants from wastewater due to its large surface area, high adsorption capacity, inherent physical properties, microporous structure, and surface reactivity (Kurniawan et al. 2006).

In a study, carried out in 1995, GAC was used for the removal of the level of COD and the results showed that 91% of COD was removed (Initial concentration: 940 mg/L). It was also found that along with film diffusion, the rate of adsorption

and the internal surface diffusion on the solid surface of the adsorbent greatly affected the kinetic rate of adsorption (Morawe et al. 1995).

GAC, granular activated alumina (GAA), and/or ferric chloride were used separately for the treatment of LL and of the three adsorbents examined, GAC was most effective in the heavy metal removal such as cadmium, chromium, manganese, lead, and zinc. About 80–96% of heavy metal at a pH range of 6–7.7 was removed with 2 g/L of GAC. It was reported that GAC adsorption was represented by Freundlich isotherm (Wasay et al. 1999).

A relative study for NH3-N removal from leachate with an initial ammoniacal nitrogen concentration of 1909 mg/L using GAC and/or lime was carried out (Abdul Aziz et al. 2004). About 40% removal was achieved using 42 g/L of GAC, whereas, with 52 g/L of lime, only 19% NH3-N was removed under the same concentration. Even though lime was less effective, it was more cost-effective when compared to GAC for the removal of NH3-N.

By varying the concentration from 0.2 to 10 g/L of PAC for the treatment of SL, it was found that 95% of COD was removed using 6 g/L of PAC whose initial concentration was 5690 mg/L (Diamadopoulos 1994). Here, Freundlich isotherm was applicable for adsorption and thus indicating the occurrence of multilayer adsorption on the surface of PAC (Imai et al. 1998).

Other than GAC and PAC, other locally available nonconventional materials such as industrial by-products or agricultural waste can be chemically altered and used as low-cost adsorbents (Babel and Kurniawan 2003; Kurniawan and Babel 2015). By converting waste into activated carbon which can be used in the treatment of wastewater there is an increase in the economic value. It also helps the industry in reducing the cost of waste disposal and also acts as a budget-friendly substitute for commercially available high-cost adsorbents (Babel and Kurniawan 2004; Heavey 2003). Coconut shell (Kurniawan and Lo 2009) and zeolite (Kargi and Pamukoglu 2004) are examples of low-cost adsorbents that are employed for the removal of COD from LL.

Overall, the usage of activated carbon (GAC or PAC) as adsorbent is a virtuous technique for the removal of nonbiodegradable compounds from LL. However, this cannot be applied for NH3-N removal since it is less efficient when compared to other techniques. With concentrations ranging from 940–7000 mg/L, around 90% COD removal was achieved. In spite of this, recurrent regeneration of activated carbon and high cost of GAC act as limiting factors for the treatment of LL.

7.2.2 Biological Processes for LL Treatment

Biological treatment also known as bioremediation of municipal LL involves the use of microorganisms such as bacteria, fungi, and some protozoa to degrade the organics present in the LL. Biological treatment is one of the most economical, efficient, and reliable method for the removal of BOD as the microorganism uses the organic matter present in the leachate as their carbon source and converts them into simpler, less toxic substances (Di Iaconi et al. 2006). Due to their growth,

adaptability to the given environment, and pliability, municipal LL treatment depend on microbes to degrade the organic compounds and produce clear effluent water by utilizing the ability of microorganisms to challenge the source of larger issues like degrading odor (Luo et al. 2019). They offer major advantages over alternative treatment strategies. The biological treatment process can be broadly classified into aerobic and anaerobic based on the requirement of oxygen by the microorganism used for the treatment (Renou et al. 2008).

7.2.2.1 Aerobic Biological Treatment Processes

The treatment of municipal LL under aerobic condition involves the use of aerobic microorganism that converts the complex organic substance into H2O and CO2 in the presence of O2. Aerobes use oxygen to oxidize the substrate, that is, the organic portion of the leachate to obtain the energy required for their metabolism. This process is known as cellular respiration in which oxygen acts as a terminal electron acceptor (Mohd-Salleh et al. 2020).

Aerated Lagoon (AL)

Aerated lagoons also known as aerated pond is an economical and simple leachate treatment system wherein the leachate is added to a basin and artificial aeration is provided to promote biological oxidation. It can be used for in situ treatment of the LL. In a study, it was found that the leachate with a low COD and high ammonium concentration of 1241 mg/L treated with four connected aerated lagoons lead to 75% and 80% removal of COD and Ammonium concentration, respectively (Mehmood et al. 2009). When a two-staged anaerobic/facultative lagoon system is used for leachate with COD of 5050 mg/L and 1670 mg/L of TN, removal efficiencies equal to COD 40%, BOD 64%, NH_4^+-N 77%, $NO3^-$-N 63%, TN 77%, P 42%, $SO4^{2-}$ 44%, Mn 44%, and Fe 30% was achieved (Frascari et al. 2004). In an aerated lagoon about 80–88% phenol can be removed (Orupõld et al. 2000).

The process of lagooning is not a satisfactory operation for leachate treatment as it is unable to meet the environmental regulations. One of the significant limitations of the process is that it is temperature-dependent which may hinder microbial activity.

Activated Sludge Process (ASP)

Activated sludge is a concoction of bacterial biomass which can utilize the organic matters present in the leachate and convert them into H2O, CO2, and minerals along with the generation of new microbial biomass via the aerobic respiration process (Fig. 7.4) (Luo et al. 2014).

Activated sludge process can be adapted for the co-treatment of municipal LL and sewage. When an anaerobically pretreated municipal LL with COD 270–1000 mg/L and $NH4^+$-N 53–270 mg/L is subjected to an activated sludge process with the addition of plastic carrier material in a laboratory-scale reactor with temperatures varying from 5–10 °C, the COD reduced to 150–500 mg/L, less than 7 mg/L BOD, and less than 13 mg/L $NH4^+$-N (Hoilijoki et al. 2000). In a research, the treatment efficiency of aerobic granular sludge and activated sludge systems for a young LL were compared and it was found that 99% of partial nitrification took place in

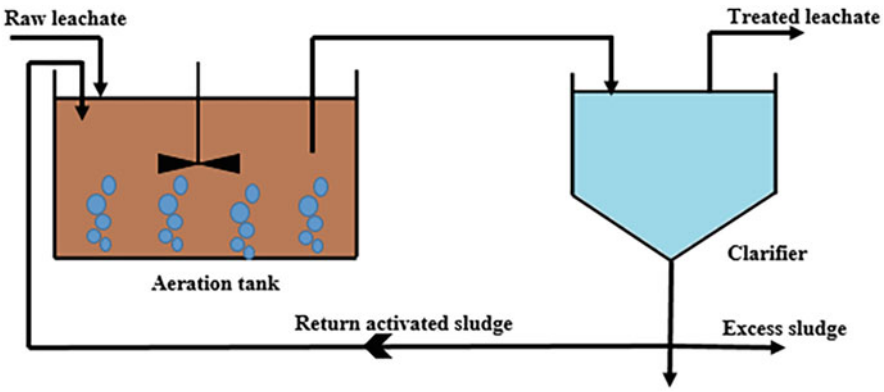

Fig. 7.4 Schematic diagram of activated sludge process in landfill leachate treatment

granular sludge and 77 ± 10% in activated sludge system. COD removal was also more efficient in granular sludge when compared to activated sludge. The removal of phosphorus was the same in both cases (Renou et al. 2008). Researches also show that the nitrification process during the activated sludge process can be improved with the mixing of powdered activated carbon into the activated sludge reactors (Özgür Akta 2001).

Although the activated sludge system is effective in eliminating the organic load and ammonia content in the municipal LL, this method has several downsides like generation of a huge quantity of sludge leading to investments in sludge disposal, high energy consumption, longer aeration time and inhibition of microbial growth due to high ammoniacal nitrogen concentration (Renou et al. 2008; Torretta et al. 2017). Hence, more efficient technologies should be developed for the removal of COD and nitrogen.

Sequencing Batch Reactor (SBR)

Sequencing batch reactor also known as a sequential batch reactor is a type of activated sludge system. The SBR is an array of tanks that operates based on a fill-and-draw mechanism. The SBR is operated in four steps, that is, the tanks are filled, aerated for a specific period, contents can settle, and finally the supernatant is decanted (Liu et al. 2005). The municipal LL can be treated through concurrent oxidation of organic matter and nitrification.

Researches show that the rate of removal of COD is reduced with an upsurge in the influent ammonium concentration when the leachate is treated with granular sludge SBR (Wei et al. 2012). In order to meliorate the performance of SBR for leachate treatment, ultrasonic pretreatment is carried out due to which 90% COD and 70% ammonia are removed (Neczaj et al. 2005). To completely utilize the organic load present in the leachate (COD/TN ratio = 1–4, $NH4^{+}$-N = 1000 ± 50 mg/L), a modified SBR that operates at anaerobic-aerobic-anoxic mode has been developed. When this was operated for 70 days, efficient nitrogen removal up to 10 mg/L was

achieved at a C/N ratio of 4 (Miao et al. 2015). When coagulation process, fenton system and SBR were combined, COD was removed up to 97.3% (100 mg/L), and 99% (3 mg/L) ammonia was removed (Li et al. 2009). When a PAC-SBR was operated with optimum conditions like 1 L/min of aeration and 5.5 h of contact time, 64.1% COD, 71.2% $NH4^+$-N, and color has been removed (Aziz et al. 2011).

Therefore, when compared to other leachate treatment technologies SBR process is more flexible for municipal LL treatment because of its high degree of variability in terms of both quality and quantity (Kennedy and Lentz 2000).

Rotating Biological Contractor (RBC)

Rotating biological contractor, also known as rotating biological filters, is an alternate to the traditional activated sludge process. They are made up of rotating discs that are fixed on a horizontal shaft (Fig. 7.5). A mechanical motor or a compressed air drive is used for the continuous rotation of the shaft. These are partially or completely submerged (Cortez et al. 2008), and it rotates as the wastewater flows through it. A biofilm is formed on the surface of the rotating disc by using biofilm-forming microorganism which oxidizes the organic matter present in the wastewater. The revolution of the disc facilitates the transfer of O2 to maintain the biomass under aerobic conditions. It also offers turbulence in the mixed liquor surface and simplifies the elimination of extra solid from the media (Patwardhan 2003; Rodgers and Zhan 2004).

A study investigated the feasibility of applying RBC system and anaerobic system for the treatment of municipal LL (Castillo et al. 2007) reported that about 65% removal of COD for an influent leachate 2500–9000 mg/L can be achieved.

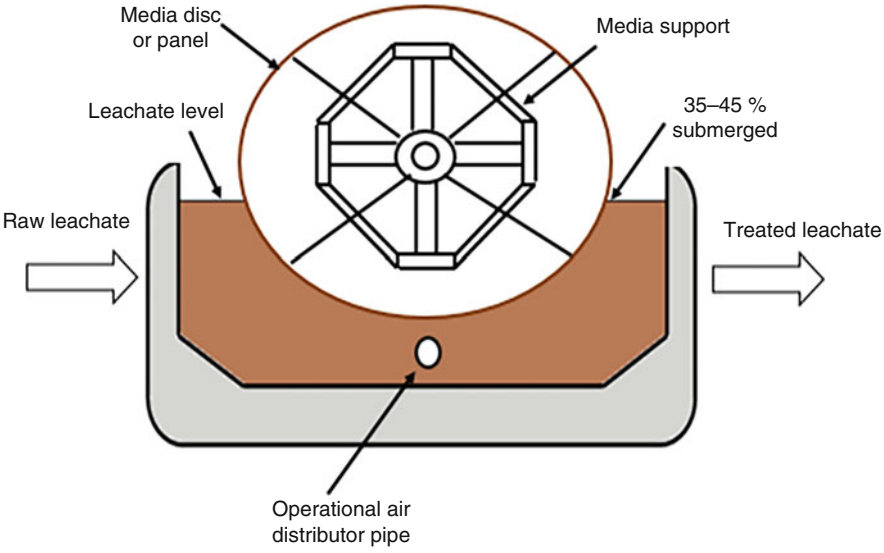

Fig. 7.5 Schematic diagram of rotating biological contractor in landfill leachate treatment

Aresearch which studied the efficiency of removal of nitrate from a mature landfill with nitrate load of 530 mg/L using the RBC system reported that nearly 100% nitrogen-nitrate removal could be achieved (Cortez et al. 2011). When the same was investigated for the removal of nitrite in the leachate, it was observed that the RBC system is insensitive to high nitrite load (up to 100 mg/L of $NO2^{-}$-N) which resulted in only a temporary decrease in the removal efficiency (Cema et al. 2007).

The RBC system is suitable for the remediation of leachate with low organic content (Kurniawan et al. 2010). However, it is inefficient in treating the high-strength leachate because of the clogging caused due to biomass deposition. The efficiency of the RBC system can be improved by coupling it with other technologies like biological treatment or physical-chemical processes (Castillo et al. 2007).

Trickling Filter (TF)

A trickling filter, also called as biofilter or biological filter is an aerobic treatment method which consists of a bed of rock or other coarse material through which the wastewater is trickled or sprayed that enables the microorganism present in the wastewater to attach themselves to the bed of rocks in the form of biofilm (Matthews et al. 2009). When the microorganism encounters wastewater, it utilizes the organic matter and converts it to CO2 and water. This process is facilitated by diffusing or forcing air through the bed (Torretta et al. 2017). A study reported that in a laboratory-constructed pilot-scale crushed brick trickling filter with loading rates of 100–130 mg/L/day at 25 °C and 50 mg/L/day at temperatures 5–10 °C was able to 90% of nitrification (Jokela et al. 2002). Another study which used bench-scale TF and SBR processes for leachate treatment found that there was a decrease in suspended solids (73.17%), turbidity (71.96%), COD (49%), BOD (76.69%), and $NH4^{+}$-N (59.50%) in TF process. This technology can be applied only for the treatment of mature leachates and not for young leachates due to high organic load in them (Aluko and Sridhar 2013). A different study that used pilot-scale submerged aerobic biofilter for co-treatment of sewage and municipal LL reported 98% BOD, 80% COD, 90% suspended solids, and 90% $NH4^{+}$-N removal (Ferraz et al. 2014).

Moving-Bed Biofilm Reactor (MBBR)

The moving-bed biofilm reactor system is a category of attached growth system that consists of an aeration tank in which the sludge is collected onto recyclable plastic carriers. These carriers have a huge internal surface area in which a biofilm can grow. The system is supplied with aeration to keep the carriers with biomass in motion to have enough interaction between the wastewater and aerobic microorganisms. The biomass consumes the organic matter in the wastewater and produces new biomass along with water and carbon dioxide. The excess sludge will come out from the carrier, flow with the treated water, and removed in the final separator as represented in Fig. 7.6.

A research investigated the execution of MBBR system for the removal of COD and ammonium from a municipal LL with an organic loading rate of $4.08–15.70 \times 10^{3}$ mg/L/day COD. It reported that 92–95% of COD and 97% of ammonium were removed (Chen et al. 2008). When granular activated carbon

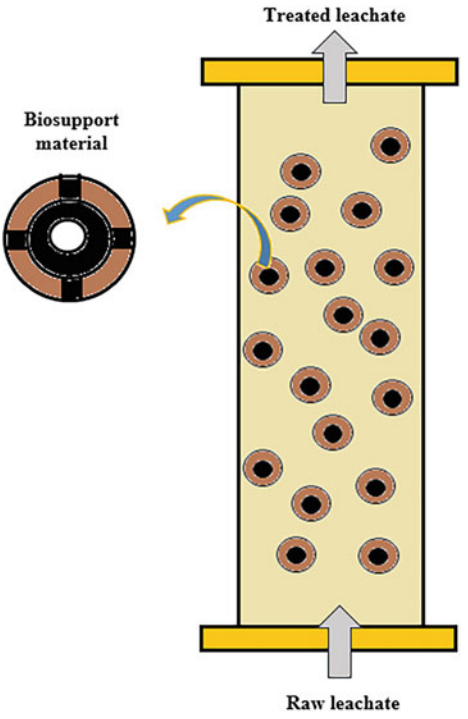

Fig. 7.6 Schematic diagram of moving-bed biofilm reactor (MBBR) in landfill leachate treatment

MBBR system was used to treat a LL, 85–90% ammonia and 60–81% of COD were removed (Loukidou and Zouboulis 2001). In a study, the membrane bioreactor and MBBR process were combined to treat the LL. It resulted in 95% oxidation of total nitrogen with effluent ammonium nitrogen concentration less than 50 mg/L (Canziani et al. 2006).

The advantages of MBBR process over traditional activated sludge process is that MBBR system has higher biomass concentration due to the large surface area provided by the carriers, they are less sensitive to toxic compounds, less sludge settling time, and removal of high ammonia concentration in a single process.

Fluidized-Bed Biofilm Reactor (FBBR)

In a fluidized-bed biofilm reactor, the reactor is packed with beads in which the microorganisms attach and grow as a biofilm on the surface. The fluidization of the beads in the column can be achieved by the recirculation of wastewater or sparging air from the bottom of the column (Fig. 7.7). When the air is sparged, the beads start to flow, and the microorganisms oxidize the organic matter when it encounters the wastewater (Bello et al. 2017). Depending on the treatment process the reactor can be run in a single or double column system. A study which evaluated the reliability and commercial viability of LL treatment using integrated liquid-solid circulating FBBR reported 85% removal of COD with loading rate of 2150 mg/L/day, 80% removal of nitrogen with loading rate of 700 mg/L/day, and 70% removal of

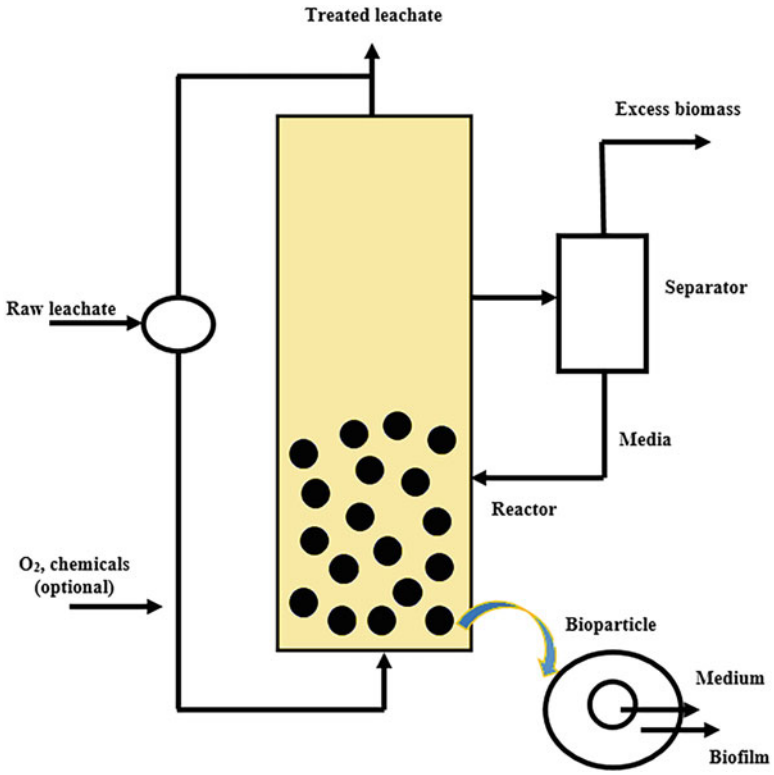

Fig. 7.7 Schematic diagram of fluidized-bed biofilm reactor (FBBR) in landfill leachate treatment

phosphorus at a loading rate of 14 mg/L/day, respectively (Eldyasti et al. 2010). Another study showed that, in the treatment of acid mine drainage using high rate FBBR, the LL can be used as an inexpensive soluble carbon source for sulfate-reducing bacteria (Sahinkaya et al. 2013). In FBBR process, the biomass grown of the expanded bed can be easily harvested as there is no filtration of solids that are from the passing flow (Torretta et al. 2017). Hence, it is considered as a feasible system for the treatment of municipal LL.

Membrane Bioreactor (MBR)

Membrane bioreactor is a wastewater treatment technology which integrates the biological treatment process like conventional activated sludge system with a semi-permeable membrane like microfiltration (Alvarez-Vazquez et al. 2004). The biological process helps in the oxidation of organic matter and the semipermeable membrane separates the biosolids/microorganisms from the treated effluent. Based on the position of the membrane, the membrane bioreactor can be classified into submerged membrane and outer membrane as represented in Fig. 7.8 (Xue et al. 2015).

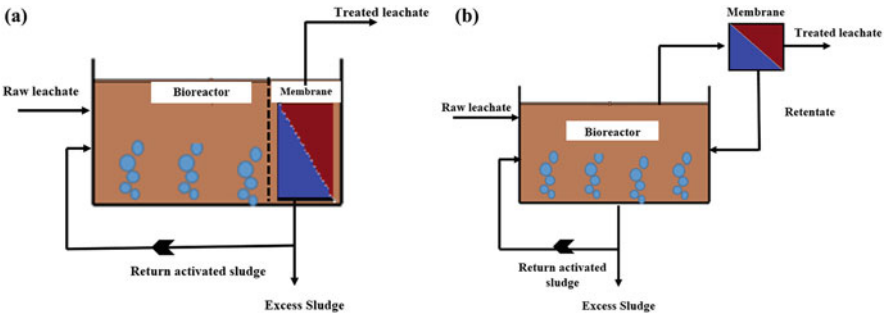

Fig. 7.8 Schematic diagram of membrane bioreactor (MBR) (**a**) submerged membrane, (**b**) outer membrane MBR in landfill leachate treatment

The mature LL is characterized by a small BOD/COD ratio contributing to the low biodegradability of the leachate (Saleem et al. 2018b) and hence MBR has a great potential in treating the mature LL. The MBR could remove 90% of BOD and ammonia and 75% of COD when compared to conventional treatment technologies, regardless of the landfill age (Ahmed and Lan 2012). When a lab-scale submerged pre-anoxic and post-aerobic bioreactor configuration like the dynamic membrane bioreactor is used for municipal LL treatment, it was observed that 98% of ammoniacal nitrogen and 90% of total nitrogen could be achieved (Saleem et al. 2018a). The combination of the MBR system with electrochemical oxidation is considered feasible for the treatment of LL as it is efficient in the reduction of several parameters such as COD (85%), TN (94%), and color (99%) with organic loading rates of 1900 and 2700 mg/L/day COD (Feki et al. 2009). In a study when MBR alone is used for the treatment of mature LL with an organic load rate of 1200 mg/L/day COD resulted in 63, 35, 98, and 52% removal of COD, TOC, $NH4^+$-N, and phosphorous (Zolfaghari et al. 2016). MBR is known as a versatile biological technology for the treatment of LL as it can be used to treat both young and mature LL.

Constructed Wetlands

Constructed wetlands are artificially constructed systems that include natural geological, chemical, and biological processes in the ecosystem for the remediation of wastewater. The constructed wetlands are mainly made of three components: an impervious layer which prevents the infiltration of wastewater into the groundwater, a grit layer where the biological treatment and denitrification occurs, and the above-ground vegetation layer that contains the plant species (Kivaisi 2001). For a municipal LL treatment, constructed wetlands have been developed in a lab-scale, pilot-scale, and full-scale system with greater removal efficiencies (Nivala et al. 2007). The toxic contaminants like phenol, bisphenol A (BPA), and 4-tert-butylphenol (4-t-BP) present in synthetic young and mature can be removed using a lab-scale vertical flow CWs. The percentage removal of phenolic compounds is in the following order: phenol (88–100%) > 4-t-BP (18–100%) ≥ BPA (9–99%) (Dan et al. 2017).

A removal efficiency of 72% $NH4^+$-N and 46% TN can be obtained by using pilot-scale vertical flow CWs planted with *Canna indica* (Camaño Silvestrini et al. 2019). When a pilot-scale sub-surface flow CW system planted with *Cyperus haspan* was used, it was able to remove 39–86.6% of turbidity, 63.5–86.6% of color, 39.2–91.8% of COD, 60.8–78.7% of BOD, 29.8–53.8% of $NH4^+$-N, and 33.8–67.0% of TN (Akinbile et al. 2012). A research reported that full-scale hybrid CW system can remove >90% of PPCPs, EDCs, ARGs, and antibiotic-resistant genes from mature land (Yi et al. 2017). The same researchers also employed a full-scale tropical CW system for the removal of perfluoroalkyl and polyfluoroalkyl substances (PFASs) from the leachate and observed that around 61% of total PFASs and 50–96% of individual PFASs can be removed (Yin et al. 2017).

CWs can be low-cost, easy handling, and maintaining technology for the remediation of municipal LL, especially in developing countries but these are not being extensively applied in developed countries due to its poor performance in the winters and large area requirement (Kivaisi 2001).

Myco-Remediation

Myco-remediation is a fungal-based form of bioremediation that uses fungi and their extracellular enzymes for the degradation or sequestration of contaminants in wastewater. Myco-remediation is beneficial for municipal LL treatment because the fungal mycelium is capable of secreting extracellular enzymes and acids that can degrade both non-stabilized organic matters like cellulose, hemicellulose, and lignin in young LL and stable refractory organic matters like humic and fulvic acids in mature LL (Ghosh and Thakur 2017; Zavarzina et al. 2004). Some fungi also act as hyper accumulators which are capable of absorbing and concentrating heavy metals on their fruiting bodies. The white-rot fungus *Dichomitus squalens* could grow on mature LL and consume the organic matter present in it as its carbon source. A study showed that the treatment of landfill leachate with the fungus *Aliivibrio fischeri* resulted in 60% removal of DOC and COD along with a reduction in the toxicity levels (Kalčíková et al. 2014). For a 50% diluted leachate with COD = 4500–45,000 mg /L and $NH4^+$-N = 640–4990 mg/L, about 79% and 68% of COD removal was attained when two strains of white-rot fungi *Trametes trogii* and *Phanerochaete chrysosporium* was used. Another selected stain *Bjerkandera adusta* along with glucose and cellulose as co-substrates was used to treat a mature LL in which, 63% of COD was removed with glucose as co-substrate and 54% COD was removed with cellulose as co-substrate (Bardi et al. 2017). Hence, myco-remediation is an efficient technology for the treatment of municipal LL and its application should be further studied.

Phytoremediation

Phytoremediation is a type of bioremediation process which uses plants and its associated microorganisms to remediate contaminated wastewater (Lavagnolo et al. 2016). The different types of phytoremediation process include phytoextraction, phytostabilization, phytodegradation, phytostimulation, phytovolatilization, and rhizofiltration. These processes have the ability to degrade

and detoxify the potentially harmful compounds present in the municipal LL (Kim and Owens 2010). Irrigation of willow and short-rotation coppice are some of the popular phytoremediation techniques used for the treatment of LL (Aronsson et al. 2010; Justin et al. 2010). When, 126 kg/ha/year of N, 6.7 kg/ha/year of P, and 707 kg/ha/year of TOC were supplied to willows grown on clay, about 93.8% of N, 99.8% of P, and 92.5% of TOC retention could be attained. Due to the irrigation properties of compounds present in LL, it had the ability to significantly improve the biomass production of *Salix* and *Populus* plants (Justin et al. 2010).

Phytoremediation is considered as a cost-effective, less harmful, and environmentally acceptable method of leachate treatment when compared to other traditional biological treatment processes. Furthermore, researches and studies need to be established to completely understand the mechanisms of phytoremediation for municipal LL so that effective and efficient remediation models could be developed (Justin et al. 2010).

7.2.2.2 Anaerobic Biological Treatment Processes

Anaerobic treatment is a type of biological treatment which employs anaerobic microorganisms to degrade the organic load in the wastewater (Kurniawan et al. 2010). The anaerobes consume the organic contaminants in the wastewater as their carbon source and convert them into carbon dioxide and methane gas as well as produce new biomass in the absence of oxygen (Smaoui et al. 2017). Anaerobic treatment occurs in four stages. The first stage is called hydrolysis in which the complex organic substances are hydrolyzed to simpler compounds by hydrolytic bacteria, the second stage is acidogenesis during which the simpler organic compounds are converted to acids which are then converted to acetic acid in the acetogenesis stage (Kurniawan et al. 2010). Finally, the acetic acids through the action of methanogenic bacteria are converted to CH4 and CO2 (Begum et al. 2018). When compared to aerobic process, anaerobic process is more beneficial due to lower sludge production, reduction of malodours, reduction in volatile content of sludge, and its ability to degrade recalcitrant compounds (Kheradmand et al. 2010; Smaoui et al. 2017).

Anaerobic Filter (AF)

An anaerobic filter is a fixed-bed biological reactor that consists of an array of filtration chamber. When the wastewater flows in the chamber, the particles greater than the size of pores in the filter get trapped and are used up by the microorganism that is grown on the filter membrane. To prevent the washout of the biomass, the anaerobic filter is usually operated in an up-flow mode with a hydraulic retention time (HTR) of 12–36 h. In a study, anaerobic filter made up of reticulated polyurethane foam was used to study the feasibility of treating alkaline sulfate-rich leachate. It reported that around 90% and 73% of COD could be removed from the influent leachate with a loading rate of 0.76 and 4.58 kg m^3/day COD, respectively (Wang and Banks 2007). Sulfate removal (88%) was also achieved when AF was used for the treatment of partially SL with COD 3750 mg/L and BOD/COD = 0.3 and from a relatively new LL with COD 14,000 mg/L and BOD/COD = 0.7, about 90% removal of COD could be achieved at room temperature with HRT of 24–96 h

(Torretta et al. 2017). Due to its high COD removal efficiencies at shorter HRTs for high organic loading rates, it is considered as a suitable technology for the treatment of highly polluted wastewater.

Up-Flow Anaerobic Sludge Blanket (UASB) Reactor

Up-flow anaerobic sludge blanket reactor or UASB reactor is a type of anaerobic digester in which a layer or sheet of granular sludge is suspended in the reservoir. As the wastewater drifts upwards, the anaerobes utilize the organic matter as their carbon source and convert them into CH4 and CO2 as represented in Fig. 7.9. The up flow of the wastewater coupled with the effect of gravity, suspends the sludge blanket with the help of flocculants.

When a sequential batch UASB reactor was used for the treatment of municipal LL at a loading rate of 0.6–19.7 g/L/day COD, 71–92% of removal efficiency was achieved whereas when a continuous flow USAB reactor was used, around 77–91% of COD was removed (Kennedy and Lentz 2000). At mesophilic conditions, 82.4% COD could be removed from influent leachate with COD 70,390–75,480 mg/L and loading rate of 12.5 kg m^3day^1 (Ye et al. 2011). A study investigated the co-digestion of LL along with septage using UASB reactor and reported that 68.2%, 73.4%, 44.3%, 47.8%, 53.7%, and 44.4% of total COD, soluble COD,

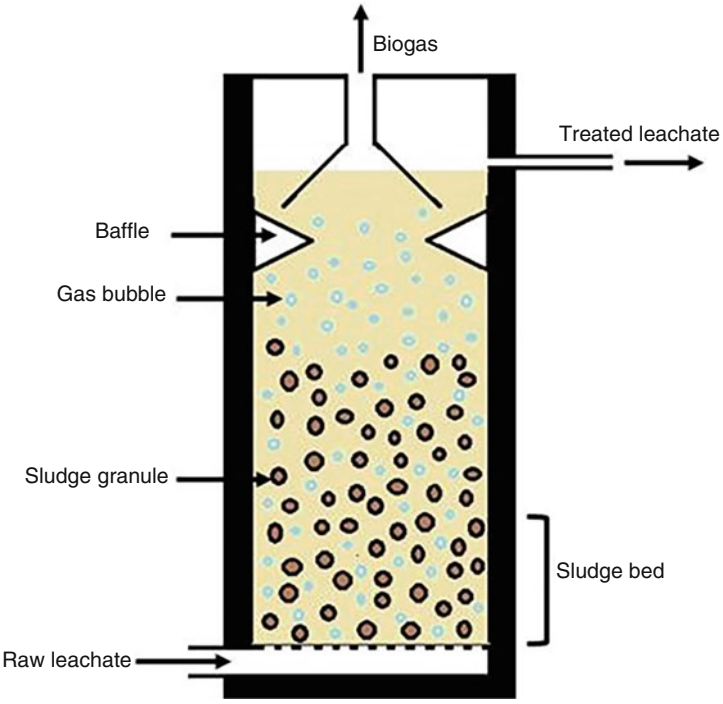

Fig. 7.9 Schematic diagram of up-flow anaerobic sludge blanket reactor (UASB) in landfill leachate treatment

total solids, volatile solids, total VFA, total phosphorus, $NH4^+$-N, carbohydrate, and protein, respectively, could be removed at a hydraulic retention time of 1.5 days (Lin et al. 2000).

UASB reactor is mostly used along with other technologies for the treatment of LL (Wu et al. 2015). For example, 98% of COD and 99.6% of $NH4^+$-N can be removed by using a two-stage sequential UASB/aerobic completely stirred tank reactor (Ağdağ and Sponza 2005). When a two-stage UASB-SBR system was used, around 96.7% of COD removal and 620 99.7% of $NH4^+$-N removal at a low temperature of 14.9–10.9 °C was achieved (Sun et al. 2010). In order to remove COD, BOD, chloride, and $NH4^+$, the efficiency of the hybrid system UASB reactor-RO was also investigated (Bohdziewicz and Kwarciak 2008). One of the major disadvantages of UASB reactor is that it is sensitive to the toxic substances present in the LL.

Anaerobic Ammonium Oxidation (ANAMMOX)

Anaerobic ammonium oxidation, also known as Anammox, is a part of the N2 cycle in which the anammox bacteria under anaerobic condition converts ammonium and nitrogen dioxide into nitrogen gas and water (Shalini and Joseph 2012; Wang et al. 2016). The bacteria achieve the degradation by using ammonium as its electron donor and nitrite as its electron acceptor (Eqs. 7.2, 7.3, 7.4, and 7.5) (Gao et al. 2015; Renou et al. 2008).

$$NH4^+ + NO2^- = N2 + 2H2O \left(\Delta G^\circ = -357 \text{ kJ mol}^{-1}\right) \tag{7.2}$$

$$NO2^- + 2H^+ + e^- = NO + H2O \left(E^\circ = +0.38 \text{ V}\right) \tag{7.3}$$

$$NO + NH4^+ + 2H^+ + 3e^- = N2H4 + H2O \left(E^\circ = +0.06 \text{ V}\right) \tag{7.4}$$

$$N2H4 = N2 + 4H^+ + 4e^- \left(E^\circ = -0.75 \text{ V}\right) \tag{7.5}$$

When continuous flow nitration and anammox process were applied for the treatment of a mature LL, nearly 94% of total nitrogen and 62% COD removal was reported when the influent ammonia was 1330 mg/L and COD was 2250 mg/L (Wang et al. 2016). Another treatment process that used two-stage anammox system with a sequencing biofilm batch reactor reported that 95% of total nitrogen was removed with an influent ammonia concentration of 3000 ± 100 mg/L at 35 °C for 107 days (Miao et al. 2016). A novel combined process which consists of a partial nitritation reactor, an anammox reactor, and two underground soil infiltration systems was used for the treatment of leachate with high 643 ammonium and organic matter concentration. About 97% $NH4^+$-N, 87% TN, and 89% COD 644 removal was reported with influent leachate compositions of 1430–2720 mg/L $NH4^+$-N, 1524–2912 mg/L TN, and 1165–2599 mg/L COD (Liang and Liu 2008). Combined partial nitritation and anammox process can be used to achieve high nitrogen

removal up to 93 ± 1% and 81 ± 1.2% at nitrogen loading rates of 4.2 kg/m^3/day TN and 8.3 kg/m^3/day kg/m^3/day, respectively (Nhat et al. 2014).

Anammox is an efficient technology for the remediation of municipal LL with high ammonia concentrations as this does not require an organic carbon source for nitrification (Wu et al. 2018). It also produces less sludge, no aeration, and decreased carbon dioxide emission. Hence, Anammox is preferred for the treatment of leachates which contain nonbiodegradable COD and high nitrogen content. Moreover, in detail research on the complete Anammox process and its optimum conditions are required to develop new Anammox reactors for the treatment of LL.

7.2.3 Combined Treatment Processes for LL Treatment

7.2.3.1 Combination of Two or More Physicochemical Treatments

Research was carried out to study the effect of coagulation-flocculation and Fenton oxidation in combination with GAC adsorption on SL treatment (Zamora et al. 2000). The outcome showed that pretreatment with Fenton oxidation enhanced the GAC adsorption thus leading to improved COD removal at pH 4.0. This is because of the oxidation by-products formed by the conversion of organic compounds that had smaller molecules which made them enter the micropores of GAC easily.

Photooxidation was carried out for the treatment of SL by UV-vis irradiation at the wavelength of 313 nm and by that, about 31% of COD was removed. But while using coagulation and photooxidation in combination, 64% of COD removal was achieved at the same concentration (Wang et al. 2002). This implies that the treatment can be effective when a combined technique is used rather than using individual processes.

About 48% of COD was removed while using coagulation alone for the treatment of SL from a landfill in South Korea (Yoon et al. 1998). But it was increased to 73% when a coagulation-Fenton treatment was used. This indicates that the addition of the coagulation process improved the Fenton oxidation process thus helping in the increased removal of organic compounds.

Ozonation-coagulation combination treatment was used for the removal of organic pollutants present in SL (Monje-Ramirez and de Velásquez 2004). Pretreatment using Fe (III) as a coagulant was found to be effective. The initial COD concentration was 5000 mg/L and about 78% of it was removed at pH 4–5 using a two-step treatment using ozonation.

NF and PAC adsorption were used in combination for the treatment of leachate that has been pretreated biologically and the results showed that combined treatments removed 97% COD whose initial concentration was 1450 mg/L (Meier et al. 2002). This recommends that combined treatments provide better treatment results than separate processes.

An integration of biologically activated carbon and UF where the cross-flow filtration unit is integrated with the adsorption of organic matter was used for the treatment of YL from the landfill in the USA. With a COD initial concentration of

3050 mg/L, 97% of COD was removed. Permeate flux deterioration was mitigated by the addition of PAC due to membrane fouling (Pirbazari et al. 1996).

Coagulation, flocculation, and Fenton oxidation were employed in a sequence for the removal of colloidal particles from the leachate. Using 0.8 mg/L FeCl3 at pH 8.5, around 90% of COD was removed whose initial concentration was 7400 mg/L (Zamora et al. 2000).

Overall, using different physicochemical techniques in combination improves the removal of recalcitrant compounds and enhances the treatment efficiency. However, the use of each technique and the order of operation have to be analyzed to justify the combined treatment technology.

7.2.3.2 Combination of Physicochemical and Biological Treatment

RO and Activated Sludge (AS) combination was adopted for the treatment of YL where COD and NH3-N were almost completely removed having initial concentrations of 6440 and 1153 mg/L, respectively, which suggests that using a physicochemical and biological treatment in combination was effective in the removal of organic compounds as well as ammoniacal nitrogen (Baumgarten and Seyfried 1996).

For the treatment of SL with higher ammonia concentration, a combination of GAC and nitrification was carried out and about 93% of ammoniacal nitrogen and 55% of COD were removed whose initial concentrations were 830 and 2450 mg/L which shows that the GAC-nitrification combination is not successful in the removal of organic load from the leachate (Horan et al. 1997).

Up-flow anaerobic sludge blanket (UASB) reactors and reverse osmosis were used in combination for the treatment of SL from a landfill in the Netherlands. The pretreatment of leachate was done using UASB reactor and the effluent has been discharged to surface water since all of the recalcitrant compounds contributing to COD and ammoniacal nitrogen have been completely removed (Kurniawan et al. 2006).

With a combination of GAC adsorption and aerobic treatment, a comparative study was carried out which showed that about 65% of COD and 97% of NH3-N were removed whose initial concentrations were 1980 and 130 mg/L, respectively. The treatment process was capable of meeting the discharge standards and thus released into surface water (Schwarzenbeck et al. 2004).

A dual-step process for the treatment of YL from Turkey was carried out using UASB and precipitation using struvite with the stoichiometric ratio of Mg: NH4: PO4 = 1:1:1 (Altinbaş et al. 2002). Around 83% of COD and 85% of ammoniacal nitrogen were removed at pH 9.2 whose initial concentrations were 8900 mg/L and 2240 mg/L, respectively, with a total treatment cost of US$ 0.9/m^3.

A three-step process constituting aerobic pretreatment, adsorption using GAC, and coagulation was explored for SL treatment from Germany and about 92% of COD was removed whose initial concentration was 1400 mg/L. Due to chemical consumption, the treatment cost was higher (US$ 2.3/m^3) than other processes.

Due to the synergistic effect of two individual processes, combined treatment technology was found to be more effective and efficient than individual process and

also helps in overcoming their limitations. Thus, combined treatment is undeniably effective in improving the quality of the wastewater with lower operational cost and minimal residue generation.

7.2.4 Miscellaneous Treatment Technologies

7.2.4.1 Ion Exchange

It is a reversible exchange between solid and liquid phases where there is no permanent change in the solid's structure. This technique is successful in efficiently eliminating the traces of metal contaminants to meet more stringent discharge standards in certain countries. However, the leachate has to be subjected to biological treatment before ion exchange. Though the treatment of LL using ion exchange has not been extensively studied, it has got a fair interest in Germany for the removal of humic -ontaining nonbiodegradable compounds from leachate (Fettig 1999).

The results of the study on the treatment of SL using ion exchange resins like Amberlite XAD-8, XAD-4, and Amberlite IR-120 and/or granulated activated carbon adsorption was evaluated and it was shown that among all the adsorbents GAC was able to remove 93% COD followed by 53% and 46% removal of Amberlite XAD-8 and XAD-4, respectively (Kurniawan et al. 2006). Amberlite IR-120 showed the least removal (31%) at the initial COD concentration of 5108 mg/L for all adsorbents. There is a competition with the heavy metals in leachate for the binding site and the effect of this competition made GAC more effective in COD removal than other synthetic resins.

A study comparing the effect of ion exchange and ozonation on the removal of ammonium from LL was carried out (Lin and Wu 1996). Ozonation can convert nitrite into nitrate, but it is not very effective in converting ammonia into nitrate. On the other hand, ion exchange is capable of reducing the concentrations of both nitrate and ammonium ions to the preferred level and it was stated that at the pH range of 7–9, 500 bed volume (BV) of ammonia was removed whose initial concentration was 20 mg/L using ion exchange alone. In the case of ozonation, only 250 BV of ammonia was removed at the expense of 0.29 mg of $NH4^{+}$/mg of ozone at the same pH range (Lin and Wu 1996).

Other than the removal of ammonia and organic compounds, adsorption using kaolinite was also been used for the removal of heavy metals like Ni (II) and Cd (II) from leachate (Majone et al. 1998). Ninety-nine percent of Ni (II) and 90% of Cd (II) were removed whose initial concentrations were 0.94 mg/L and 0.002 mg/L, respectively, and it was found that the two metals were removed when came in contact with kaolinite. The ion exchange technique is effective in the removal of heavy metals based on the type of the prevailing contaminant and the resin that has been employed for ion exchange. Ion exchange can achieve excellent metal removal from effluent after a proper aerobic pretreatment. However, the main limitation is the requirement of suitable pretreatment processes like the removal of suspended solids present in the leachate prior to ion exchange.

7.2.4.2 Electrochemical Treatment

Treatment using an electrochemical technique like electrodialysis has been employed in environmental protection. In Brazil, electro degradation by the application of a flow electrochemical reactor of SL was investigated and the highest COD and NH3-N removal of 73% and 49%, respectively, were obtained using a flow rate of 2000 L/h for 180 min and a current density of 1160 A/m^2. Thus, electro-degradation was found to be an alternate for the treatment of LL. However, this technology has not been widely explored for the treatment of leachate because of its high cost and energy consumption (Moraes and Bertazzoli 2005).

7.3 Conclusion

Municipal landfill leachate poses a substantial risk to the environment due to the occurrence of toxic substances such as refractory organic compounds, ammonia nitrogen, and heavy metals. To meet stringent discharge standards for release into water bodies, various individual and/or combined sustainable technologies have been recommended and evaluated for their efficiency in leachate treatment. Depending on various factors like location of the landfill, composition of the leachate, and the concentration of various components (COD, BOD, and NH3-N), different physicochemical, biological, and combined treatment technologies have been adopted. This comprehensive chapter summarizes the recent advancement in understanding the role of treatment technologies in leachate treatment. It was concluded that though various physicochemical and biological technologies have been used for the removal of heavy metals, refractory organic and inorganic compounds from landfill leachate, a combination of technologies has been found to be more effective than individual techniques. Combining two or more physico-chemical processes or physicochemical and biological treatments is necessary for more effective removal of toxic contaminants from leachate. Typically, a pretreatment of leachate by physicochemical processes best complements the biological treatment. Of all the physicochemical and biological processes discussed, chemical precipitation, membrane filtration, adsorption, and up-flow anaerobic sludge blanket (UASB) reactor are most commonly used and applied worldwide for the removal of refractory organic compounds present in the landfill leachate. Over 95% of COD removal is achieved through both nanofiltration and activated carbon with an initial concentration ranging from 5000–17,000 mg/L and about 98% of NH3-N with an initial concentration varying from 3300–5618 mg/L was removed through chemical precipitation using struvite. A combined physicochemical and biological treatment is required for the effectual removal of both COD and NH3-N from landfill leachate.

Acknowledgment The authors are highly thankful to "The Department of Science and Technology, Ministry of Science and Technology, Government of India," for funding the project under the Scheme "Scheme for Young Scientists and Technologists (SYST)-Science for Equity and Empowerment Division (SEED)"; Project No: SP/YO/2019/1360(G). DST-INSPIRE Fellow Maseed

Uddin [DST/INSPIRE Fellowship/2019/IF190897] acknowledges the Department of Science and Technology, Ministry of Science and Technology, Government of India for providing the fellowship and financial support to carry out the research.

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Fungal Bioremediation of Soils Contaminated by Petroleum Hydrocarbons

8

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8.1 Introduction

Irrespective of the regional barrier, environmental pollution is considered one of the major challenges faced by living beings. The repercussion of environmental pollution was highly alarming, which are reflected by threatening more than 1141 species globally (International Union for Nature conservation). Due to the instant and direct effect of air and water pollution, more emphasis was given by both scientific and nonscientific communities. Apart from nuclear disasters such as Chernobyl in 1986 and Fukushima Daiichi in 2011, a predominant focus on soil pollution was not given globally (Friedman 2011). On the other hand, the experience from the toxicity effect on more than 20,000 people in and around sites of worst soil polluted lands, such as the dumpsite for the e-waste processing in Ghanaian capital Accra, Dzershinsk, Russia for dioxins, Hazaribagh, Bangladesh for tannery waste, and Niger river delta for petroleum hydrocarbons, diverted the several scientists to address the problem. Acting as a sink for several pollutants, the hazardous effects of soil pollution is very immense which include several health-related issues such as cancer, premature birth,

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_8

mental retardation, impotency, and miscarriage. In spite of several remediations of the pollutants, the diversified properties of the soil along with their unique interactions with the pollutants difficult to devise a universal remediation strategy for soil treatment.

To understand the transport, distribution, and fate of pollutants in the soil, it is mandatory to have a comprehensive knowledge of the intricate functionality of the soil (Van Breemen and Buurman 2002). In general terminology, soil can be termed as a dynamic naturally occurring compound on the earth's surface, which is formed from the earth crust by the action of living organisms and weather agents. Depending on the accessibility of the parent rock and climatic conditions, the duration for the formation of soil ranges (Van Breemen and Buurman 2002). On the other hand, the living habitat of great diverse groups of organisms majorly dictates the physical, chemical, and nutrient profile of the soil, which results in the variation of the soil composition from place to place. Although the soil properties are very diverse with more than 50% of solid fraction from parental earth crust, the base composition of the soil is dictated by the mineral content, organic matter, water, and air. The combination of these compounds decides the chemical profile, color, porosity, and texture of the soil. The large constituent of soil-mineral fracture consists of silicate minerals, quartz, and feldspars with particle size $> = 0.05$ mm along with secondary minerals (0.05–0.002 mm), which results from the weathering of primary minerals. The sand particles from 0.05 to 2 mm have a negligible role in the interaction with pollutants however the secondary minerals silt (0.05–0.002 mm) and lower than 0.002 mm clay minerals have a prominent role in the soil–pollutant interaction (Van Breemen and Buurman 2002).

On the other hand, the organic fraction due to their negative charge sequesters key positively charged compounds, such as potassium, sulfur, nitrogen, and carbon, and acts as a major sink for the plant macronutrients. The source of soil organic matter (SOM) fraction includes detritus stages of decomposed living organisms such as microorganisms, plants, and animals (Senesi and Loffredo 2018). However, the reversible masking of the pollutant by the SOM due to hydrophobic interactions limits the utilization of the conventional treatment process. The majority of the pollutants adsorb on the surface of the soil organic matter by hydrophobic interaction, pi–pi interaction, and are largely dependent on the soil's physiological conditions such as pH, temperature, redox condition, surface area, and available chemical species (Lehmann and Kleber 2015).

The humic substance present in the SOM attributes an overall negative surface charge due to the presence of –OH, –COOH, –SH, and –C=O– functional groups and acts as a key player in sequestering the pollutants in soil by exhibiting high affinity for cationic pollutants in soil (Rivero et al. 2004). Apart from the SOM, the presence of inorganic ions such as NO_3^-, Cl^-, HPO_4, and SO_{42}^- along with both natural and synthetic chelating agents such as citric acid, fulvic acid, gluconic acid, oxalate, and ethylene diamino tetracetic acid (EDTA) affect the remediation

efficiency in soil. Hence, despite the lower percentage of SOM in the soil, the presence of SOM claimed to have a substantial influence on pollutant interaction with soil, making them a prime target in the soil remediation.

8.2 Pollutants in Soil and their Classification

The vast classes of the soil pollutants can be grouped into two major groups, the organic pollutants and inorganic pollutants. Irrespective of the grouping both the groups have both natural and anthropogenic origins with diverse chemical properties (Bernes 1998). In spite of the ability of the indigenous microorganism for the degradation of pollutants, the hydrophobic nature, and structural stability of a few pollutants elevates the toxicity of the soil and paralyze the natural remediation efficiency of the soil microbiota. The majority of organic pollutants consist of carbon and hydrogen backbone and can be categorized into petroleum hydrocarbons (PHC), pharmaceutical active compounds and its derivatives, pesticides, and chlorinated compounds (Zuloaga et al. 2012). On the other hand, inorganic compounds include heavy metals, radioactive compounds, salts, and nutrients. Among the inorganic pollutants, the presence of heavy metals and metalloids are of greatest concern due to their potential toxicity to living organisms. Being resistant to degradation, the heavy metals can be remediated by biotransferring to lesser toxic compounds rather than degradation. Although these two categories of pollutants differ in structural and chemical properties, their interaction with soil due to their hydrophobic nature and their persistence nature along with hazardous toxicity properties make them unique and of greatest concern.

Depending on the persistence nature and the severity of toxicity in terms of teratogenicity, carcinogenic, and mutagenicity, environmental protection agency (EPA), Clean water act 1977 grouped 126 pollutants as priority pollutants (Stout et al. 2015). However, a recent increase in the number of anthropogenic pollutants in an environment, having the properties of disrupting the reproductive and endocrine system in micro concentrations, initiated EPA to categorize the compounds under a special category of "contaminants of emerging concern" (Vidal-Dorsch et al. 2012). These compounds are not completely understood due to their non-monotonic toxicity nature with similar properties to that of priority pollutants. Most pharmaceutical compounds and pesticides come under this category.

8.2.1 Petroleum Hydrocarbons

Petroleum hydrocarbons are a group of several hundreds of compounds consisting of crude oil or derivative of crude oil (Riser-Roberts 1998). Depending on the source of the occurrence the physical and chemical properties of crude oil vary. Due to this diversity, crude oil is generally classified based on the density or sulfur content or the geographical location of the production. However, conventional crude oil is classified either as light crude oil (low density) or heavy crude oil (high density) (Yasin

Table 8.1 Classification of total petroleum hydrocarbons (TPH) according to the equivalent carbon chain length (EC) [(a) according to ATSDR and (b) according to US-EPA]

Primary fraction	Secondary fractions	Examples	Surrogate
Aliphatic	EC5 to EC8 (low carbon range)	*n*-hexane	*n*-hexane[a,b]
	EC >9–EC16 (medium carbon range)	Kerosene, dearomatized petroleum stream, JP-7	JP-7, dearomatized petroleum[a]
	EC >16–EC 35 (high carbon range)	White mineral oil	Mineral oil[a,b]
Aromatic	EC 6–EC 9 (low carbon range)	Benzene, toluene, ethylbenzene, xylene	Toluene[a,b]
	EC >9–EC 16 (middle carbon range)	Isopropyl benzene, naphthalene, 3-methylnaphthalene.	Naphthalene[a]
	EC >16–EC 35 (high carbon range)	Fluorene, fluoranthene, benzo (a) pyrene	Pyrene[a,b]

et al. 2013). In terms of the chemical structure, crude oil represents a complex mixture of carbon and hydrogen backbone along with small quantities of sulfur, nitrogen, oxygen, and other metals (Yasin et al. 2013).

These combinations and unique chemical constituents in crude oil makes them one of the most complex naturally occurring compounds so far analyzed. Due to the practical difficulty in analyzing the properties and toxicity of every compound present in the soil-contaminated crude oil, environmentalist considered the usage of total petroleum hydrocarbons (TPHs) as the best indicator to define the levels of toxicity present in the petroleum-contaminated soil (Kuppusamy et al. 2020a). However, the quantification of TPH resulted in the gross toxicity of the soil rather than defining the individual contribution of the compounds in elucidating the toxicity. As a result for a better understanding based on the carbon chain length, scientists divided the TPH into fractions and designated them as EC (Equivalent Carbon number index) (Yang et al. 2015). Further, the toxicity levels of each fraction were assessed with the reference to the presence of a selected component in the fractions called "surrogate" in accordance with ATSDR (Agency for Toxic Substances and Disease Registry) (Table 8.1).

8.2.2 Toxicity of Petroleum Hydrocarbons

The toxicity of petroleum hydrocarbons present in soil depends mainly on their type and concentrations. Besides, environmental effects and the presence of co-contaminants such as heavy metals sometimes decide the toxicity of petroleum hydrocarbons (Khan et al. 2018). Exposure to these contaminants results in short-term health effects such as eye irritation, skin allergy, nausea, and diarrhea (Kuppusamy et al. 2020b); however, continuous exposure leads to long-term chronic health issues. Being lipophilic, most TPH compounds specifically aromatic

hydrocarbons tend to adsorption in the adipose tissue and cause damage to the internal organs. Irrespective of their presence in soil or water, the TPH and their epoxides exhibit high toxicity to mammals, amphibians, reptiles, and microorganisms. It has been reported that these compounds are commonly carcinogenic and mutagenic, also, recent discovery holds their responsibility for being immunotoxin, endocrine disruptor, genotoxic, and teratogenic. Exposure to polycyclic aromatic hydrocarbons during pregnancy reported to affect the progeny's mental ability and reproductive system (Agarwal et al. 2018). Despite wide research, the toxicity data are limited to either whole petroleum hydrocarbons or by considering key components in them, which are relatively heterogeneous on applying to actual petroleum hydrocarbon exposures. In addition, variability in the composition of crude oil due to variation in the refining process and the presence of impurities makes it difficult in assessing the eco-toxicity levels of petroleum hydrocarbons.

8.2.3 Fate of Hydrocarbons in Soil

Remediation of TPHs from the environment can be achieved either by removing or completing degradation of the TPHs (Kuppusamy et al. 2020c). However, the hydrophobic nature of the TPHs along with their structural stability, complete removal or degradation of these compounds is considered to be a major challenging task (Aguelmous et al. 2019). The fate of the TPHs in soil depends on several factors including the chemical and physical properties of the soil, aging of soil, and the presence of co-contaminants (Fig. 8.1) (Adams et al. 2015). Majority of low molecular weight TPH either evaporates or oxidize or is degraded by the action of microorganisms. However, persistent compounds tend to be adsorbed onto the soil organic matter or leached into water bodies. In nature, TPHs are removed from the environment by various processes such as photooxidation and chemical oxidation (Adams et al. 2015). On the other hand, the overall contribution of natural

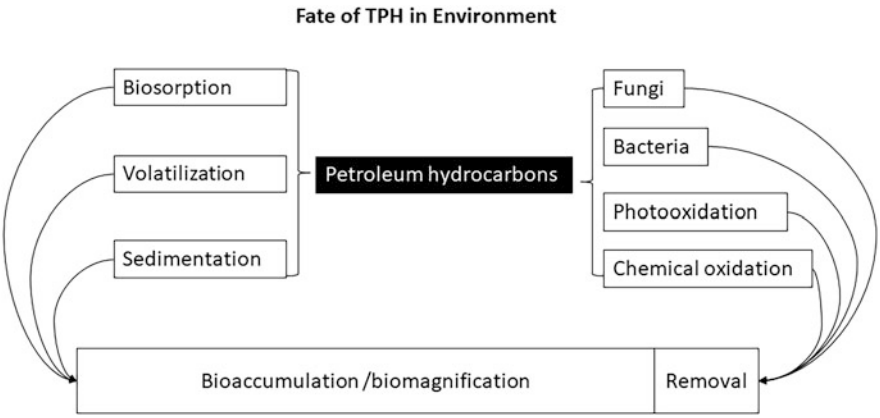

Fig. 8.1 Fate of total petroleum hydrocarbons in soil

remediation for the removal of the TPHs was limited to low molecular weight TPHs, which urges for the development of technologies for the degradation of persistent petroleum hydrocarbons.

8.3 Remediation of Hydrocarbons

With the advancement in technology, various treatment strategies were introduced for the effective treatment of TPHs in the soil. To generalize the treatment technologies were divided into physical, chemical, and biological methods. In addition, the utilization of chemicals for remediation such as surfactants was found to be non-ecofriendly and in turn act as a toxic compound to the soil microbiota. In this context, the utilization of green technologies for pollutant removal by the usage of bacteria or fungi as a bioagent is considered the most cost-effective and eco-friendly efficiency method (Shah et al. 2019).

The term remediation refers to the degradation, removal, or transformation of contaminants to less toxic or harmless substances. The method includes restricting the mobilization of pollutants and preventing spreading to uncontaminated sites or degradation. Bioremediation is a process, where the goals of remediation were achieved with biological agents (Microbial or enzymatic). This widely used technique for the treatment of TPHs contaminated soil is believed to be invasive and cost-effective. Bioremediation involves either conversion of contaminants to less toxic compounds or mineralization of the contaminants to inorganic compounds such as carbon dioxide (CO_2) or water (H_2O) (Saha et al. 2019). The bioremediation process is classified into two strategies in situ and ex situ methods (Fig. 8.2). The former strategy involves remediation of the TPH at the site of contamination whereas ex situ involves the excavation of soil and treating the soil elsewhere (Adams et al. 2015). In situ treatment is considered as low cost and low maintenance along with environment-friendly process for the remediation of soil. On the other hand, ex situ treatment was reported to be an efficient remediation strategy in less time compared

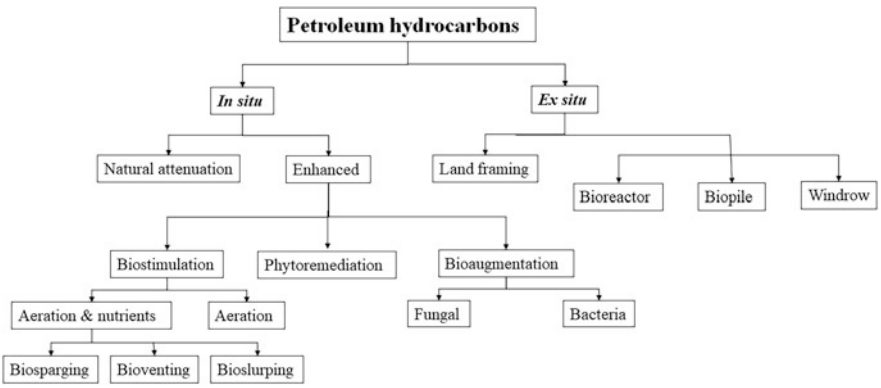

Fig. 8.2 Various strategies for cleanup of soil contaminated with petroleum hydrocarbons

to in situ, which is considered a major advantage (Shahsavari et al. 2017). However, the involvement of excavation and transport of soil makes the ex situ treatment costly. In addition, a variation of soil properties along with the influence of co-contaminants present in the soil makes the in situ treatment inconsistent inefficiency and outcome. On the other hand, with the advancement in the soil chemistry and biology, several in situ technologies were reported, which include biostimulation, bioaugmentation, and phytoremediation. Bioaugmentation and phytoremediation are of great interest due to their efficacy and easy handling.

8.3.1 Bioaugmentation

Since the early 2000s addition of microorganisms as a supplement to the contaminated soil has been proven to be an alternative strategy for the bioremediation of the TPHs in soil (Wu et al. 2016). This strategy is commonly used when indigenous microorganisms failed to degrade the contaminants or the soil become abiotic in condition due to the high toxicity of the contaminants (Adams et al. 2015). Other conditions for bioaugmentation are also considered when the hydrocarbon-degrading microbial population is low. The success of the strategy depends on the ability of the microorganism to survive in a hostile and foreign environment by overcoming the competition of indigenous microorganisms. In addition, the presence of the contaminants and their concentrations along with several environmental factors play a decisive role in judging the outcome of bioaugmentation (Cunningham and Philp 2000).

Microbial degradation of the TPHs in the soil is considered an ultimate natural mechanism by which the cleanup of soil can be done in an environmentally friendly way. The commonly used bacteria include *Bacillus sp*. (Das and Mukherjee 2007), *Acinetobacter sp*. (Mishra et al. 2004), *Burkholderia sp.* (Jasmine and Mukherji 2013), *Arthrobacter sp*. (Poi et al. 2017), *Mycobacterium sp., Rhodococcus sp., Pseudomonas* sp. (Das and Mukherjee 2007), and *Sphingomonas sp.* (Tyagi et al. 2011), which were isolated from the contaminated sites with TPHs. Different microorganisms have different mechanisms for degrading the TPHs in the soil. Some microorganisms depending on their metabolism prefer aromatic or alkane hydrocarbons, while others jointly degrade both.

Although protozoa and algae are important contributors to the microbial community in both terrestrial and aquatic ecosystems, reports on their involvement in the degradation of TPHs are limited (Adams et al. 2015). On the other hand, bacteria are the commonly preferred microorganism that is used as a bioaugmenting agent for the remediation of contaminants. However, lack of broad contaminant range alone makes fungi a suitable alternative, specifically for treating the TPHs contaminated soil. Further, easy adaptation to extreme conditions and their ability to grow in a wide range of pH made fungi suitable bioremediation agent (Schadt et al. 2003, Rousk et al. 2010).

8.3.2 Fungal Bioaugmentation

The fungal biomass is unique and diverse, having the ability to survive in a range of heterogeneous environments. The ability to utilize soil organic matter by colonizing on both biotic and abiotic surface act as an additional advantage for mycoremediation. The mycelia formation of the ability of fungi other than yeast aids them in easy colonization of soil and helps in the translocation of key nutrients and water (Pérez-Armendáriz et al. 2010). Also, the ability of the fungi to grow in petroleum or crude oil spill site, by utilizing them as a sole carbon source makes them potential organisms for hydrocarbon degradation. Apart from the several filamentous fungi genera, *Aspergillus* and *Trichoderma* were the commonly used Ascomycetes and white-rot fungi in basidiomycetes.

The most studied fungi for bioremediation are basidiomycetes, specifically, that are saprotrophic and biotrophic fungi. Saprotrophic basidiomycetes are the fungi that grow on dead organic matter, among which white-rot fungi exhibit a high potential for bioremediation of TPHs (Bosco and Mollea 2019). These fungi can utilize phenol backbone lignin compounds present in the dead plant matter as a sole carbon source until complete mineralization. On the other hand, biotrophic basidiomycetes (Ectomycorrhizal fungi) obtain the nutrient by having a mutualistic plant partner, where fungal mycelia envelopes the hair root and penetrate the cells of the cortex.

8.3.3 White-Rot Fungi

White-rot fungi (WRF) are one of the major naturally available bioremediation agents in nature. This Hymenomycetes class fungi grow on the woody plant, having the ability to degrade lignin or lignin-like compounds (Pointing 2001). WRF degrades the lignin molecules in the woody plant, less affecting the cellulose and hemicellulose. These degraders are specifically active under nitrogen starvation by activating their secondary metabolism for the production of a variety of ligninolytic enzymes (Esterhuizen-Londt et al. 2019). The secretion of the ligninolytic enzymes (lignin peroxidase, manganese peroxidase, aryl-alcohol oxidase) along with laccase, make them a potential bioremediation agent (Manavalan et al. 2015). The genera of *Trametes* sp., *Phanerochaete chrysoporium*, *Pleurotus* sp., and *Bjerkandera* are the four common WRFs used for bioremediation studies. The enzymes secreted by the white-rot fungi have the ability to transform a variety of organic pollutants such as PAHs, pesticides, and pharmaceuticals in wastewater and soil (Ellouze and Sayadi 2016).

The ability of WRT to sustain highly toxic compounds such as organic pollutants and hydrocarbons makes them a suitable bioaugmentation agent in comparison with bacteria (Table 8.2). The nontarget specific nature of the ligninolytic enzymes makes this WRT ideal for the remediation of a wide range of contaminants that include dyes, polycyclic aromatic hydrocarbons Pharmaceutical active compounds (PhAC), and pesticides (Mir-Tutusaus et al. 2018). Significant degradation of the contaminants can be achieved in combination with biostimulation; however, the

Table 8.2 Bioremediation of petroleum components present in soil by basidiomycetes fungi

Contaminants	Basidiomycetes	Conditions	Degradation (%)	References
Diesel fuel	*Pleurotus sp.*	5% level of contamination	55.53%	Ogbo et al. (2010)
		10% level of contamination	35.53%	
Benzo(a)pyrene	Ozonation + Spent Mushroom Compost	10 min pre-ozonation treatment	>75%	Russo et al. (2012)
			82%	
Bunker C fuel oil—C10 alkane	*Trametes versicolor*	180 days of growth on pine media	98.10%	Young et al. (2015)
Bunker C fuel oil—C14 alkane			48.60%	
Bunker C fuel oil—Phenanthrene			76.40%	
Bunker C fuel oil—C10 alkane	*P. Strigosozonata*	20 days of growth	99%	Young et al. (2015)
Anthracene	*Pleurotus ostreatus*	110 days incubation	96.00%	Acevedo-Sandoval et al. (2018)
Pyrene			86.00%	
Chrysene			98.00%	
Benzo(a)anthracene			98.00%	
Diesel's F2 fraction (C10-C16)	*Pleurotus ostreatus*, *Salix planifolia*		69–73%	Robichaud et al. (2019)
Hydrocarbons	*A. bisporus*	Soils:25–28 degrees Celsius; 60% water holding capacity	71.50%	Mohammadi-Sichani et al. (2019)
Hydrocarbons	*Pleurotus pulmonarius*	Soil amended with 150 g of sawdust	90.12%	Stanley et al. (2017)
Crude oil	*Lentinus subnudus*	Incubation for 3 months	20%	Adenipekun and Fasidi (2005)
Aromatic weathered hydrocarbon contaminated soil	*Phanerochaete chrysosporium* NRRL 6361	Incubation for 30 days	94.46%	D'Annibale et al. (2005)
PAHs in soil	*Phanerochaete chrysosporium*	19 days incubation	72.77–25.50%	Wang et al. (2009)
High molecular weight polycyclic aromatic hydrocarbons	*Trametes versicolor*	180 days of incubation	71%	Lladó et al. (2013)
	Lentinus tigrinus		61.2%	
PAHs in soil	*Bjerkandera sp. BOS55*	30 days of incubation	30 mg/kg of soil	Valentin et al. (2007)
Nitrobenzene and anthracene	*Trametes trogii*	12–24 days	90%	Levin et al. (2003)

(continued)

Table 8.2 (continued)

Contaminants	Basidiomycetes	Conditions	Degradation (%)	References
PAHs	*Pleurotus ostreatus*	80 days	80–95%	Norton (2012)
Total aliphatic hydrocarbon (TAH)	*Pleurotus ostreatus*	60 days	86.80%	Covino et al. (2016)

possibility of promoting indigenous microbes and subsequently creating competition for the augmented WRT makes this approach under wide scrutiny (Wu et al. 2016). As an alternative by considering the ability of WRT to degrade lignocellulosic material, bulking agents such as rice husk are used (Adewole and Olanrewaju 2017; Meysami and Baheri 2003). The commonly used substrates include rice straw, corn cob, straw bales, sugar beet pulps, cotton waste, wheat bran, rice bran, fragmented wood, sawdust, and coffee pulp (Young et al. 2015, Harry-Asobara et al. 2018). Furthermore, these agents were coated with animal manures or nutrients to enhance effective growth. By considering the significance of extracellular enzymes produced by white-rot fungi and their role in the degradation of hydrocarbons in soil, attempts have been made to increase the activity of the enzyme cocktail by growing in solid-state fermentation in the presence of various bulking agents such as orange peels (Rosales et al. 2007).

8.3.4 Other Fungi

Apart from the white-rot fungi, several ascomycete fungi exhibit a high ability to remediate contaminants mainly by the intracellular metabolism of xenobiotic mediated by cytochrome P450 enzymes, by producing secondary metabolites such as biosurfactants, lignin modifying enzymes, or polyunsaturated fatty acids. Ascomycetes such as *Trichoderma sp*. exhibit the ability to degrade the PAHs by the secretion of laccase to a limited extent (Nazifa et al. 2018). Ascomycetes are well adapted to polluted environments and are more frequent than basidiomycetes in these environments. Marine fungus is one example. By exploiting the ability of the marine fungus to adapt to high saline conditions and pH, utilization of these fungi as bioaugmenting agent have an advantage over terrestrial fungi in degrading hydrocarbons in extreme soil conditions (Table 8.3). Besides, their ability to tolerate heavy metals, such as lead and copper, adds an additional advantage.

8.3.5 Factors Affecting the Fungal Bioremediation

Bioremediation of hydrocarbons by fungi is affected by various factors which include physical-chemical properties of soil, the presence of co-contaminants, and their interaction with contaminants (Magan et al. 2010). The major factors that affect bioremediation are mentioned below.

Table 8.3 Bioremediation of petroleum components present in soil by Ascomycetes fungi

Contaminants	Ascomycetes	Condition	Degradation (%)	References
TPH	*Scedosporium*	182 days	91.20%	Adetutu et al. (2015)
TPH	*Penicillium funiculosum*	Acidic soil	30%	Mancera-López et al. (2008)
	Rhizopus sp		36%	
	Aspergillus sydowii		17%	
Diesel oil	*Fusarium solani EH*	Growing concentration (4 mL/L); diesel oil (7.2 mg/100 mL)	90.28% (static); 93.05% (shaking)	Mohamed and El-Kassas (2010)
Motor oil	*Bionectria sp.*	8% (v/w) of inoculum; 60% moisture content; 10% (v/w) used motor oil	91%	Kota (2010)
Petroleum chain hydrocarbon	*Mucor circinelloides f. circinelloides*		61.80%	HongBo et al. (2011)
Crude oil	*A. niger*	28 days of treatment	95.00%	AI-Jawhari (2015)
Aliphatic hydrocarbons	*Pseudallescheria sp. 18A*	60 days mycoaugmentation	79.70%	Covino et al. (2015)
Diesel spiked soil: C12–40 HC fraction	Trichoderma longibrachiatum	Inoculum conc.:1 × 10^10 conidia/mL; 96 days inoculation	54.2 ± 1.6%	Andreolli et al. (2016)
	Trichoderma harzianum CCECH-Te1		47–69.1%	
Diesel oil: TPHs	*Trichoderma reesei H002*	40 days; Temp-25° C	94.78%	Nazifa et al. (2018)
TPH	*Verticillium sp.*		99.60%	Marín et al. (2018)
Crude oil	*Penicillium citrinum*		77.00%	Barnes et al. (2018)
n-Alkane			95.37%	
TPH	*Lambertella*	2 months incubation	47.60%	Becarelli et al. (2019)
Crude oil	*A. flavus*	15 days	60.00%	Al-Dossary et al. (2019)

8.3.5.1 Oxygen and Nutrient Requirements

The growth and acceleration of the bioaugmented fungi depend on the availability of carbon and nitrogen source in the soil (Quintella et al. 2019). The growth of the fungi is generally hindered if there is a shortage of organic and inorganic compounds. On the other hand, being aerobic, lignin-degrading fungi require a continuous supply of aeration and other key nutrients (Boopathy 2000). Fungi require a continuous supply of oxygen, in addition, molecular oxygen is needed for the activation of key

degrading enzymes, which include laccase and oxygenase. In addition, while degrading the hydrocarbons in the dead soil, additional minerals such as calcium, sulfur, and magnesium along with other nutrients are essentially supplied to support the growth of fungi.

8.3.5.2 pH

The growth of fungi and their stability depend on the pH of the soil (Tortella et al. 2015). Most of the soil pH range from 5.0 to 9.0, which makes the indigenous microbes to sustain. Most of the microbes tolerate pH 4 to pH 8 but preferably pH 6.5 to pH 7.5. However, fungal optimum pH varies from pH 4.0 to pH 7.0, making them a perfect choice for bioremediation of oil spills in acidic soil (pH 4.0) (Asif et al. 2017). In general, before bioaugmenting the fungi, the pH of the soil has to be adjusted either with calcium carbonate or with organic acids to bring it to neutral.

8.3.5.3 Temperature

Temperature plays a major role in affecting the outcome of the TPHs degradation in the soil by fungi. In terms of the hydrocarbons, with the increase in the temperature, a gradual reduction in the surface tension properties leads to an increase in the bioavailability of the TPHs in water (Magan et al. 2010). On the other hand, fungi are sensitive to modulation in the temperature and the optimum temperature required for these fungi ranges from 20 °C to 40 °C (Fukasawa 2018). Depending on the nature of the fungi, during bioaugmentation, a slight variation in temperature gradually reduce the degradation ability and may aid indigenous microbes to propagate. However, in tropical and temperate zones, the temperature does not act as a major limiting factor.

8.3.5.4 Water Availability

Water content plays a crucial role in bioremediation processes by influencing fungal growth and their metabolic activities (Bastos and Magan 2009). Sufficient moisture content is required both for transport of gases and contaminants and for movement and growth of microorganisms. Optimal microbial growth occurs when the moisture content is between 30% and 80% (Sangeetha et al. 2004). When the moisture content is below 10% WRF becomes less active. However, during the waterlogging condition, the moisture content reaches the above optimal limit leading to the development of the anoxic condition, which the bioremediation rates.

8.3.5.5 Other Parameters

Texture, hydraulic conductivity, and permeability are the three interrelated properties of the soil that affect the availability of nutrients, contaminants, and oxygen in the soil. Low permeability soils are ineffective in soil flushing techniques (Magan et al. 2010). The conductivity in the range of 10–4 cm/s supports the transport of nutrients and pollutants within the soil thereby increasing the efficiency of the bioremediation. The presence of organic matter in greater quantities affect the bioavailability of the TPHs and enzymes. On the other hand, the salinity of the soil

has a positive impact on the degradation ability of WRF. It was noted that an increase in the salinity along with temperature reduces the growth of WRF (Tortella et al. 2015).

8.4 Types of Bioaugmentation

Apart from the fungal selection, bioaugmentation can be classified into three types depending on the external supplements. With the advancement, several organic and inorganic supplements were reported to be included with bioaugmentation, making a huge list. To simplify the types of bioaugmentation were broadly classified into

1. Bioaugmentation and biostimulation with organic nutrients.
2. Bioaugmentation and biostimulation with inorganic nutrients.
3. Bioaugmentation with surfactants.

Among the above three strategies, bioaugmentation with surfactants for the remediation of soil, TPH is considered to enhance the efficiency of the degradation process by reducing the overall time. Surfactants are compounds that reduce the surface tension between two interfaces (Mao et al. 2015). In the soil, utilization of the surfactants reduces the interfacial tension that holds TPHs with soil organic matter and makes them release into the aqueous phase. In the aqueous, the surfactant traps the TPHs in their micelles (Goddard 2017). The micelles are surfactant aggregates that are formed in liquid colloid, with either hydrophobic "tail" or hydrophilic "head" facing the aqueous phase. The concentrations that are required for the surfactants to form micelles are called critical micellar concentration (CMC).

The most common type of surfactant that is used is an anionic surfactant or nonionic surfactant (Table 8.4). Apart from the advantage of using surfactants, surfactants exhibit the ability to adsorb to soil particles and exhibit toxicity, which is more prominent in cationic surfactants (Mao et al. 2015). On the other hand, the surfactants are complex in structure and resist degradation which further complicates the process. As an alternative, several biosurfactants were introduced, which are produced by microorganisms. These compounds are easy to degrade and exhibit

Table 8.4 Common surfactants used for the bioremediation of hydrocarbons

	Anionic	Cationic	Non-ionic
Surfactant	• Sodium dodecyl sulfate (SDS) • Perfluorooctanoic acid • Sodium dodecyl • Benzene sulfonate	• 1-dodecylpyridinium • Chloride • Didecyl dimethyl ammonium chloride	• Tween 80 • Triton X-100 • Triton X-20 • Cocamide • Brij-35
Biosurfactant	• Rhamnolipids		• Sophorolipid • Fructose lipid • Surfactin • Guar gum

similar properties to that of chemical surfactants (Lang and Wagner 2017). The commonly utilized biosurfactant is rhamnolipids, which is a widely used anionic biosurfactant in the remediation of PAHs in soil (Zhen et al. 2019).

In addition, surfactants can increase the secretion of ligninolytic enzymes by removing the enzymes that are trapped in the mycelium or hyphae of the white-rot fungi (Singh and Singh 2017). On the other hand, surfactants have the ability to ease transport of vesicles that could carry enzymes. Despite several advantages, utilization of the surfactants beyond certain concentrations causes toxicity towards the WRF (Lechuga et al. 2016).

8.5 Mechanisms of Petroleum Fungal Hydrocarbon Degradation

In most organic pollutants, complete degradation was achieved in the aerobic conditions. Oxidation of organic pollutants is considered as an initial step in the degradation process by WRF, which is followed by a series of catalytic actions of oxygenases and peroxidases leading to mineralization of the organic pollutant to either carbon dioxide or water. On the other hand, peripheral degradation pathways convert these pollutants into several central intermediate metabolism compounds.

8.5.1 Fungal Degradation of PHCs Polycyclic Hydrocarbons

Mycoremediation suitably occurs either through adsorption of the hydrocarbon or degradation of the compound by the fungi. The petroleum hydrocarbon contaminants sustain proportionate molecular structures that enable effective fungal strains to act upon them. Fungi follow two main mechanisms for breaking down recalcitrant petroleum hydrocarbons.

1. *Intracellular attack via cytochrome P450 (CYP 450)*, utilizing oxidation, reduction, hydrolysis, and dehalogenation processes. The recalcitrant compounds adsorbed into the fungal system and degraded by cytochrome P450 monooxygenase in microcosms have been reported (Das and Chandran 2011). The CYP system first catalyzes the aromatic structured contaminants to form arene oxide, which is highly reactive and carcinogenic. Further, these arene oxides are catalyzed to trans-dihydrodiols by epoxide hydrolase or rearrange nonenzymatical to phenols. Moreover, hydroxylation products undergo the detoxification process to form various intermediates, which are excreted (Prenafeta-Boldú et al. 2018).
2. *Extracellular oxidation* of petroleum hydrocarbons occurs through the secretion of extracellular enzymes by the fungi for the oxidation of the recalcitrant compound mediated by oxidative and ligninolytic enzymes.

8.5.2 Ligninolitic Enzymes

Laccases (EC 1.10. 3.2) are multicopper enzymes, which catalyze oxidation reactions coupled to the four-electron reduction of molecular oxygen to water. These high redox potential enzymes have the ability to oxidize the phenolic compounds including TPH to carbon dioxide (Prenafeta-Boldú et al. 2018). This substrate nonspecific enzyme is commonly produced by fungal ascomycetes such as *Trichoderma* and Basidiomycetes—white-rot fungi. In addition, the requirement of oxygen as co-substrate for the initial degradation makes the laccase a more popular choice for the degradation of TPH. The key intermediate product in oxidation of aromatic hydrocarbons is phenoxy radicals, which couple to quinone followed by ring fission leading to the formation of carbon dioxide and water as end products (Hwang et al. 2007).

Tyrosinase (EC 1.14. 18.1) is derived from *Streptomyces glaucescens* and the fungi *Neurospora crassa* and *Agaricus bisporus*. Recent studies suggest the ability of white-rot fungi to produce tyrosinase (Seo et al. 2003). Contrary to laccases, tyrosinase catalyzes the initial step in the formation of the pigment melanin from tyrosine (Madhavi and Lele 2009). Tyrosinases are metalloenzymes belonging to the type-3 copper protein family which contain two copper ions in the active site. Tyrosinases perform two sequential enzymatic reactions: hydroxylation of monophenols and oxidation of diphenols to form quinones which polymerize spontaneously to melanin. Tyrosinase is capable of oxidizing the phenols into insoluble substances that can be eliminated by precipitating or filtering them.

Lignin peroxidase (EC 1.11. 1.14) is also referred to as diaryl propane oxygenase. Lignin peroxidase is a heme-containing enzyme that catalyzes hydrogen peroxide-dependent oxidative degradation of lignin. Lignin peroxidase catalyzes the biodegradation of lignin using hydrogen peroxide. Lignin peroxidase oxidizes phenols to phenoxy radicals and nonphenolic aromatics to radical cations.

Compared to lignin peroxidase, manganese peroxidase follows a different mechanism of action. Manganese peroxidase (EC 1.11. 1.13) is an extracellular heme enzyme that catalyzes the peroxide-dependent oxidation of Mn (II) to Mn (III). Manganese peroxidase catalyzes the conversion of Mn^{+2} to Mn^{+3} using hydrogen peroxide. Aryl-alcohol oxidase is the third class of ligninolytic enzymes. Aryl-alcohol oxidase (EC 1.1. 3.7) belongs to the family of oxidoreductases specifically those acting on the CH–OH group of donors with oxygen as acceptor. Aryl-alcohol oxidase oxidizes a variety of aromatic benzyl (and some aliphatic polyunsaturated) alcohols to the corresponding aldehydes. In addition, aryl-alcohol oxidase participates in the oxidation of aromatic aldehydes to the corresponding acids and also has activity on furfural derivatives.

Lligninolytic enzymes exhibit a mutualistic working mechanism. Among them, laccase and AAO work through direct oxidation, by attaching to the hydroxyl functional groups and thereby breaking down large phenolic polymers. On the other hand, lignin peroxidase and manganese peroxidase act by producing reactive peroxide species, which aid in the degradation of hydrocarbon compounds. Overall, relating the by-products produced by these enzymes confirms the mutualistic role of

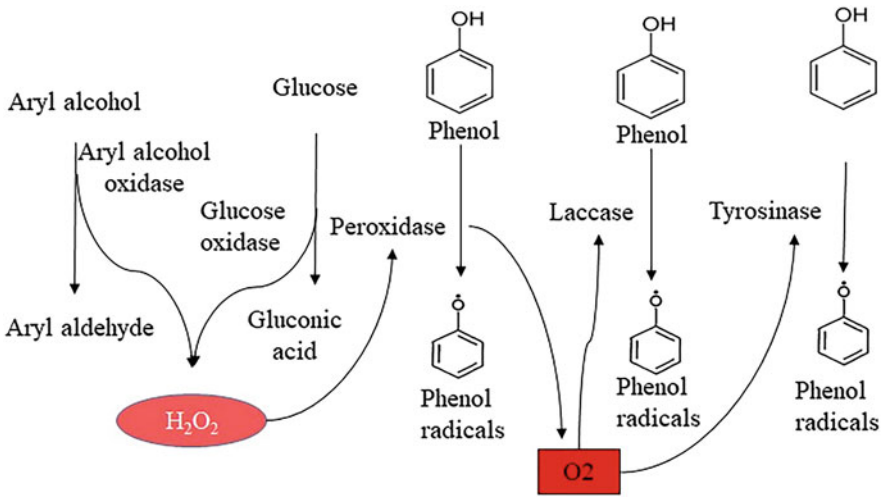

Fig. 8.3 Mutualistic mechanism of ligninolytic and oxidative enzymes in degradation of phenolic compounds

ligninolytic and oxidative enzymes in the degradation of hydrocarbons (Fig. 8.3). Being controversial on the degradation debate of pollutants by ligninolytic enzymes or laccase as a cumulative and as sole, the current results clearly shows the cumulative effect of the enzymes in the degradation of pollutants (Agrawal and Shahi 2017).

8.6 Conclusion

Bioremediation of the subsurface oil spills or the presence of petroleum hydrocarbons in the soil is of major concern due to its potential toxicity. Bioremediation processes by fungi, specifically by white-rot fungi, involve multiple dimensional studies by considering various factors that affect the efficiency. Despite various techniques to enhance the remediation, including surfactants and bulking agents to address the problem, there is still a gap for innovation needed to design industrially feasible processes. With current knowledge on the bioremediation of petroleum hydrocarbons degrading pathway and the enzymes involved, it may be concluded that white-rot fungi with surfactants can be considered as a key strategy for the cleanup of soil contaminated with petroleum hydrocarbons.

Acknowledgments Authors thank the Group RNM-270 of Environmental Microbiology. EA gratefully thank the Ministry of Economy and Competitiveness (MINECO-FEDER) RYC-2013-12,481.

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9 Microbial Biosurfactant in the Removal of Hydrophobic (Oily) Pollutants Laden Industrial Wastes

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9.1 Introduction

The exponential growth of industrial sectors in the past few decades made the lifestyle more comfortable and luxurious. But on the other side, it has been negatively impacted by the rapid increase in environmental pollution and other environmental hazards. Owing to this, petroleum pollution is one of the most prevailing causes of hydrocarbon contamination on soil and water, ever since the industrial revolution showing harmful effects on all living organisms particularly the microbial population (Karlapudi et al. 2018). The discharge of hydrophobic hydrocarbons/oily wastes from industries into the environment is hastily increasing every year due to rapid urbanization and industrial developments (Affandi et al. 2014). The toxic hydrocarbon laden wastes generated by the industries are difficult to treat due to their complex hydrophobic nature. In the past, researchers have approached with several physiochemical techniques to tackle these prevailing issues in which the chemical methods include the use of surfactants, polymers, acids, bases, and solvents for remediation of hydrocarbons/oil-laden industrial wastes (Gudiña et al. 2012). Due to the fact that most of these methods are chemical-based, their application, in the long run, would in turn create secondary pollution since the accumulation of by-products are harmful to the environment. Therefore, the search and demand for

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_9

an efficient and environment-friendly alternative for oil/hydrocarbon remediation are increasing among the scientific community. Studies on the microbial community that are prevalent in hydrocarbon-contaminated sites led to the discovery of biosurfactants as an extracellular amphiphilic compound (Ahmadi-Ashtiani et al. 2020).

Biosurfactants are natural surface-active molecules that decrease the surface and interfacial tension of the liquid-liquid, solid-liquid, or air-liquid medium (Kandasamy et al. 2019). They are produced by microorganisms such as bacteria, fungi, and algae found in environments with a high level of hydrocarbon content. These molecules are mainly isolated from microorganisms which are found in marine environments, oil fields, or hydrocarbon-contaminated industrial wastelands (Pacwa-Płociniczak et al. 2011). The mechanism of action is very similar to chemical surfactants. Like chemical surfactants, biosurfactants too have a hydrophilic head and a hydrophobic tail that attaches to the hydrocarbon (oil) molecule and aid in its mobilization which is followed by their solubilization (Urum and Pekdemir 2004). Biosurfactants are mainly classified into four categories, based on the structure of their hydrophilic part, i.e., (i) glycolipid, (ii) fatty acid, (iii) lipopeptide, and (iv) polymer (Mnif et al. 2017). Due to their high biodegradability and environmental safety, biosurfactants started to be viewed as an alternative to chemical surfactants. As compared to their chemical counterparts, biosurfactants have high environmental tolerance and vast substrate availability which make them commercially more suitable (Mnif et al. 2015). The main focal theme of this chapter is to describe the wide application of biosurfactant flushing technique for the remediation of hydrocarbon/oil-laden wastes generated from industries like petroleum, food, and textile. In addition to this, this chapter describes the detailed mechanism of biosurfactant flushing strategies adapted for the treatment of petroleum industrial wastes have been described.

9.2 Nature and Types/Classification of Biosurfactants

Biosurfactants are generally divided into two main aspects: a) Combination of chemical elements; b) Microbial origin. Further, the biosurfactants are classified into four categories such as Glycolipid biosurfactants; Lipopeptide and lipoproteinsbiosurfactants; Fatty Acids, Phospholipids, and Neutral Lipid biosurfactants; Polymeric biosurfactants; and Particulate biosurfactants.

9.2.1 Glycolipid Biosurfactants

These are the most common forms of biosurfactants, due to their structural diversity and facile production strategies (Mnif et al. 2018). These are rooted as fatty acids of hydroxy fatty acids linked to carbohydrate by glycosidic bond. The backbone being carbohydrate and lipid structure offers a wide diversity in their structure and classification within glycolipids (Daverey and Pakshirajan 2009). Glycolipids are subdivided into the following:

9.2.1.1 Rhamnolipids

This group consists of one or two rhamnose groups bound to β-hydroxydecanoic acid, synthesized namely as 1-Mono rhamnolipid and 2-Di rhamnolipid. They are derived from *Pseudomonas aeruginosa.* They acquire several biological activities like hemolytic, anticancer, antifungal, antibacterial, and antiviral due to which they are used as biocontrol agents. Rhamnolipids are recognized based on characteristic features like higher emulsification and surface-active properties which mediate in enhancement of hydrocarbon solubilization and biodegradation (Abdel-Mawgoud et al. 2010; Vijayakumar and Saravanan 2015).

9.2.1.2 Trehalose Lipids

These are the disaccharide trehalose that is linked to mycolic acids having long-chain α-branched-β-hydroxy fatty acids. They are derived from gram-positive bacteria of *Actinomycetes* such as *Mycobacterium*, *Nocardia,* and *Corynebacterium*. Their surface activity makes them part of various environmental applications as improvers of hydrophobic solubility and desorption from contaminated soil for enhanced oil recovery. They also have several biological properties hence used in the biomedical field (Vijayakumar and Saravanan 2015).

9.2.1.3 Sophorolipids

Sophorolipids incorporate carbohydrate sophorose bonded to long-chain hydroxyl fatty acid through glycosidic linkage. These are mainly derived from yeast variants such as *Candida bombicola, C. magnoliae, C. apicola,* and *C. bogoriensis*. Lactonic and anionic sophorolipids are worn to shrink the surface and interfacial tension. These are not used as emulsifiers because they are not potent at stabilizing water-in-oil emulsions. Sophorolipids are fruitful in various industries like cosmetics and pharmaceuticals and have wide applications as surface-active components (Shoeb et al. 2013).

9.2.2 Lipopeptide and Lipoprotein biosurfactant

These are basically cyclic lipopeptides affixed to fatty acid chain which have exceptional surface-active properties. In the spotlight, the cyclic lipopeptide Surfactin, which is produced by *Bacillus subtilus* is considered as one of the potent and active biosurfactants (Hamley 2015). They have diverse biological activities and also help in reducing surface tension. Iturin which is produced by *Bacillus subtilis* is also an active surface agent with antifungal and antimicrobial activity (Banat et al. 2010).

9.2.3 Fatty Acids, Phospholipids, and Neutral Lipids Biosurfactant

Few bacterial and yeast variants bring out enormous amounts of phospholipid and fatty acid biosurfactants during growth in a culture medium accommodating

n-Alkanes. Numerous forms of fatty acids and lipids are often seen as extracellular products of a microbial cell found to have surface activity. Few organisms growing on hydrocarbons are required for certain fatty acids or neutral lipids' extracellular production by bacterial strains. This suggests that they play a vital role in Hydro carbon Emulsification. Saturated fatty acids (in the range C12–C14) are required for lowering the surface and interfacial tensions. Phosphatidylethanolamines produced by *Acinetobacter* sp. HO1-N is a vesicle-forming emulsifying agent (Ahmadi-Ashtiani et al. 2020).

9.2.4 Polymeric Biosurfactants

A vast number of bacterial species from diverse genera fabricates exocellular polymeric surfactants composed of mixtures of various biopolymers such as lipoproteins, lipopolysaccharides, and proteins. These include Emulsan, Liposan, Alasan, Polysaccharide protein complex, Biodispersan, and mannoproteins. The main property of these surfactants is they act as emulsifying agents. They are mainly produced from the strain of *Acinetobacter*. Biodispersan produces from *Acinetobacter calcoaceticus* is an anionic heteropolysaccharide which acts as a nondialyzable dispersing agent. Liposan from *Candida lipolytica* is a water-soluble emulsifying agent (Cortes-Sánchez Alejandro 2011; Vijayakumar and Saravanan 2015).

9.2.5 Particulate Biosurfactants

Particulate biosurfactants split up extracellular membrane vesicles from forming a microemulsion that exerts impact on alkane uptake in microbial cells. *Acinetobacter, Calcoaceticus, Cyanobacteria,* and *Pseudomonas* are a few microorganisms that produce these types of biosurfactants (Dhote et al. 2010).

9.3 Sources of Biosurfactants

Microorganisms use a wide range of organic compounds as a source of carbon and energy for their growth and metabolism. Researchers have reported that when the carbon source is immiscible like a hydrocarbon, microorganisms tend to make possible diffusion of these compounds into the intercellular unit by secreting a variety of extracellular substances such as biosurfactants (Sharma et al. 2020). Microorganisms used for the industrial application for the removal of waste products usually are isolated from hydrocarbon pollutant laden areas such as oil fields, wastelands, wastewater discharge points, and marine environments. Extremophiles, i.e., microorganisms that are found in extreme living conditions such as extreme temperature (thermophiles), pH (halophiles), and pressure (barophiles) are also viewed as good sources for biosurfactant production (Schultz and Rosado 2020).

Biosurfactants from such extreme environments show higher stability at a broad temperature and pH range. Apart from the type of microorganism used, the type of substrate used also greatly influences the quantity and quality of the biosurfactant production. Carbon source is an important factor affecting the production of microbial biosurfactants (Das et al. 2009). Likewise, the concentration of other nutrients such as Nitrogen, Phosphorus, Manganese, Magnesium, and Iron also affects the biosurfactant production rate. The selection of microorganisms for the use of microbial-enhanced oil recovery is based on varying conditions which they use such as temperature, pressure, pH, and salinity must be given priority (Shekhar et al. 2015). It is reported that there is a clear correlation exists between the type of surfactant and the type of hydrocarbon/oil that gets degraded (Karlapudi et al. 2018). Some of the widely used sources for biosurfactant production are listed below.

9.3.1 Bacterial Biosurfactants

Bacteria play a major role in biosurfactant production. Bacteria produce biosurfactants in the form of biofilm. Biofilm interacts with an interface between the immiscible substrate and the cell wall of bacteria and alters the surface properties such as wettability and surface tension to facilitate the uptake of nutrients. Different strains of bacteria are observed to excrete different types of microbial surfactants. *Pseudomonas* is the dominant genus involved in biosurfactant production (Rufino et al. 2007). It is known for producing rhamnolipids, ionic biosurfactants which emulsify CxHy substance in the growth medium. Various *Mycobacterium* sp. and *Enterobacter* sp. produces lipopolysaccharide biosurfactants to change the structure of their cell wall (Jonas 2002; Abdel-Mawgoud et al. 2010). *Acinetobacte*r spp. due to its versatile nature is a good source of emulsan, a lipopolysaccharide (Schultz and Rosado 2020). *Bacillus Subtilis*, a marine bacterium is used for producing lipoprotein biosurfactants such as surfactin and subtilisin (Fenibo et al. 2019).

9.3.2 Fungal Biosurfactants

Unlike bacteria, the field of production of biosurfactants from fungi is not so well explored. Relatively fewer fungi are known to produce biosurfactants. Many of these are known to produce biosurfactants on low-cost raw materials, lowering the production cost. Majorly glycolipids biosurfactants are produced by fungal strains (Vijayakumar and Saravanan 2015; De Giani et al. 2021). *Candida* sp. is the most commonly available fungal species for biosurfactant production. Many authors have reported other fungal species for biosurfactant production, for example, *Candida bombicola* for the production of sophorolipids and *Yarrowia lipolytic* is well-known fungi for the production of lipid carbohydrate protein-based emulsifiers (Schultz and Rosado 2020). Some Yeast are preferred to bacteria as sources for biosurfactant production, mainly due to their GRAS status for environmental and health safety reasons (Daverey and Pakshirajan 2009; Rocha e Silva et al. 2017).

*Ascomycetes*such as *Aspergillus, Penicillium,* and *Fusarium*, among others, are also regarded as good fungal biosurfactant producers due to their ability to utilize a high number of resources as substrates for their growth (Sachdev and Cameotra 2013; Andrade et al. 2018).

9.3.3 Algal Biosurfactants

Due to the structural and functional diversity, biosurfactant production from algae has been an interesting area for researchers. Algal biosurfactants are mostly lipid in nature and classified as glycolipids, phospholipids, lipopeptides, natural lipids, fatty acids, and lipopolysaccharides. Macro and microalgae are reported to be good producers of exopolysaccharide (EPS) type of biosurfactants (Rahman et al. 2019). The production of biosurfactants by marine algae is still an area with limited literature. To broaden its scope, extensive research is required for better algal sampling techniques, effective screening, and identification and characterization techniques for algal-based biosurfactants.

9.4 Properties and Environmental Fate of Microbial Biosurfactant

The unique properties of biosurfactants which stand out from their chemically synthesized equivalents made them appropriate for a wide range of industrial applications (Kandasamy et al. 2019). The major high spots of the properties are discussed below.

9.4.1 Surface and Interfacial Activity

The biosurfactants aid in diminishing the surface strain and interfacial pressure. They also show exceptional efficiency compared to chemical/synthetic surfactants. As they are surface-active agents, they reduce the surface tension of water exceptionally. They work even in extreme temperature conditions also called extremophiles. Their CMC level ranges from 10 to 40 times lesser than chemical surfactants. Hence, little of it goes a long way (Karlapudi et al. 2018). Sophorolipids produced by *T. bombicola* reduces the surface tension. Trehalose lipids from *Rhodococcus erythropolis* and *Arthrobacter* sp. lowered the surface and interfacial tension in culture broth from 25 to 40 and 1–5 mNm, respectively (Ahmadi-Ashtiani et al. 2020).

9.4.2 Temperature and pH

Few biosurfactants fit into both of these factors as thermostable and pH stable. They can be adapted to any extremities and show resistance to natural factors like temperature and pH. The numerous easy adaptabilities shown by biosurfactants, function as a way for huge commercialization in industries. The activity and stability of a glycolipid bio-emulsifier produced by Streptomyces spp. SS 20 was effective over a broad range of conditions: pH range of 3–7, temperature range of 30–100 °C, and NaCl concentration of up to 3% w/v. (Sobrinho et al. 2014). Research studies suggest that lichenysin, which is produced by *B. licheniformis* was less affected by pH (4.5–9.0), temperature (up to 50 °C), and NaCl as well as Ca concentrations (Karlapudi et al. 2018).

9.4.3 Biodegradability

In the present era where chemicals are overused in many aspects, biosurfactants are believed to have a significant effect to rescue the environment. They are easily degraded without showing any adverse effects. While the chemical surfactants possess a greater threat, the degradability of biosurfactants is exceptional with respect to cutting down the environmental threats (Abdel-Mawgoud et al. 2010). They are easily degraded by the microorganisms in nature making them appropriate for bioremediation and waste treatment. Their capability to degrade harmful pollutants is used in numerous industries like petrol, pharmaceutical, cosmetics, and food. Biosurfactants that are produced by *pseudomonas* have a wide range of applications in industries due to their nontoxic and environment-friendly nature. Many studies suggested that the toxicity levels are exceptionally less than other synthetic surfactants. Their ability to form emulsions and degrade toxic pollutants by increasing surface area and eventually leading to upgrade bioavailability is proven to be phenomenal (Sobrinho et al. 2014).

9.4.4 Low Toxicity

Biosurfactants are highly commercialized these days due to their basal factor of being less toxic. They are considered to be the most suitable organisms industrial-wise in various employments. As we know that the use of synthetic/chemical surfactants poses a great threat to the environment, switching to biosurfactants for their various considerable properties is a better option. As we know there are a number of harsh chemicals coming down from various industries leading to water pollution, soil pollution, and pollution of many resources. We need stringent and sustainably most acceptable forms to treat these pollutants. Chemical surfactants are composed of many harsh chemicals which in turn pollute the environment again in spite of using them for finding the solution. Low toxicity of biosurfactant,

sophorolipids from *Candida bombicola* made them useful in food industries (Daverey and Pakshirajan 2009).

9.4.5 Biosurfactants as Emulsifiers

Biosurfactants have the ability to act as emulsifiers and de-emulsifiers (Banat et al. 2010). Emulsifier helps to shoot up the stability of a compound by reducing the speed of chemical reaction. An emulsion is a heterogeneous foundation of incompatible compounds like Oil in water and Water in oil. Bio-emulsifiers are good alternatives to chemical/synthetic emulsifiers which are sometimes harmful. They have a number of unique notable features like good efficiency, biocompatibility, and high adaptability at different pH, temperature, and salinity. Mannoprotein bio-emulsifier is a glycoprotein within the cellular wall of *Saccharomyces* sp. and *Kluyveromyces marxianus* of yeast. In addition, the yeast strain *Rhodotorulaglutinis* produces an extracellular emulsifier (Ahmadi-Ashtiani et al. 2020).

9.4.6 Anti-Adhesive Agents

Biosurfactants act as a good anti-adhesive agent. They alter the hydrophobicity of the microorganisms which is the main pillar for the surface adhesion in a biofilm. A Biofilm is regarded as a cluster of microbes secured over a surface. There are a number of factors that contribute to maintain connection with the surface such as Charge of surface, Environmental status, External polymers of microbe, and Hydrophobicity. Biosurfactants are utilized in altering hydrophobicity value which regulates their adhesive connection. The anti-adhesive activities of the biosurfactants were seen against *Staphylococcus aureus* ATCC 29523, *Salmonella typhimurium* ATCC 19430, and *Bacillus cereus* ATCC 11778 (Banat et al. 2010).

9.4.7 Availability

Biosurfactants are broadly available and widely used in many industries. They are comparatively much more easily available than synthetic surfactants. Due to their huge beneficial roles to the environment and eco-friendly aspect they are used around due to easy availability (Colores et al. 2000).

9.5 Existing Physicochemical Technologies in Oil Flushing and their Drawbacks

For more than a decade, the primary focus of environmental experts has been to adopt risk-based management approaches to cleanup the total petroleum hydrocarbons (TPH) polluted sites that pose potentially destructive ecological

consequences. This attention led to the development of several physicochemical, thermal, and biological technologies that are widely implementable (Atteia et al. 2013).

9.5.1 Centrifugation

It is a purely physical method for extracting oil from contaminated fields. The oil sludge is heated to facilitate the pumping and then transferred to the storage tank. The storage tank consists of a mixture of inorganic compounds, sand, wax, and heavy crude oil. A three-phase centrifuge is used as shown in Fig. 9.1, to separate the water phase, oil phase, and solid phase. This process normally leaves residual solid waste containing 6–15% hydrocarbon and is able to recover almost 90% of crude oil (Ramadan et al. 2018).

9.5.2 Soil Flushing

Soil flushing is an in situ remediation technique for extracting hydrocarbon pollutants from the soil by applying water with the appropriate flushing agent to the soil. Pollutants get dissolved in the flushing solution and are leached into the groundwater and then extracted. Sometimes, the flushing solution is injected directly into the groundwater. This results in the rise of the water table into the capillary fringe just above the surface of the water table, where high concentrations of pollutants are found. The biological effects of low-pressure saltwater flushing of a thin oil layer use of seawater flushing as a method for cleaning up gross oil pollution on sediment shores, surfactants such as SDS (sodium dodecyl sulfate) and AMA (sodium hexyl sulfosuccinate) are used as flushing agents to enhance the contaminant solubility. Surfactants are capable of forming aggregates known as micelles

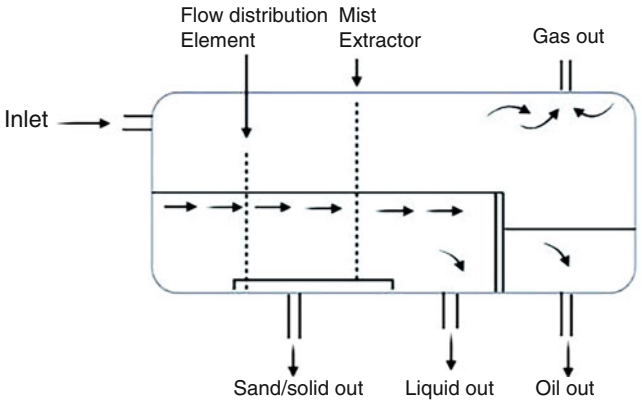

Fig. 9.1 Illustration of three-phase centrifugation method for oil extraction

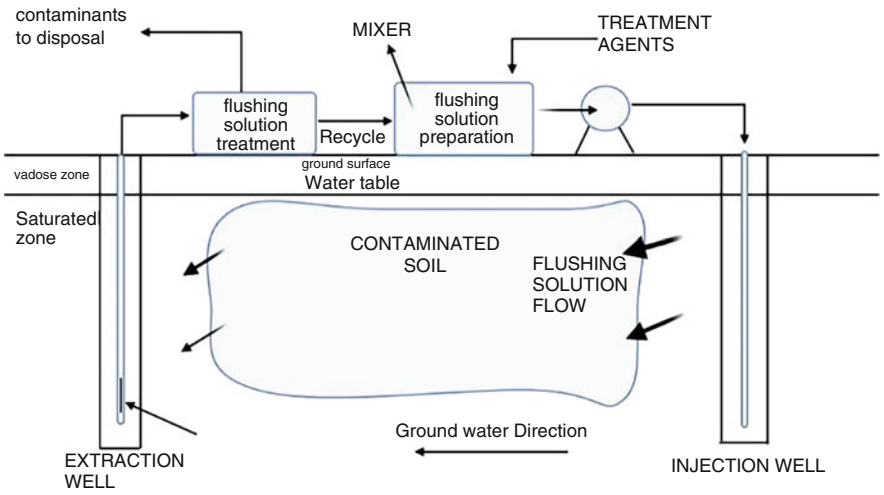

Fig. 9.2 Soil flushing in situ remediation technique for extracting hydrocarbon pollutants from contaminated soil

thus solubilizing hydrophobic organic compounds (Abdel-Moghny et al. 2012). The use of surfactant alone gives efficiencies of about 80–85% in laboratory experiments, but the amounts of product to be injected are very important, which does not seem to be economically sustainable (Atteia et al. 2013). Apart from surfactants, some other molecules are also used as flushing agents. Alcohols can also increase the solubility of organic compounds, albeit in a somewhat different manner. Rather than forming aggregates with nonpolar interiors, water-miscible alcohols lower the polarity of the aqueous phase thereby increasing the aqueous concentration of sparingly soluble organic compounds. Although, much higher alcohol concentrations as compared to that of surfactants are required to achieve high contaminant solubility (Perfumo et al. 2010). Solubilization agents (such as cyclodextrin) increase solubility in water but do not modify the behavior of the organic phase. Studies using cosolvents in soil flushing demonstrated its ability to transform a dual-phase system (oil/water) into a single phase that will be a mixture of all compounds. Other flushing agents such as polymers increase viscosity in high permeability areas and thus tend to favor flushing of low permeability areas and thus highly contaminated zones, foams have similar behavior, leading to homogeneous displacement fronts (Atteia et al. 2013). To implement this method efficiently, the soil permeability must be high enough (e.g., 102 cm/sec) to allow water to be pumped through the soil, also soil homogeneity must be uniform so that the entire contamination zone is treated without short-circuiting (Fox 1996). Since the aid of chemical surfactants and other inorganic molecules is required to enhance the efficiency of this method, it is not environmentally feasible to practice for the long term. A schematic representation of the soil flushing technique is shown in Fig. 9.2.

9.5.3 Chemical Extraction

Extraction using a chemical solvent is one of the most common methods applied to solve the oily sludge problem. The chemicals used non-specifically oxidize the organic pollutants present in the contaminated site. Many oxidants have been studied till now for the bioremediation of crude oil through chemical oxidation. Among the most common oxidants used in soil, remediation is ozone, hydrogen peroxide, hypochlorite, chlorine, permanganate, and persulfate (Ramadan et al. 2018). This technology uses oxidizing agents to transform organic contaminants into non-hazardous or less toxic, mobile, or inert compounds. Also, coupling this technology with other conventional methods such as in situ soil flushing has been proven to be very useful and efficient. Though this method is very effective in treating oil sludge, it has its shortcomings. Firstly, the chemicals used as oxidizing agents are very expensive so it is a very costly method. Secondly, sometimes the product of chemical oxidation treatment also is toxic. This method is not compatible with the environment hence cannot be practiced frequently. An overview of the process of chemical extraction is shown in Fig. 9.3.

9.5.4 Soil Vapor Extraction

Soil vapor extraction, also known as soil venting or vacuum extraction, is a relatively simple method for remediation of total petroleum hydrocarbons (TPHs) contaminated soils. This technique uses a vacuum pump process to treat gases and volatile and semi-volatile organic compounds from contaminated soil petroleum hydrocarbons (Ramadan et al. 2018). For this, vertical and/or horizontal wells are installed in the area of soil contamination. Huge fans used as air blowers are used to aid the evaporation process. Vacuum is then pumped into the wells near the source of contamination to evaporate the volatile hydrocarbons of the contaminated mass and its vapors are collected in the extraction well. Extracted vapors are then treated commonly with carbon adsorption before being released into the atmosphere (Ingle et al. 2014). The major drawback of this method is it consumes a lot of time. The duration to achieve cleanup of oily waste ranges from months to years. Also, this

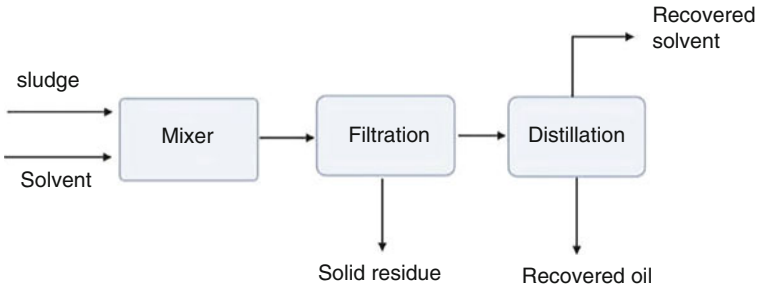

Fig. 9.3 General flow sheet for the treatment of oily sludge using chemical solvents

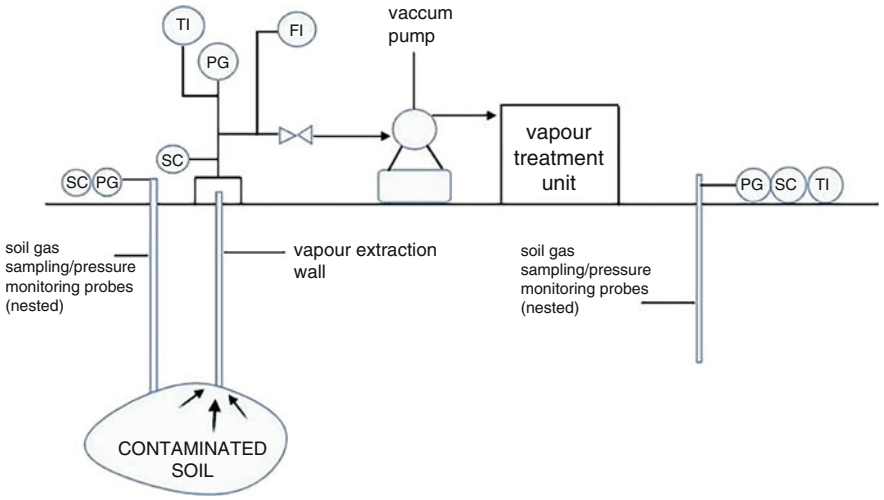

Fig. 9.4 Overview of process flow sheet of soil vapor extraction method for the removal of hydrocarbons/oil from contaminated soil

method is specific for volatile hydrocarbons, such as methane. Long-chain hydrocarbon pollutants cannot be efficiently removed by this method. An overview of the soil vapor extraction process is shown in Fig. 9.4.

9.6 Importance of Microbial Biosurfactant in Oil Flushing

Oil is one of the most highly demanded resources which is leading the development from the front. As the oil demand is increasing day by day the greed and need for it are not any less. As important is their presence in this growing development, so it is one of the harmful environmental pollutants. Various contributions and inventions have been developed in recent years in order to fix this problem (Maudgalya et al. 2005). But fixing oil reservoirs with the help of chemical surfactants, polymers, etc., can eventually lead to environmental impact again. Oil spills are critical environmental concerns that need fixation. Biological fixation by biosurfactants is the most acceptable method for this problem. The action of biosurfactants for oil flushing is widely used as it does not impact the environment while it does not intake huge energy, is cost-effective, has low toxicity, etc.(Chandankere et al. 2014). Based on few studies, it is already reported that few strains of biosurfactant-producing bacteria have the capability to degrade oils and such contaminants. Many reports mentioned these methods either based on biosurfactant produced externally (extracellular) or internally (intracellular). Biosurfactants increase pseudo-solubility due to their specificity and degradability (Karlapudi et al. 2018). Oil flushing is one of the methods conducted for oil recovery which flushed out the hydrocarbons that are hugely

volatile and help make them light and more valuable which further continues for oil recovery methods.

9.7 Mechanism of Microbial Biosurfactants on Hydrophobic Substrates

Biosurfactants are a structurally diverse group of surface-active molecules, produced extracellularly by microbes found in hydrocarbon-laden environments such as wastelands, marine environments, and oil fields to facilitate the uptake of nutrients in such conditions. Just like the chemical surfactants, biosurfactants are also amphiphilic molecules consists a hydrophilic head and a hydrophobic tail. A hydrophilic group consists of mono-, oligo- or polysaccharides, peptides or proteins, and a hydrophobic moiety usually contains saturated, unsaturated, and hydroxylated fatty acids or fatty alcohols (Pacwa-Płociniczak et al. 2011). Due to their amphiphilic structure, biosurfactants decrease the interfacial tension at air-water and water-oil interfaces, increasing the bioavailability of such substances and changing the properties of the bacterial cell surface. As the interfacial tension is reduced and the aqueous surfactant concentration increases, the monomers aggregate to form micelles. The concentration at which micelles first begin to form is known as the critical micelle concentration (CMC) and it corresponds to the point where the surfactant first shows the lowest surface tension (Urum and Pekdemir 2004). An efficient surfactant should reduce the surface tension of water from 72 mN/m to 30 mN/m and have a low critical micelle concentration (CMC). CMC is defined as the concentration of surfactants above which micelles form, and all surfactants subsequently added to the system become micelles (Kandasamy et al. 2019). The biosurfactants' action greatly depends on the CMC as represented in Fig. 9.5a, b. Biosurfactants act on the hydrophobic substrates in two phases: (a) Mobilization: Occurs at the Critical micelle concentration (CMC) range of surfactant. At this stage,

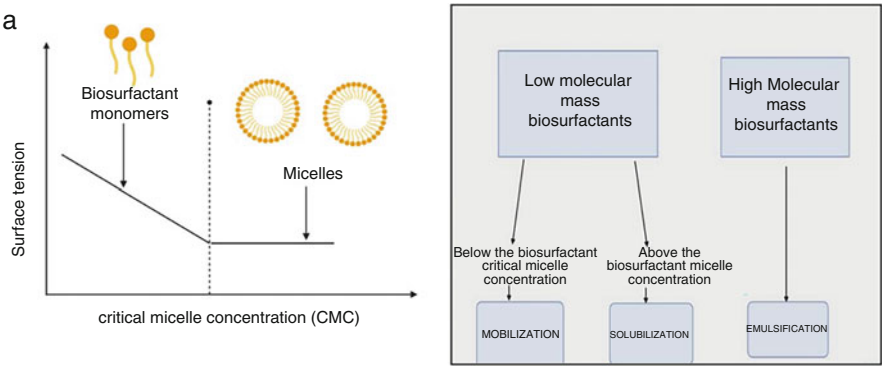

Fig. 9.5 (**a**) Relation between (**a**) CMC concentration and surface tension (**b**) CMC and action of biosurfactants on hydrocarbon/oily wastes

surfactants reduce the surface and interfacial tension between air/water, oil/water, and soil/water systems. The interfacial force holding the soil and oil together decreases and biosurfactants in contact with the soil/oil system increase the contact angle. Since its adsorption, this process highly depends on the charge of the biosurfactants. Mostly biosurfactants are non-ionic or Anionic in nature. (b) Solubilization: As the concentration becomes above CMC, the solubility of oil increases dramatically due to the aggregation of surfactant micelles. The hydrophobic ends of the biosurfactant molecules cluster together inside the micelle structure, trapping the oil molecule with the hydrophilic ends exposed to the aqueous phase on the exterior. Now, the hydrophobic organic compounds are easily solubilized in the inner environment of the micelle and are washed off effortlessly. As mentioned earlier too, unlike chemical surfactants biosurfactants are classified based on their mass. The Low-molecular-mass biosurfactants (Rhamnolipids, Trehalose lipids, sophorolipids, lipopolypeptide, etc.) are efficient in lowering surface and interfacial tensions, whereas high-molecular-mass biosurfactants (Emulsan, Alasan, etc.) are more effective at stabilizing oil-in-water emulsions. The high molecular weight biosurfactants are efficient emulsifying agents (Pacwa-Płociniczak et al. 2011).

9.8 Efficiency of various types of microbial biosurfactants on the removal of hydrophobic pollutants

Degradation of hydrophobic pollutants by biosurfactants is the most novel and effective method. We have observed that there is interdependence between the type of biosurfactant and the type of hydrophobic pollutant it degrades. A good Bioremediation method lies in the effective degradation of the mentioned pollutants. They are either registered in-site or off-site based on the specificity of pollutants (Cameotra and Makkar 2010). Hydrophobic compounds become toxic pollutants when bound with soil in heavy mounts as they are very difficult to separate. A good degradation for this requires proper solubilization of hydrocarbons prior to degradation. As surfactants are surface-active agents they reduce the surface tension and emulsify hydrocarbons which make it easy for recovery. Several bacterial and yeast strains which produce surface-active substances were considered for this process. *Aeruginosa* UG2, isolated from an oil-contaminated soil, produces high extracellular emulsifying activity when grown on several hydrocarbons and non-hydrocarbon substrates (Van Dyke et al. 1993). In another study, the hydrocarbon-contaminated soil was coupled with *Acinetobacterhaemolyticus* and *Pseudomonas* ML2. Careful observation on hydrocarbon degradation concluded that there is a huge impact in reduction which is about 39–71% by *Acinetobacterhaemolyticus* and *Pseudomonas* ML2 for about 11–71%.

As the studies progressed, it narrowed down to two mechanisms of surface-enhanced soil washing which is based on the concentration of the surfactants. The first one occurs below CMC (Critical micelle concentration), where the surfactants upgrade the contact angle between hydrophobic pollutant and soil which enhance the separation of pollutants from the soil and finally results in separation. While the

second process of solubilization occurs above CMC. Solubilization is mainly based on interaction, origin, and type of surfactant used. This process is widely used in in situ soil washing (Cameotra and Makkar 2010; Pacwa-Płociniczak et al. 2011).

9.9 Methods Involved in the Biosurfactant Flushing in Petroleum Industrial Waste

9.9.1 Petroleum Oil Spill-Contaminated Soil

Petroleum oil spills can occur by any accidental leakage, transport leakage, oil tank corrosion, etc., petroleum spills are one of the major environmental concerns these days and the contaminated soil is also affected. The remediation of this oil spill-contaminated soil can be done either by physical, chemical, mechanical, or biological method. Physiochemical methods might remove the contamination from that site and transfer it to the other but this does not solve our problem (Varjani 2017; Patel and Patel 2020). We need a solution in such a way the spilled oil in the soil is completely eradicated and purified. Biological methods involve the use of biosurfactants which are originated from microorganisms and these do not pose threat to the environment any further so called the best alternative for purification. The hydrocarbon pollutants constitute several forms such as alkane, cycloalkane, monoaromatic hydrocarbons (MAHs), polyaromatic hydrocarbons (PAHs), resins, asphaltenes, and heavy metals (Fenibo et al. 2019). A Biosurfactant produced by *Candida sphaerica* for the remediation of motor oil from soil and seawater established removal rates of 75% and 92% from clay and salty soil, respectively (de Silva et al. 2014). Biosurfactants enhance the bioavailability of contaminants to the microorganisms which makes them easy for their bioremediation. Several properties in biosurfactants make the contaminants easily available for degradation. This process includes modification of cell surface, solubilization, and remediation of pollutants. The contact angle increases between soil and contaminant which eventually reduces the binding force between them both and leads to their dispersion. The formation of micelles which increase the solubility of hydrocarbons makes them available for degradation. The comparison of biosurfactant types, their origin, and applications is provided in Table 9.1. The application of a biosurfactant which is produced by *Candida tropicalis* for removing motor oil from sand and achieved removal rates around 78–97%, demonstrating considerable potential with regard to soil bioremediation (Karlapudi et al. 2018). Luna et al. evaluated a new biosurfactant, denominated Lunasan, produced by *Candida sphaerica* UCP 0995. This biosurfactant has the capability to remove 95% of motor oil adsorbed into the sand, showing considerable use of them for treating the toxic pollutants present in soil (Luna et al. 2012). These are the various studies and experiments conducted regarding the effect of biosurfactants over petroleum oil spill-contaminated soil.

Table 9.1 Biosurfactant producing microorganisms and its application in the bioremediation of oil-contaminated environments

Microorganisms	Type of Biosurfactants	Applications	References
Rhodococcus erythropolis 3C-9	Glucolipid and trehalose lipid	Oil spill cleanup operations	de Silva et al. (2014)
Pseudomonas aeruginosa S2	Rhamnolipid	Bioremediation of oil-contaminated sites	Abdel-Mawgoud et al. (2010)
Bacillus subtilis BS5	Lipopeptide	Bioremediation of hydrocarbon-contaminated sites	Mazaheri Assadi and Tabatabaee (2010)
Pseudomonas aeruginosa BS20	Rhamnolipid	Bioremediation of hydrocarbon-contaminated sites	Fenibo et al. (2019)
Micrococcus luteus BN56	Trehalosetetraester	Bioremediation of oil-contaminated environments	Banat et al. (2010)
Pseudomonas cepacia CCT6659	Rhamnolipid	Bioremediation of marine and soil environments	Olkowska et al. (2012)
C. glabrata UCP1002	Protein-carbohydrate-lipid complex	Oil recovery from sand	Moldes et al. (2013)
C. sphaerica UCP0995	Protein-carbohydrate-lipid complex	Removal of oil from sand	Perfumo et al. (2010)
C. guilliermondii UCP0992	Glycolipid complex	Removal of petroleum derivate motor oil from sand	Toru et al. (2012)
C. tropicalis UCP0996	Protein-carbohydrate-lipid complex	Removal of petroleum and motor oil adsorbed to sand	Edwards et al. (2003)
C. lipolytica UCP0988	Sophorolipids	Removal of petroleum and motor oil adsorbed to sand	Franzetti et al. (2006)

9.9.2 Petroleum Refinery Wastewater

Several forms of contamination are piled by pollutants and many harmful chemicals in our day-to-day life. These might be the result of some harmful reactions from industries like chemicals, petroleum, cosmetics, etc. One of the harshest pollutants from this is petroleum refinery wastewater which is produced as a result of activities from petroleum industries (Pacwa-Płociniczak et al. 2011; García-Reyes and Yañez-Ocampo 2016). As we all know that petroleum industry plays a very huge role in delivering pollutants into the environment, we can see a varied range of pollutants in their refineries such as wastewater containing leakages from machinery, high contaminant hydrocarbons, surface spills, water resulting from crude and product storage, roof drains, and runoff (Pugazhendi et al. 2017). Petroleum refinery wastewater is one of the major pollutants of aquatic life leading to its accumulation on the

grounds and resulting in high COD. These toxic compounds if not treated with suitable materials might lead to serious ecosystem damage and might leave zero resources for the future. The use of biosurfactants that ensures the safety of the environment and enhance the bioremediation of these pollutants is the major duty of present researchers. Biosurfactants have the capability to emulsify and comprise hydrophilic and hydrophobic moieties leading to a remarkable decline in surface tension and also enhance the bioavailability of pollutants for microbes leading to considerable bioremediation. Henceforth, biosurfactants are crucial components for this remediation. Lab-based degradation of petroleum refinery wastewater is conducted in a controlled bioreactor under continuous shaking conditions along with wastewater and bacterial culture. The consortium under optimized hydrodynamic conditions of the reactor obtained encouraging results. At the end of a specific incubation period, tests are performed to know the degradation. A study which showed that Strain S1VKR-26 produces 5.15 g L^{-1} of biosurfactant, possesses a CMC of 30 mg L^{-1} and reduced surface tension to 30.5 mNm^{-1} could prominently regulate the removal of PAHs, TPH, phenolic compounds, and nitrogen content with a reduction in BOD and COD. PAH (Polycyclic aromatic hydrocarbon) is also present in petroleum refinery wastewater. Different forms such as NPH, PHE, FLU, and PYR present in the untreated wastewater had concentrations of about 11.66, 9.2, 5.34, and 1.81 mg L^{-1}, respectively. The degradation of these forms was observed in 3 weeks. The first week had relatively less degradation due to various tough conditions. Eventually, the degradation process stood fast by the second week showing a boom in degradation of NPH, PHE, and PYR which is due to various enzymatic factors and the availability of hydrocarbon to the biosurfactant. While the final week showed a degradation of 93, 86, 92, and 98.3% for NPH, PHE, FLU, and PYR, respectively (Patel and Patel 2020).

9.9.3 Tank Bottom Oil Sludge

Oil sludge generally occurs by pollutants present in it where the oil solidifies at high temperatures. The deposition of oil sludge layer by layer can eventually penetrate the surface on which it is residing. If the sludge occurs on the earth's surface this eventually penetrates soil leading to severe damage and contamination of soil, groundwater, and resources by hydrocarbons (Toru et al. 2012). This mainly occurs due to oxidation of heavy organic materials present in crude oil along with variations in climatic conditions or it can also be due to inorganic substances such as soil, dust, and other materials. Various developments and technology inventories have been developed to resolve this situation such as thermal evaporation, soil vapor extraction, and excavation. But a biological and environmentally friendly method is always chosen over other synthetic methods (Toru et al. 2012). Hence, several strategies were developed including biosurfactants and this gives a huge percentage of sludge degradation as biosurfactants increase the rate of biodegradation of the organic compounds by increasing their solubility by emulsification. This can be done by

micelle formation and gradually decrease in angle between pollutant and source leading to dissociation by the effect of microbes (Karlapudi et al. 2018).

In a recent study regarding Degradation of Ratnagiri crude oil sludge under field conditions showed that the addition of biosurfactants to the depleted areas affected by hydrocarbons showed a significant increase in bioremediation levels of depletion after 4 weeks of treatment and it was significantly higher. Also, the crude oil degradation from Faridabad showed similar results as Ratnagiri field studies. A number of bacteria were isolated from the contaminated site, of which P2, P7, and SSC2 were chosen as members of a consortium to degrade crude oil. But here the depletion was observed after 8 weeks. The areas which are treated with biosurfactants along with consortium showed significantly higher depletion compared to consortium alone. Hence, biosurfactant-producing hydrocarbon degraders are preferred for remediating crude oil sludge hydrocarbons rather than bacteria relying only on their degrading capability (Cameotra and Makkar 2010).

9.9.4 Petrochemical Waste Effluent Stream

The waste effluent stream is waste runoff exiting an industry after following various steps and indulged with various chemical effluents, chemicals, runoff water, leakage water, and treatment results. In the petrol industry, the resulting effluent exiting the outlet may lead to many natural resources, lakes, or any natural body resulting in serious depletion as they further enter into deep layers of soil by continuous exposure, polluting aquatic life which might pile up harmful and deleterious chemicals on the sea bed leading to problems in exchange of air between layers and for the aquatic beings (Patel and Patel 2020). As we know there might be a number of pollutants in the petrochemical effluent stream such as High COD, turbidity, phenol, hydrocarbons, and grease resulting from petrochemical wastewater.

Various methodologies were developed such as electrochemical technologies, advanced treatment systems, physical, and chemical methods. But instead of having chemical remedies for such major problems, it is always helpful to upgrade to a sustainable process involving biological methods such as anaerobic treatment, autotrophic denitrification, and the use of biosurfactants (Iqbal et al. 2007). As the effluent stream consists of various harsh chemicals using biosurfactant which is a surface-active agent and helps emulsify them and reduces surface tension by making them available for degradation. In this way biosurfactants are used in the treatment of petrochemical waste effluent stream (Zheng et al. 2011). Pacwa et al. tested levels of BOD, COD, and hydrocarbon waste and the results obtained after treating them with biosurfactants are as follows. The concentration of total petroleum hydrocarbons was 802 g/m^3. BOD and COD were high, and reached 155 g/m^3 and 275 g/m^3, respectively. The major bacterial genera that were used in this study for biosurfactant production include *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Rhodococcus*, *Arthobacter*, *Staphylococcus,* and *Flavobacterium*. These are resulted to be a good

option for the process of bioremediation of petroleum effluent (Pacwa-Płociniczak et al. 2011).

9.10 Application of Biosurfactant in Food Industry Oily Waste Bioremediation

The wastes from the food industry include fresh fruits, vegetables, and dairy products, including discharges from food processing industries, agriculture waste, and effluents from the edible oil industries such as palm oil (Sharma et al. 2020). Food and Agriculture Organization (FAO) estimated that worldwide, approximately 1.3 billion tonnes of wasted food is generated annually. This corresponds to one-third of the total food produced globally, whose production utilizes 28% of the agricultural area, i.e., 1.4 billion hectares of the world's fertile land (Parthiba Karthikeyan et al. 2018). Due to the massive increase in food waste in the past few decades, its valorization for the production of value-added products is gaining a lot of attention from researchers worldwide (Sharma et al. 2020). Biosurfactants due to their versatility regarding the use of substrates and surface-active properties are very potential candidates for the remediation and valorization of wastes from food industries (De Giani et al. 2021). Wastes from food industries are rich in nutrients. One of the ways for remediation of wastes from the food industry is its utilization as substrates for the production of bioactive molecules such as biosurfactants themselves. The use of various cheap and renewable waste substrates from dairy, agriculture, food processing industry, oil processing mills, etc., lowers the cost of biosurfactant production, making the process commercially viable. Many studies have been done regarding the use of food waste as substrates for biosurfactant production in the past. One of the studies reported a microorganisms strain, *L. pentosus*CECT-4023 as a strong biosurfactant-producing strain on whey cheese (Sarubbo et al. 2015). So this is an innovative way of the utilization of wastes from the food industry. Palm oil milling industries have grown very much due to their high demand due to which palm oil mill effluent (POME) has become a topic of concern (Moldes et al. 2013). Biosurfactants due to their surface-active properties are used for their bioremediation of contaminated sites. Since POME is high in nutritional value too, it can also be used as a substrate for biosurfactant production (Affandi et al. 2014).

9.11 Biosurfactants in the Removal of Pollutants in Textile Industries Waste Effluent

In this twentieth century, there is a phenomenal growth in various industrial sectors. One among them includes the growth and establishment of the textile industries. But, the modern updates and trends only tend to invest in chemical mixtures, enzymes, dyes, softeners, etc. Chemical usage is seen in a wide range of applications concerned with the textile industry (Montoneri et al. 2009). This is only leading to

harmful extractions and environmental death by including huge chemical pollutants that are posing a deleterious impact. The industrial effluents which flow to the environmental resources might also harm other living beings. With providing a large deleterious effect on the environment it needs a robust cleaning strategy. Sustainable technologies are needed to be developed which show a positive effect. Using biosurfactants that are derived from microorganisms has some specific potential. As biosurfactants have a great potential for degrading incompatible components and are a more conventional method compared to chemical surfactants. As we know, biosurfactants have wider types and properties contrasting with chemical surfactants (Kesting et al. 1996). The textile industry produces waste either during production or postproduction which mostly includes textile materials which are unfit, damaged, or stained. The stained textile materials can be remediated by a few following methods mediated by biosurfactants. Biosurfactants are proficient in reducing the surface tension of textile effluents, natural lipophilic substances of the fabric (mineral oils, natural oils, and waxes), and acquired lipophilic substances (Mnif et al. 2015). A recent study where they used *C. echinulate* to remove the pollutants of fabric followed a washing procedure where they used a cotton cloth stained with engine oil, this was immersed in an aqueous solution of the biosurfactant (1.5%) washing occurred at a stirring speed of 150 rpm for 1 h. A commercial detergent was also included as a positive control in cleaning and degreasing. The cotton fabric was rinsed twice with 50 mL of distilled water for 30 min with stirring followed by drying at room temperature. It is concluded that *C. echinulate* has a greater surface tension reduction potential (32 mN/m) (Andrade et al. 2018).

9.12 Current Strategies and Advancements in the Application of Biosurfactant Flushing in Bioremediation of Toxic Contaminants (Oil/Hydrocarbons)

Since the discovery of the first surface-active biomolecule, microbial biosurfactants have come a long way and have enabled themselves as an effective approach for the bioremediation of hydrocarbon pollutant laden contaminated soil. Due to the ability to mobilize and solubilize hydrocarbon present in contaminated soil, they are starting to be perceived as environmental cleanup agents (Olasanmi and Thring 2018). Through their action on contaminated soil, they make the contaminants available for microorganisms' growth hence performing bioremediation. Till now countless studies have been done and various biosurfactants have been tested for removing hydrocarbon products from contaminated water and soil. It is found that *Pseudomonas* sp., *Bacillus* sp., and *Candida* sp. are among the major biosurfactant producers used in soil remediation (Sachdev and Cameotra 2013). Out of all the surfactants, rhamnolipid has gained great success in decontamination processes (Fenibo et al. 2019). Microbial-enhanced oil recovery (MEOR) is one of the most attractive applications of biosurfactants in the scientific community currently. MEOR methods are used to recover oil remaining in the reservoir. Biosurfactants through their amphiphilic nature can reduce the oil/water and oil/rock interfacial

tensions thereby reducing the capillary force which hinders the flow of oil through the pores of the rock, reduction in surface tension caused by microbial surfactants can also be used to separate oil from the bottom of tanks (Ahmadi-Ashtiani et al. 2020). Not only does biosurfactants application but also its production helps to aid the bioremediation of hydrocarbon industrial waste. Various studies have led to development of new techniques for the production of biosurfactants using industrial hydrocarbon wastes as substrates for biosurfactant production. This not only helps in the fixation of hydrophobic toxic pollutants from industrial wastes but also makes the production of biosurfactants cheaper. Recent studies have even led to the production of nanoparticles with the aid of biosurfactants. A study demonstrated the production of Nickel oxide (NiO) nano-rods by water-in-oil microemulsions (Mulligan et al. 2014). Nowadays, biosurfactants are also being used for polynuclear aromatic hydrocarbons (PAHs). Exposure to PAHs has long been identified as an environmental concern. Due to their poor solubility and aromatic nature, biodegradation has always been an issue for researchers. Application of biosurfactants can improve PAH bioconversion processes by increasing PAH bioavailability and mass transfer rates to cells so that microorganisms can easily degrade it (Cameotra and Makkar 2010). So without a doubt biosurfactants have come a long way in terms of applications, especially when it comes to the bioremediation of toxic hydrocarbon pollutants.

9.13 Conclusion

Circumambient with various degrees of chemical pollutants like oil-contaminated soil, petroleum refinery waste effluents, palm oil sludge and wastewater, textile industrial waste stream, dairy waste, etc. The development of various chemical methods for treating these pollutants specifically resulted in worsening the environment even more. To reduce this impact, environmentalists are looking towards the application of biosurfactants that are relatively less toxic with environment tolerance, which has effective biodegradability and surface—interfacial activity on hydrophobic pollutants. In this paper, various applications of biosurfactants in the dairy, petroleum, and textile industries and its methodologies developed for the bioremediation of toxic pollutants were described. Also, the importance of microbial biosurfactants and its significant role in the removal of hydrophobic (oily) pollutants laden industrial wastes have been highlighted in this chapter. The comparative description of different types of biosurfactant on the remediation of hydrophobic/oily pollutants revealed that the Trehalose lipids biosurfactant from *Rhodococcus erythropolis and Arthrobacter* sp. lowered the surface and interfacial tension from 25 to 40 and 1–5 mNm, respectively. Hence, it is concluded that biosurfactants are better alternatives for chemical surfactants in the process of hydrophobic/oil contaminants flushing and strategies involving bioremediation of toxic contaminants.

Acknowledgment DST-INSPIRE Fellow Swathi Krishnan Venkatesan [DST/INSPIRE Fellowship/2019/IF190218] acknowledge the Department of Science and Technology, Ministry of Science and Technology, Government of India for providing the fellowship and financial support to carry out the research. The authors are highly thankful to the Department of Biotechnology, Ministry of Science and Technology, Government of India for funding the project under the Scheme "Biosystems and Bioprocess Engineering scheme" (No. 677 BT/PR20297/BBE/117/193/2016).

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Hazardous Organic Pollutant Contamination in Indian Holistic Rivers Risk Assessment and Prevention Strategies

10

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10.1 Introduction

A plethora of synthetic organic compounds is being constantly used by anthropogenic activities for multiple purposes that include food production and preservation, and animal and human healthcare. The fate of these organic pollutants ultimately culminates in increasing contamination in the terrestrial and aquatic environment. Thus, the toxicity and environmental fate of these pollutants have been a center of focus among researchers (Lapworth et al. 2012; Daughton and Ternes 1999; Schwarzenbach et al. 2006; Kümmerer 2009). Besides these persistent pollutants, the advancements in analytical methods enabled the detection of emerging organic contaminants recently (Petrovic and Barceló 2006; Richardson and Ternes 2011). These emerging pollutants include personal care products, pharmaceuticals, cosmetics, food colorants, and veterinary products. Agricultural runoff, wastewater from industries, and domestic and municipal dumps became a major source of contaminants finally entering into rivers, lakes, ponds, and estuaries (Pal et al. 2010) due to which the rivers became vulnerable to pollution (Fig 10.1). Industrial and municipal wastewaters are a constant polluting source and the surface runoffs seasonally add to the burden of sewage treatment and disposal systems (Singh et al. 2005b). Seasonal variation in surface runoff, groundwater flow, precipitation, water

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_10

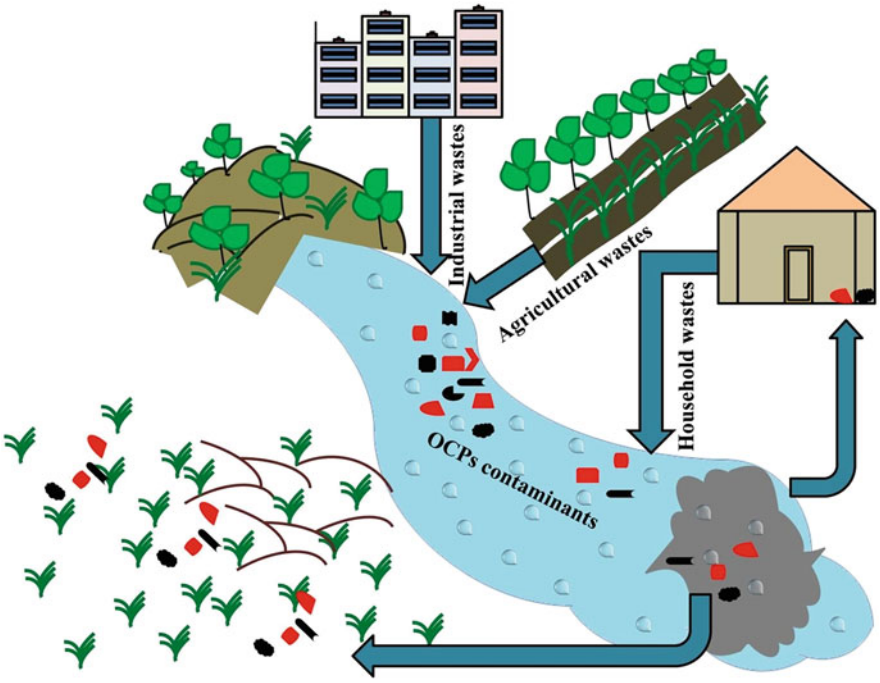

Fig. 10.1 Figure representing the spread and origin of contaminants

interception, and abstraction exhibit strong effect on the level of pollutant concentration in river water (Vega et al. 1998).

Many researchers have reported the presence of organic contaminants in river water (Stuart et al. 2012; Vasquez et al. 2014; Radović et al. 2015; Dwivedi et al. 2018; Jani et al. 2018; Sinha et al. 2018). Both organic contaminants and pharmaceutical residues contributed largely affecting the aquatic as well as terrestrial ecosystems (Stuart et al. 2012; Vasquez et al. 2014; Radović et al. 2015; Dwivedi et al. 2018). As rivers are the main source of water for irrigation, industrial, and domestic purposes, it is vital to control and find remedial measures to maintain river water quality. This chapter summarizes the sources of pollutants generated from various sources, their effects on aquatic life, and human and environmental health, and outline some plausible mitigation strategies.

10.2 Sources of Pollution

10.2.1 Agriculture

Due to the extensive and continuous use of pesticides for pest management, the agricultural soil has been polluted and lost its fertility affecting crop productivity (Liu et al. 2015). A huge amount, i.e., 4.5 and 0.44 million tons of HCHs

(Hexachlorocyclohexane) and DDT (Dichlorodiphenyltrichloroethane), respectively, is reported to be produced during the period 1950s–1980s (Cao et al. 2007). According to WWF (World Wide Found 2005), HCH-isomers, Endosulfan, and Methoxychlor were considered as potential persistent organic pollutants (POPs). Furthermore, the United Nations Environment Program (UNEP) in the Stockholm Convention listed some organochlorine pesticides like DDT, HCB, heptachlor, aldrin, and dieldrin as POPs. DDT having a half-life of ~15 years was the most commonly used pesticide until the early 1980s which is hazardous and persistent in nature (Augustijn-Beckers et al. 1994) and is considered as the first insecticide used globally since the 1940s (Mansouri et al. 2017). DDT was used in vast quantities to control the vector-borne diseases like malaria and typhoid and to kill the insects in agricultural crops (Wurl and Obbard 2005). Approximately 175,000 tons per year worldwide consumption of DDT has been estimated by the United Nation Environment Programme (UNEP) between the years 1950 and 1963. In the year 1940s–1980s, dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) were extensively used as organochlorine pesticides to control pests. Due to their hydrophobic nature, DDT and HCH have been reported to be retained in the soil system for a long time (Nam et al. 2008). In the year 2001, the Stockholm Convention terminated the use of DDT and HCH for agricultural purposes (Wang et al. 2016). According to the USEPA, hexachlorocyclohexane (HCH) is considered as a primary contaminant which accumulates in the agricultural soil that can adversely affect the environment and human health (UNEP 2003). Organochlorine residues in agricultural soil can cause a serious risk to human and animal health by entering food chains (Fantke and Jolliet 2016). Many vegetables and cereals such as rice, maize, wheat, carrot, and cabbage have been detected to contain organochlorine pesticides (Mekonen et al. 2015). Tao et al. (2005) stated that organochlorine pesticide accumulation in fibrous roots is more than in the tuberous root system. Although aerobic and anaerobic rotation in paddy fields favor the degradation of organochlorine pesticides (Hao et al. 2008), it has been estimated that the paddy soil collected from the Pearl river delta had a low concentration of HCHs and DDTs in comparison to vegetable soils (Li et al. 2006) (Fig. 10.1).

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic in nature and hence tend to be retained in the soil (Terashima et al. 2003). It has been reported that PAHs are responsible for the changes in the functional genes of the soil-harboring microbiota (Han et al. 2014). The USEPA identified 16 PHAs as priority pollutants and in the control list out of which seven PAHs, i.e., Benz(a)anthracene (BaA), benzo(b)fluoranthene (BbF), chrysene (Chr), dibenz(a,h)anthracene (DahA), indeno (1,2,3-c,d)pyrene (InP), benzo(k)fluoranthene (BkF), benzo(g,h,i)perylene (BghiP), and benzo(a)pyrsne (BaP), were found to be carcinogenic in nature (Harvey 1991).

Stockholm Convention in 2001 declared halogenated aromatic compounds such as PCBs as POPs (Zhang et al. 2008). Some congeners of PCBs like PCB-12, 77, 81, 105, 114, 118, 126, 156, 157, 167, 169, and 189 were highly toxic and hence considered as dioxin-like PCBs. However, there are some factors like soil composition and environmental parameters including the organic carbon, porosity and size of the soil particles, relative humidity, and temperature which are responsible for the

exchange of PCBs between soil and air (Sun et al. 2018). Other synthetic insecticides such as organophosphate, carbamates, pyrethroids, herbicides, and fungicides were applied into agricultural fields after the year 1960s. In India, pesticides are mainly used in rice fields, wheat, and 45% of cotton crops; this large application of pesticide has affected the flora and fauna of the ecosystem (Agrawal and Sharma 2010). Wheat flour contaminated with parathion led to the death of approximately 100 people in India after which a committee by the Indian Council of Agricultural Research (ICAR) was constituted. This committee suggested the remedial measures that can be taken to overcome pesticide toxicity in agricultural crops (Wadhwani and Lall 1972).

A study by the World Health Organization (WHO) showed that 80% of all pesticides were used by developing countries (Jayaraj et al. 2016). Pimentel (1995) reported in his study that 0.3% of total introduced pesticides go into the target pest whereas the rest of 99.7% enters into the environment. Some residues of pesticides are volatile and hence enter the environment while others remain stable bounded to the soil particles causing more persistent risk to human and animal health. The introduction of pesticideswas found to alter the biochemical, physiological, enzymatic, and nonenzymatic antioxidant systems in the plants. These changes may reduce the growth and yield of edibles and also affects the nontarget organisms. The impact of herbicides on the germination and seedlings growth of *Oryza sativa* L. and *Hemp sesbania* L. was studied by Hirase and Molin (2002). Furthermore, Wang (1994) studied the toxicity of paraquat, 2, 4-D, glyphosate, and bromacil in *Oryza sativa* L. seed. On the other hand, the study also revealed that exposure to fipronil at a concentration of 2000 mg/L for 4 days significantly impaired the germination in rice (Parween et al. 2016). Mishra et al. (2008) investigated the reduction in the length of root and shoot of the plants over the application of the insecticide dimethoate. Glover-Amengor and Tetteh (Glover-Amengor and Tetteh 2008) observed the suppression in the yield of garden eggs, tomatoes, and okra due to applied concentrations of 156.0, 244.0, and 312.0 g/ha lindane, 125.0, 187.5, 250.0 g/ha unden, and 166.6, 209.8, 333.3 g ha^{-1} of dithane and karate.

10.2.2 Sewage

Rivers have turned into receiving sink for waste and untreated sewage for several years now. According to the Central Pollution Control Board (CPCB), India, 30% of municipal sewage is treated whereas 68.5 million cbm (cubic meter) of industrial waste and sewage were dumped in rivers (Agoramoorthy 2012). In the last decade, various changes have been made to save the declining rivers across India, for example, north India based a popular nonviolent crusade "Save Ganga Movement" (Agoramoorthy 2015). The curiosity in the cleanup of the river Ganges is due to the importance of this river as "Mother Ganga" by millions of Hindu devotees (Das 2014). It has been reported that holy rivers in India, viz., Ganges, Yamuna, Godavari, Krishna, Sone, Cauvery, Damodar, and Brahmaputra are highly polluted with a number of organic and inorganic contaminants along with some other substances like solvents, oils, grease, plastics, etc., due to direct disposal of sewage

and industrial effluents in a water body without any treatment (Agrawal and Sharma 2010). In many countries studies have been performed addressing river contamination in the last few decades (Cai et al. 2003, 2004; Mascolo et al. 2010; Lindholm-Lehto et al. 2017).

Organic matter recycled from the primary and secondary treatment of wastewater which came from domestic and industrial areas is referred as bio-solids. Due to adsorption capacity, hydrophobicity, and persistent nature a significant amount of PCBs and organochlorine pesticides are reported to be present in the influent (Gibson et al. 2005; Semblante et al. 2015). The main sources of organic contamination in sewage are agricultural runoff and domestic and industrial wastes (Blanchard et al. 2001; Katsoyiannis and Samara 2004; Harrison et al. 2006; Agamuthu and Narayanan 2013). Due to carcinogenicity, mutagenesis, toxic and persistent characteristics of organic contaminants, a number of investigations have been carried out to demonstrate the contamination in sewage sludge (Stevens et al. 2003; Abad et al. 2005; Wang et al. 2007; Clarke et al. 2010; Sánchez-Brunete et al. 2008; Zennegg et al. 2013). Sewage sludge usually contains various persistent organic pollutants that adversely affect the microbial diversity in the soil and ultimately growth and development of the plants (Ahmad et al. 2004). Some of the chemicals in the sludge like polycyclic aromatic compounds (PAHs), OCPs, or PCBs have ability to bind with solid particles due to their lipophilic nature (Berset and Holzer 1999; Lazzari et al. 1999; Tyagi and Lo 2013). Persistent polar compounds were found to be less absorbed by the soil and hence responsible for the contamination of groundwater (Klöpffer 1996).

Unfortunately, the Ganga is considered as an extremely polluted river with toxic wastes. USD 4 billion project has been started by the government to overcome calamity and to restrict the entry of untreated sewage/industrial runoff in 2500 km long river by the year 2020.

Throughout the whole course of approximately 2525 km, effluents of about 300 small, medium, and big industrial units are discharged into river Ganga (Agoramoorthy 2015). The Ganga receives effluents from industries and pollutants from various nonpoint sources, municipal wastes from 23 Class II (population between 50,000 and 100,000) and 29 Class I (population over 100,000) cities and 48 towns which results in the pollution of water bodies. As mentioned in the audit report, according to NRCD (National River Conservation Directorate) per day 5044 million L of sewage is generated in the towns situated along the Ganga and its tributaries. The Central Pollution Control Board report of 2001 estimates the generation of 6440 million L of wastewater per day in the Ganga basin. Most of the towns situated on the bank of the Ganga are highly industrialized. Many industries dispose wastes directly into the rivers without any treatment. It has been estimated that 151 tanneries in Kanpur located at Jajmau discharge 5.8–8.8 million L wastewater per day along the southern bank of the Ganga (Agrawal and Sharma 2010). The Yamuna is another sacred river which has become yet another highly polluted water resource of India. As it is considered the primary source of drinking water for Delhi and adjacent towns and villages of the states of Uttar Pradesh, Uttaranchal, and Haryana, many local government agencies and nonprofit organizations have

committed to overcome the issue and clean up the river Yamuna which is highly polluted in Delhi and Ghaziabad area. It has been reported that approximately 515,000 kL of wastewater from sewage is disposed in the river Yamuna per day. Furthermore, approximately 1500 medium and small industries discharge a large amount of nontreated and partially treated effluents directly to the river Yamuna every day. On the other hand, between Wazirabad and Okhla, the National Capital Territory (NCT) of Delhi, the river has been receiving a huge amount of partially treated and nontreated wastewater (Agrawal and Sharma 2010).

Another holy river Cauvery situated in South India is also facing trouble receiving textile and other chemical wastes. Local communities in Karnataka have launched a campaign to save the river from further damage (Agoramoorthy 2015). Nongovernmental agencies in "The Save Narmada Movement" constructed large dams and fought for the rights of millions of displaced people.

10.2.3 Industrial Wastes

Pollutants coming from industrial wastes are the main cause of river water pollution. Although pesticides usage is a continuous trend in developing countries and most of the industries rely on freshwater, viz., lakes, rivers, and wetlands in order to direct wastewater and industrial wastes, it can lead to a harmful impact on human health and environment (Jain et al. 2003; Hines 1967; Rajput et al. 2017). Direct discharge, wet or dry deposition, runoff of industrial wastes from nonpoint sources, and other means are the ways for the addition of organic pollutants in the water bodies. Unfortunately, in India, there is a huge production and consumption of organochlorine pesticides, especially DDTs and HCHs for agriculture purposes. It has been estimated that more than 70% of the gross tons of pesticides applied in the agricultural fields in the year 1990s are banned or restricted in the east and west regions (Subramanian et al. 2007). Furthermore, insecticide consumption in India increased from 22,013 tons to 51,755 tons during the period of 1994–1995. During the course of 2005–2006, pesticide industries in India were ranked second in Asia which is behind China and 12th on the global market due to 82,000 metric tons production of pesticides. According to the Green Peace report, 90,000 metric tons of pesticide production by Indian industries was reported in the year 2008. Due to the thirst for industrial production of pesticides, people have contributed to the life of plants, animals, and microorganisms. However, the predominant POP contaminants like HCH, DDT, and its metabolites were reported to be found along the Indian coast (Sarkar et al. 2008) and in major rivers of India, viz., Gomti River (Malik et al. 2009) and Tamiraparani River (Kumarasamy et al. 2012a).

Various industrial units constitute thermal power plants, textiles, wood and jute mills, sugar mills, pulp and paper factories, electro-processing industries, distilleries, dairies, coal washeries, dying units, pesticide factories, synthetic rubber industry, and tanneries oozed a number of wastes in the Ganga river. Furthermore, out of these industries, 956 units are only in Uttar Pradesh (Down To Earth 2014). It has been estimated that 2500 MLD wastewater from industries is disposed to the entire Ganga

basin (Trivedi 2010). According to CPCB (2016), it has been evaluated that most of the grossly polluting industries were situated in Uttar Pradesh out of which 764 industries were reported to discharge their effluent directly into the river Ganga without any treatment. The region from Kannauj to Varanasi records the highest concentrations of pollution in the river Ganga due to the addition of industrial wastes. Varanasi, Kanpur, and Allahabad are the main sites in Uttar Pradesh for the manufacture of carpets, locomotives, tanneries, and engineering processes, respectively. Among grossly polluting industries the highest proportion, viz., 58% comprised of tannery sector only. Out of three major hubs of tanneries in India, two are situated in Kanpur, Uttar Pradesh, and Kolkata, West Bengal at the bank of river Ganga. However, other minor tanneries were also located at Ganga basins which are directly or indirectly responsible for the contamination of metals in the Ganges. In addition, apart from tanneries partially treated or untreated toxic pollutants from other industrial wastes also lead to the contamination of Ganga water (Rai et al. 2010; Aktar et al. 2010; Bhattacharya et al. 2008; Dwivedi et al. 2006; Gupta and Raghubanshi 2002).

Kanpur is one of the vital industrial towns in Uttar Pradesh. In Kanpur, Ganga travels the Northern alluvial plain in a sinuous flow and migrates from one bank to another. The major clutch of tanneries in Kanpur is situated at Jajmau (the southern part of Kanpur). In addition to Kanpur, some tanneries are also located in the Unnao district. Out of the 150 tanneries situated in sector Kanpur or Unnao, 20 are located at Unnao and the rest at Jajmau. In the tanning process, spent chrome liquor (SCL) and vegetable tanyard waste (VTW) are the two major sources of pollution. Without any primary treatment of the wastes some of the industries directly dispose their effluent in water bodies that could help to get information about the pollution load caused by tannery wastes (Khwaja et al. 2001).

The chemical, fertilizer, pharmaceutical, tanneries, oil, textile and paper mills plants, refineries, and electronic industries situated on the Ganga River basin also contribute to the increasing contamination level. Near Kanpur city, leather industries are the main sources of water pollution and carry a high content of chromium and other toxicants in their effluents hence untreated industrial and sewage wastewater are considered as the primary source of water pollution. Besides metals, the incoming wastewater contains a large amount of pesticide residues, organic contaminants, and metals. Annually an estimated amount of approximately 9000 tons of pesticides in the Ganga River basin has been estimated for public health and agricultural purposes (Ghose et al. 2009). Recently, a level of 21,000 tons has been reported (NGRBA 2011). Kumari et al. (2001a) reported that moderate content of hexachlorocyclohexane (HCH) has been found in the Bihar stretch of river; however, studies by Ray (1992) and Halder et al. (Haldar et al. 1989) indicated a moderate level of dichlorodiphenyltrichloroethane (DDT) and its analogs. Furthermore, a moderate extent of endosulfan compounds has also been reported. HCH residue levels were up to 5000 ng/L in water bodies however the pollutant receiving areas reported higher values of 1000–5000 ng/L. In the case of DDT 0–5000 ng/L and 0–3000 ng/L for endosulfan were reported (Samanta 2013). The use of endosulfan for agricultural purposes enhanced to a high extent in the Uttar Pradesh stretch of

the river while in the uppermost stretches glacial melting is the source of pesticides. High contamination of the Bhagalpur stretch with aldrin and related compounds has been observed (Mutiyar and Mittal 2012).

Takeoka et al. (1991) stated approximately 99.6% removal of HCH to the air applied to the paddy field in coastal south India and 0.4% drainage to the estuary. However, 20% flux removal from estuary into the air has been found and hence only 0.1% HCH drainage to the sea has occurred. Gajbhiye and Agnihotri (1991) also reported similar data, i.e., soil-incorporated treatments of lindane showed 13.5–62.6% and 6.7–24.0% volatilization in the Ganga basin of the NCR region. Thus, a low proportion of organochlorine pesticide residue is retained in the aquatic system. The recorded range for HCH is 0–100 ng/g however a study showed a high content of HCH in the river Gomti (the tributary of River Ganga) (Singh et al. 2005a). The presence of DDT and aldrin were found in the range of 0–500 ng/g. For instance, the occurrence of Aldrin residues in sediments is more frequent than in water. On the other hand, Endosulfan (0–72.6 ng/g) was reported in the sediments of River Gomti (Singh et al. 2005a). Senthilkumar et al. (1999) reported 0.1–49 ng/g of chlordane in the sediment of River Ganga (Samanta 2013).

10.2.4 Religious Activities

In the Hindu religion, people have a high faithful association with the rivers which can be seen from cradle to cremation. Although the Ganges is the site for religious activities, watering and washing of animals, disposal of human bodies, and cremation discharges of industrial wastes, etc., it has become highly polluted with toxic wastes (Agoramoorthy 2015). A number of historical towns, viz., Haridwar, Allahabad, Rishikesh, Varanasi, Gangasagar, Garhmukteshwar, Kannauj, and Mirzapur are considered as sacred sites for the religious activities on the bank of river Ganga, throughout the year. Millions of people visit these places during the festive seasons to take a holy dip in the river known as "Ganga Snan." A variety of materials such as flowers, sweets, coins, and lighted earthen lamps are offered to the Ganga by the devotees. However, people also contribute to the contamination of the Ganges due to the disposal of idols and old holy books in the rivers. As the part of the last ritual, many communities dispose the remains of bones and ashes after the cremation and also dead bodies directly to the rivers in India. Allahabad (Prayag), Haridwar (Har Ki Pauri), Ujjain (Shipra Ghat), and Nashik (Godavari Ghat) are the main places for the people to take bath for massive and large ritualistic events like "Kumbh." According to Hindu mythology, the elixir of immortality was spilled over these four places during the battle of demons and devtas which took place for Amrit, recovered during the "Samudra Manthan" (churning of the ocean). In the year 2010, it has been estimated that 41.6 million people took bath during the period of "Kumbh" at Haridwar. However, an official data from Govt. of Uttar Pradesh, 2013, shows that 88.7 million people took bath during the Kumbh at Prayag in the year 2013 within 45 days. Furthermore, the largest gathering was found on February 10, 2013, on the auspicious day of Mauni Amavasya, when 30 million devotees and

Rishis took a holy dip in the Ganga in a single day. Due to the incautious and massive use of shampoos, detergents, soaps, and the disposal of food, flower, leaves, milk, curd, ghee, polythene, coins, etc., as offering to river Ganga, water quality is being deteriorated severely. Therefore, such ritualistic practices had their own contribution to contaminate the holiest water of the Ganga. An estimate shows that the water quality of river Ganga was remarkably altered which was evaluated by a rapid increase in BOD, COD, and total and fecal coliform counts during the period of Ardh Kumbh at Haridwar in the year 2004. An altered physicochemical and microbial property (decreased pH and increased chlorine, TDS, TSS, SPC, and bacterial counts) of Ganga water due to mass bathing at Haridwar during Maha Kumbh in the year 2010 was reported by Arora et al. (2013). Srivastava et al. (2013) estimated that the water quality of the river decayed after the period of massive bath in the Ganges. In other studies, it has been reported that the water quality of the river at Allahabad was most terrible during the Maha Kumbh 2013 than the time period of Ardh Kumbh in the year 2007 and was also unsuitable for swimming or bathing. In the year 2013, a crucial augment of *Salmonella typhi* was found in the water and sediment of the river Ganga during Maha Kumbh at Allahabad. An epidemiological study by Tyagi et al. (2013) showed a significant rise in the prevalence of gastroenteritis, fever, and skin diseases (water-borne infections) due to bathing events during Ardh Kumbh at Haridwar in 2010. Worship is a traditional and every day practice related to many religions and cultures in Asian countries including India. A huge number of carcinogenic pollutants such as respirable particulate matter, nitrogen and sulfur oxides, and volatile organic compounds (VOCs), viz., formaldehyde, styrene, ethyl benzene, xylene, toluene, benzene, 1,3 butadiene, chloroform, etc., are produced due to burning of incense sticks on several religious and ritual places which cause detrimental injuries to our health. The exposure of temple workers to a higher concentration of 1,3-butadiene (11.29 μg/m^3) and benzene (45.90 μg/m^3) than those of control workers have been reported (Navasumrit et al. 2008). In India, various combustion materials are used for ritual and religious purposes which is different from those used in other countries where usually synthetic materials are preferred for these activities. Hence, religious combustion activities assign a distinct way of emission in India. Furthermore, biomass combustion from household activities like cooking attributes a different characteristic than those of religious activities. In addition, various natural and synthetic organic biomaterials are burnt in several proportions to perform many religious and ritual activities in India. Interestingly, scattering of liquid ingredients such as holy waters and oils over these combustion materials in religious activities like flaming and smoldering of wood, clarified semifluid butter, cow urine, cow dung cakes, hawan materials, viz., vermilion powder, rice, turmeric powder, sesame, cardamom, barley, betel leaf, camphor, clove, betel nut, etc., in marriage ceremony; smoldering of incense sticks, candles, and styrax benzoin, in Muslim graveyards and Buddhist temples; and also the flaming of clarified semifluid butter, vegetable oil, vermilion powder, and cotton in Hindu temples results in the frequent alteration in flaming and smoldering events). Approximately ~3.0 million religious places of worship are there in India where ten million marriage ceremonies are held every year. The couple transits seven circuits around a holy fire fueled by the materials

(wood, cardamom, clarified semifluid butter, cow dung cakes, rice, cow urine, barley, vermilion powder, turmeric powder, clove, sesame, betel leaf, betel nut, camphor, and so on) in the Hindu wedding ceremony (Dewangan et al. 2013).

Idol immersion also comes in the category of religious activities which contributes to generate numerous pollutants and deteriorates the water quality of rivers including the Ganga. A number of religious activities take place around the year in India where Durga Puja is one of the most important festivals celebrated in Bihar, Uttar Pradesh, and West Bengal. Additionally, idols of Durga, Lakshmi, and Ganesha sizes up to 40 feet are made each year during the festivals, viz., Dussehara, Lakshmi Puja, and Ganesh Chaturthi and immersed in the river Ganga at the end of the episode. The materials used to construct and adorn the idols include small iron rods, plaster of Paris, clay, bamboo, cloths, and different paints such as water colors, varnish, plastic, and polystyrene, respectively, which can lead to remarkable changes in the water quality of rivers after immersion. However, some known carcinogens such as As, Cd, Cr, Hg, and Pb are also present in these paints (WHO 2008). Furthermore, the plaster of Paris contains gypsum, sulfur, phosphorus, and magnesium hence submerging these idols leads to an increase in the acidity and high content of heavy metals in the water and makes it noxious. It has been estimated that 5000 L of paint and 100 kg of plaster of Paris and other hazardous materials in the form of idols has been immersed in the river. Due to idol immersion in the river Ganga during the festive season of Durga Pooja in West Bengal, a significant increase in temperature, pH, BOD, COD, conductivity, chloride, phosphate, total alkalinity, and total hardness has been reported by Sarkar (2013). Furthermore, a huge amount of vermilion powder (Sindoor) sprinkled during idol immersion contributes to enhance the level of Pb and Cr in the river.

10.3 Organic Contaminants in Indian Rivers

Worldwide most of the economic development depends on rivers for many centuries, mainly due to the source of freshwater, irrigation requirement, dams on rivers support hydropower production, tourism, and transportation, which is necessary to support a huge population. Therefore, the population increase and continuous development of our society dramatically increase the discharge of the municipal, hospital, and industrial waste. This waste release in the ecosystem increases the load of organic and inorganic pollutants in the river ecosystem and deteriorates the water quality. The uncontrolled discharge of such pollutants into the environments at high or trace quantities (i.e., ng/L or g /L, recognized as micropollutants) contributes to the accumulation of these pollutants in our food chain and adversely affects the animal and human health (Stuart et al. 2012; Vasquez et al. 2014; Radović et al. 2015; Dwivedi et al. 2018). Without water treatment, pollution control mainly depends on natural runoff and natural biodegradation that dilutes pollutants to reduce downstream effects. The load of organic pollutants in rivers is generally expressed in terms of biochemical oxygen demand (BOD). Regardless of the substantial self-cleaning capability of rivers, the number of individuals affected by organic

pollutants (BOD >5 mg/L) is predictable to increase from 1.1 billion in the year 2000 to 2.5 billion by the year 2050 (Wen et al. 2017). Therefore, developing countries with high waste generation and less treatment were excessively affected. These river basins are continuously polluted due to anthropogenic activities therefore regularly reported to exhibit an increased level of pollutants such as herbicides and pesticides, waste and sewage generated by industry, domestic, pharmaceuticals, veterinary medicines, industrial compounds, lifestyle compounds, endocrine disrupting substances, phthalates, and other persistent organic pollutants in river water as discussed in Sect. 10.2 (Jani et al. 2018; Sinha et al. 2018; Dwivedi et al. 2018).

Pharmaceuticals and hormones are emerging organic pollutants that are frequently detected in different ecosystems due to their mass production and consumption to support the huge population. The waste generated from domestic sewage and pharmaceutical manufacturing facilities contains a high level of pharmaceuticals which pollute the rivers and deteriorate the water quality (Larsson et al. 2007; Mutiyar and Mittal 2014; Prabhasankar et al. 2016). Some of the pharmaceuticals are biologically active at very low concentrations and due to their nature they can be easily accumulated in aquatic organisms and through them pass to humans (Brausch and Rand 2011; Tanoue et al. 2015). The availability of these pharmaceuticals in the river ecosystem could promote antibiotic resistance in microbes, which can affect the human microbiome and can contribute to the generation of antimicrobial resistance in the microbial community, which potentially impacts human health (Szekeres et al. 2018). Indian pharmaceutical sector growing prominently in the past few decades occupies 20% share globally in generic medicines supply. India has third rank worldwide for production by volume (Department of Pharmaceuticals, Government of India 2018). Due to easy availability, pharmaceuticals are widely used in developing countries like India, China, and Pakistan for the purpose of intensive animal farming, hospitals, and processed food and feeds. Therefore, wastewater generated from the above sites also contributes to the increased load of pharmaceuticals directly and indirectly to the river water. Scientific literature are available for the detection of pesticides, metals, microorganism (bacteria), etc., in rivers, but very few studies pertaining to the availability of pharmaceuticals in river water were performed. In recent years, the United States Environmental Protection Agency (USEPA) and the United States Geological Survey (USGS) have focused and mentioned a serious concern, on monitoring the availability and fate of emerging pollutants in diverse water sources.

Ganga basin is the largest river basin in India and the fourth largest in the world with an 8,61,404 sq. km catchment area and 2525 km total length. Ganga supports about 43% of the Indian population (448.3 million as per the 2001 census) with an average density of 520 persons/km^2, which is the most deeply populated area in the world. Recent studies showed that the Ganga river water quality continuously depreciates at several locations along with the upper stretch, where the water is not appropriate for domestic purpose (Dwivedi et al. 2018). In the river Ganga, organic pollutants play a crucial role in deteriorating water quality. These contaminants are mainly contributed by municipal, agricultural, and industrial sources. In the Ganga

river, sewage wastewater discharge was mainly responsible for 75% of its pollution which includes the addition of huge metric liter water per day in the vicinity of the Ganga basin (Das 2011). CPCB identified 138 drains along the Ganga river basin, mainly situated in Uttar Pradesh (Daughton and Ternes 1999) and West Bengal (Ejaz et al. 2004). Around 6087 MLD of wastewater, 80% of which is untreated sewage with 500 MLD of toxic industrial waste is directly dumped into the river (CPCB 2016). Therefore, CPCB observed the huge gap between the wastewater generation and their treatment capacity in India ((CPCB) 2013).

India is one of the largest producer and consumer of organic pesticides until now. India produces roughly 85,000 MT of pesticides and consumes about 60,000 MT yearly (Mohapatra et al. 1995; Kumar et al. 2013; Sampathkumar 2014). As the Ganga has the largest basin therefore maximum consumption culminated to addition in the Ganges basin mainly. After agricultural use, the remaining pesticides runoff with water goes into the river through runoff streams and tributaries. Organophosphorus pesticides and organochlorine are the most commonly used pesticides in India which are termed as Persistent Organic Pollutants (POPs) due to their highly persistent nature. Indian Government has withdrawn the use of DDT, aldrin, and HCH in agriculture after the years 1989, 1996, and 1997, respectively, where DDT was barred for agricultural usage in India, but the use of pesticides for public health sector was exempted under the Stockholm Convention. Under the National Malarial Program, 3750 tons of DDT was used in the year 2001 (Devi and Raha 2013). But these pesticides are still used due to the lack of better alternative, low cost, and easy availability (Abhilash and Singh 2009; Vijgen et al. 2011). The conventional treatment processes are generally seen as inefficient in removing antibiotics from wastewater.

Previous study report that the Ganga river upper stream Narora, Kachla, Fatehgarh, and Kannauj has a significant level of Organochlorine such as α-BHC (1380–3010 ng/L), DDT (1360–5330 ng/L), DDD (880–2410 ng/L), dieldrin (410–4110 ng/L), aldrin (950–2810 ng/L), and organophosphorus pesticides like methyl parathion (160–500 ng/L) and dimethoate (200–560 ng/L). Narora site possess a low level of pesticide contamination (Rehana et al. 1995, 1996). Furthermore, Mutiyar and Mittal in the year 2010–2011 analyzed the presence of organochlorine pesticides in large no of samples (Harvey 1991) which cover almost 72% area of the Ganga River. The study reported the presence of DDT (0–12.3 ng/L); HCH (0.1–17.6 ng/L); endosulfan (0–85.4 ng/L); and heptachlor (0–11.8 ng/L). In the year 2017, National Environmental Engineering Research Institute (NEERI), Nagpur assesses the water quality of the river Ganges and reported no detection of POPs in most of the samples. On the basis of the above studies, we can conclude that a declining trend was observed for the presence of POPs in the river Ganga water. (Central Pollution Control Board (CPCB) 2016; Dwivedi et al. 2018; CSIR-NEERI report 2017).

There is a scarcity of data on the monitoring of pharmaceuticals in the river ecosystem. Recently, Sharma et al. (2019) reported the presence of pharmaceuticals and personal care products (PPCPs) in the Ganges River. In this study out of 15 target PPCPs, they detected 14 in the river Ganges. The sum of the detection PPCPs in the

river Ganges sampling sites ranged from 54.7 to 826 ng/L. In this study, highest concentration was observed for caffeine (743 ng/L) followed by ketoprofen (107 ng/L) among the PPCPs. PPCPs high load detected in the middle and lower stretch of the river Ganges compared to the upper Himalayan region. However, the concentration of pharmaceuticals such as ibuprofen, naproxen, diclofenac, and ketoprofen in river Ganga was reported to be less compared to that found in Yamuna River in India (Fick et al. 2009; Mutiyar and Mittal 2014; Sharma et al. 2019).

Testosterone and progesterone are steroid hormones containing biologically active pharmaceuticals. Both the hormones were detected in the Ganga river and the concentration was comparatively high in the middle and downstream as compared to the pristine upstream stretches. Testosterone was detected in low concentration in river Ganges (Pre-monsoon, 30.7%), compared to river Yamuna (33.3%) and high with respect to the Narmada River (14.2%). The level of Progesterone in the Ganges middle region (Pre-monsoon, 38.4%) was found high in comparison to River Yamuna (37%), Narmada (14.2%), and downstream of River Ganga (Pre-monsoon, 12.5%) (CSIR-NEERI report 2017).

As organic contamination level is commonly indicated by the biochemical oxygen demand (BOD) level in water (Wen et al. 2017), according to CPCB report on river quality shows that Ganges' upper stretch (Haridwar), middle stretch (Narora, Kannauj, Kanpur, Allahabad, Raibareilly, Varanasi), and lower stretch (Behrampore, Dakshineswar, Howrah, Kolkata, Diamond Harbour) exhibited a high level of BOD (more than 3 mg/L) which indicated the high level of organic pollutants in the river Ganga (CPCB 2016).

Based on the study of previous BOD data it was suggested that computed BOD concentrations for the year 2050 will be very serious due to variations in urban population, intensive livestock farming, river discharge, and high-water consumption to support a growing global population (Wen et al. 2017). In all situations, this study also predicted that if the rate of wastewater treatment remains constant at their current levels, the absence of additional investments outside existing treatment capacity indicated the alert on the global sanitation crisis.

The river Yamuna is originating from the Yamunotri glacier of the Lower Himalaya in Uttarakhand and travels 1376 km in total length. Yamuna is the longest tributary in India and the second largest tributary river of Ganga. Yamuna River covers 366,220 km^2 of a total catchment area which is 40.2% of the whole Ganges Basin. After passing through the Himalayas, the river Yamuna crosses Delhi roams through Agra city and finally merges with river Ganges at Allahabad. Better water quality of River Yamuna before entering Delhi was reported (CPCB river classification). The Yamuna pass through the Delhi stretch has only 2% of the total length, but received 71% of the total waste discharged in this river (Mutiyar and Mittal 2014). Although along with this stretch the Yamuna river has the highest number of running sewage treatment plants (STPs) with the highest sewage treatment capacity in India (Mutiyar et al. 2018), that is not enough for the treatment of complete waste generated by this area.

High concentrations of pharmaceuticals such as antibiotic amoxicillin in treated sewage (ND- 62.5 ng/L) and raw (ND- 172.6 ng/L) in Delhi sewage treatment plant were detected in Yamuna river (Mutiyar and Mittal 2012; Mutiyar and Mittal 2013). Mutiyar and Mittal in the year 2014 reported ampicillin (13.8 μg/L), ciprofloxacin (1.4 μg/L), gatifloxacin (0.48 μg/L), sparfloxacin (2.1 μg/L), and cefuroxime (1.7 μg/L) antibiotics in the River Yamuna (Mutiyar and Mittal 2014). Although this detected concentration was almost 1000 times lower than those found in the Isakavagu-Nakkavagu Rivers (Mutiyar and Mittal 2014; Balakrishna et al. 2017).

Mutiyar et al. in the year 2017 evaluated the distribution of nine PhACs, viz., ibuprofen, aspirin, caffeine, paracetamol, diclofenac, carbamazepine, ranitidine, diazepam, and codeine related to different therapeutic groups in the river Yamuna at Delhi. In this study, the highest concentration was detected at Wazirabad downstream, where the Najafgarh drain joins the river Yamuna. They reported the presence of Aspirin (0.18–0.77 ng/L), Ibuprofen (0.28–1.4 ng/L), Paracetamol (0.09–1.70 ng/L) Caffeine (0.37–2.08 ng/L), Ranitidine (0.143 ng/L), Carbamazepine (0.07–0.77 ng/L), Codeine (0.05–0.131 ng/L), and Diazepam (0.04–0.10 ng/L) (Mutiyar et al. 2018). In seasonal investigation, it was observed that frequency and concentration residue of antibiotics were maximum in winters (ND-13.75 μg/L), and minimum in monsoon followed by summer season. The most possible region behind is that during the monsoon season huge freshwater comes to River Yamuna, whereas low freshwater flows in other season (Philip et al. 2018).

Persistent organochlorine pesticides are detected in earlier studies in river Yamuna, but recent studies do not detect any pesticides, or if found it is in a non-detected concentration or in a trace level which is below the permissible limit (CSIR-NEERI report 2017). ΣDDT detected 387.9 ng/L in the year 1999, 0.1.44 in the year 1995–2001, 0.12 in the year 2002, and no detection by CSIR-NEERI in the year 2017. ΣHCH detected 310.25 ng/L in the year 1999 whereas in a recent report no HCH is detected by CSIR-NEERI in the year 2017 (Sharma et al. 2014a, b; CSIR-NEERI report 2017).

The presence of organic contaminants is also reported in other rivers in India (Table 10.1). Ten different studies have reported the presence of pharmaceuticals and other organic pollutants in Indian rivers (CSIR-NEERI report 2017; Mutiyar et al. 2018; Archana et al. 2016; Subedi et al. 2012; Mutiyar and Mittal 2014; Shanmugam et al. 2014; Iyanee et al. 2013; Ramaswamy et al. 2011; Kristiansson et al. 2011; Fick et al. 2009). The presence of pharmaceuticals was confirmed by all the above studies in respective rivers. The river Cooum at Chennai city was reported to be contaminated with amphetamine (0.984 μg/L), atenolol (3.18 μg/L), a metabolite of antiplatelet carboxylic acid (1.37 μg/L), ibuprofen (2.32 μg/L), and triclocarban (6.18 μg/L) (Table 10.1).

In another study, samples collected from Patancheru Enviro Tech Limited (PETL) wastewater treatment plant near the drug manufacturer's vicinity in Hyderabad exhibited the presence of the highest level of pharmaceuticals in wastewater as compared to the reports globally. From PETL wastewater goes into the Isakavagu-Nakkavagu stream, and this stream finally merges into the Godavari

Table 10.1 Major contaminant in Indian River and their sources

S. No.	Rivers	Name of contaminants	Source	Reference
1.	Gomti River	ΣDDT, ΣHCH, Aldrin Dieldrin, Endrin	Aged and weathered agricultural soils	Malik et al. (2009)
2.	Tamiraparani river	ΣDDT, ΣHCH, Aldrin Dieldrin, Endrin	Agricultural and municipal outfalls	Kumarasamy et al. (2012b)
3.	Ganga	HCH DDT Aldrin Dieldrin Endosulfan heptachlor chlordane	Untreated effluents from industries, hospitals and urban settlements, agriculture practices	Leena et al. (2011)
4.	Yamuna	HCH DDT Aldrin Dieldrin Endosulfan heptachlor chlordane	Untreated sewage, industrial wastewater, and agriculture runoff	Samanta (2013)
5.	Gomti	HCH DDT Aldrin Dieldrin Endosulfan heptachlor chlordane	Discharge of domestic sewage, industrial wastewater, agricultural field runoff	Singh et al. (2005a)
6.	Rivers of northeast tributaries of ganga Hooghly	HCH DDT Aldrin Dieldrin Endosulfan heptachlor chlordane	Untreated sewage, industrial wastewater, and agriculture runoff	Samanta (2013)
7.	Ganga	Σ-HCH, Σ-DDT, Σ-Endosulfan, Σ-Aldrin, Σ-Hepta, 2-4D	Sewage pollution, industrial effluent, agricultural runoff, and religious activities	Dwivedi et al. (2018)
8.	Ganga	HCH, DDT, DDE, Endosulfan, Dieldrin, Aldrin	Sewage, industrial wastes, burning of corpses, and wastes	Sankararamakrishnan et al. (2005)
9.	Yamuna	ΣHCH, ΣDDT	Agriculture runoff, municipal and industrial wastes	Kaushik et al. (2008)
10.	Yamuna	Σ-HCH, heptachlor, Aldrin, heptachlor epoxide, g-chlordane, Endosulfan 1 + al-chlordane, DDE, Dieldrin, Endrin, Endosulfan 2, DDD, Endrin aldehyde,	Sewage, industrial wastewater, agriculture runoff	Pandey et al. (2011)

(continued)

Table 10.1 (continued)

S. No.	Rivers	Name of contaminants	Source	Reference
		Endosulfan sulfate+ DDT, Endrin ketone, Methoxychlor		
11.	Cauvery	DDT, HCH, Aldrin, Dieldrin, Endosulfan, Chlorpyrifos	Agriculture runoff, industrial wastes discharge, and sewage wastewater	Sarkar et al. (2008)
15.	Ghaggar	ΣHCH, γ-HCH, β-HCH, o,p′-DDT, p,p′-DDT, o, p′-DDE, p,p′-DDE, o,p′-DDD, p, p′-DDD	Agricultural practices and industrial wastes	Agarwal et al. (2015)
16.	Cauvery	p,p′-DDT, p, p′-DDE, p,p′-DDD, Endosulfan	Agricultural practices and industrial wastes	Agarwal et al. (2015)
17.	Yamuna	ΣHCH, ΣDDT	Agricultural runoff from fields and grazing lands	Rajendran and Subramanian (1997)
19.	Cauvery	ΣHCH, ΣDDT	Drainage of agricultural wastewater	Rajendran and Subramanian (1997)
21.	Ghaggar river	γ-HCH, β-HCH, ΣHCH, o,p′-DDT, p,p′-DDT, o, p′-DDE, p,p′-DDE, o,p′-DDD, p, p′-DDD, ΣDDT	Soil erosion and runoff from agricultural areas, discharge of industrial and domestic Sewage	Kaushik et al. (2010)
22.	Ganga river	α-HCH, β-HCH, γ-HCH, p,p′-DDT, o,p′-DDT, p, p'-DDE, o,p′-DDE, α-Endosulfan, β-Endosulfan, Aldrin, Dieldrin, heptachlor	Runoff from agricultural fields, discharge of wastewaters and disposal of containers, etc.	Agnihotri et al. (1994)

River. Although the concentration of these pharmaceuticals in 30 km downstream were significantly reduced compared to PETL outlet: enrofloxacin (3281 times), ciprofloxacin (1400 times), citalopram (86 times), cetirizine (22 times), ofloxacin (9 times), and metoprolol (4 times) drugs, these pharmaceuticals in wastewater will finally fall into the Godavari River and pollute it (Subedi et al. 2012).

10.4 Health Effect

Chlorinated organic compounds, viz., DDT (dichlorodiphenyltrichloroethane), polychlorinated dibenzofurans (PCDFs), hexachlorobenzene (HCB), polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated biphenyls (PCBs), and lindane (γ-HCH or gamma-hexachlorocyclohexane), are the major group of organic contaminants and have been widely used for industrial and agricultural purposes. HCH, DDT, and HCB were highly persistent and toxic in nature and hence their effects on the health of humans were studied in detail (Mrema et al. 2013; Wu et al. 2016). Although pesticides were reported to be responsible for their interference and disruption of endocrine systems, they are also considered as xenohormones (Agrawal and Sharma 2010). A rise in global pollution due to organic micropollutants is a serious threat to the environment and human health also leading to disruption of the natural ecosystem. To look over the impact of organic micropollutants on human health, phytotoxicity, surface/groundwater quality, and aquatic/terrestrial organisms, the Environmental Risk Assessment (ERA) reported a wide range of disorders caused by the exposure to organic micropollutants (Eriksson et al. 2006; van Wezel and Jager 2002; Garland et al. 2000). Serious chronic effects were reported due to the presence of chemical compounds in drinking water (Santos et al. 2010; Kidd et al. 2007) and can lead to irreversible mutations in both human beings and wildlife (Jjemba 2006; Daughton and Ternes 1999). The presence of organic compounds in aquatic systems impairs the reproductive system of fish and organisms residing in freshwater ecosystem (Barnes et al. 2008; Brian et al. 2007). Chlorinated by-products produced by aromatic organic micropollutants were reported more persistent and toxic which can adversely affect living organisms. The formation of several organic micropollutants during chlorination and the occurrence of these compounds in raw water made it carcinogenic in nature (Chan et al. 2003). Pesticides enter the human body via direct contact, viz., by the handling of pesticides, ingestion, penetration through the skin, and inhalation (Spear 1991). Although the human body has a mechanism to excrete toxin, pesticides induce health concerns by getting immediately absorbed into the circulatory system (Jabbar and Mallick 1994). The swindling of skin, throat, eyes, and nose vexation, belly pain, rashes in the skin, skin concupiscence, vertiginous, eyesight weakness, vomiting, nausea, and rarely death are the acute effects of pesticides whereas chronic effects include memory and vision loss. It also leads to asthma, allergy, and damage to the immune system. The presence of pesticide residues in the blood cells of cancer patients has been reported. The toxic effects of pesticides involve the disruption of liver, lungs, kidney failure, leukemia, brain cancer, breast cancer, birth defects, instinctive abortion, and infertility in humans (Casida and Durkin 2013; Asghari et al. 2017). Inhalation of organochlorines causes hypersensitivity to sound, touch, and light, nervousness, confusion, nausea, vomiting, and tremors and even the long-term exposure to pesticides may lead to Parkinson's and Alzheimer's disease (Casida and Durkin 2013).

Pesticides have been reported to cause cardiovascular disorders, hypertension, and other health issues in humans. Although organochlorines interfere with

molecular circuitry and function of the endocrine system, it is also referred to as endocrine disrupting chemicals (EDCs) (Ejaz et al. 2004). Neuromuscular disorders, stimulation of drugs, and steroid metabolism were reported to occur due to direct and indirect exposure to pesticides (Subramaniam and Solomon 2006). Many food items especially fatty food such as poultry, fish, meat, and dairy products can be the other mode of exposure to pesticides (Rusiecki et al. 2008). Several organochlorine molecules are reported to be carcinogenic and neurotoxic in nature (Kaiser 2000) which leads to the increased threat of hormonal cancers including lung, stomach, breast, and prostate cancer (Wolff et al. 1993). Organochlorine pesticides were reported to affect diabetes mellitus induction in humans. A study conducted in Slovakia showed that high levels of obesity markers, viz., body mass index (BMI), triglyceride, and cholesterol and also elevated blood levels in diabetes, viz., fasting glucose and insulinoccurred in large groups of males and females in highly polluted areas (Langer et al. 2014). It has also been demonstrated that exposure to organochlorine pesticides causes a potential risk for gallstones in humans (Su et al. 2012). Even low doses of organochlorine pesticides were reported to cause neurotoxic effects on early psychomotor development (Forns et al. 2012). A study showed the correlation between exposure to organochlorine pesticide and vitamin D deficiency in humans (Yang et al. 2012).

Pesticide contamination and degradation of water quality exhibited two main adverse impacts on human health. One of these includes the consumption of fish and shellfish from contaminated water sources or the direct intake of water contaminated with pesticides. Furthermore, fish economies that recline downstream of major agricultural areas can face the problem in its maintenance. Cancer, tumors, inhibition or failure of reproductive system, disruption of endocrine hormonal system, suppression of immune system, cellular and DNA damage, lesions on fish and animals, and teratogenic effects are major health hazards associated with these persistent pesticides. Physical deformities, viz., hooked beaks on birds, sickness in fish which is observed by excessive slime on fish scales and gills, the low red to white blood cell ratio, other physiological effects like shell thinning, and even the death of organisms can occur due to the exposure to pesticides. It has been reported that children and infants absorb more insect repellents, pediculocides, and pesticide residues through the skin and exhibit more toxicity than adults (Hallberg 1989). It causes alterations in the behavioral pattern and may cause several diseases such as ataxia, muscle cramps, encephalopathy, frequent urination, coma, and seizures (National Research Council 1993; Oranskey et al. 1993). Farmers get exposed to pesticides while spraying chemicals into agricultural fields. Cutaneous and respiratory systems are the main routes for the absorption and predominant contribution to the toxicity in farmers which ultimately leads to the cause of non-Hodgkin's lymphoma (Hoar et al. 1986; Agrawal and Sharma 2010).

HCB (Hexachlorobenzene) is a compound which was used in the manufacture of electrodes as a porosity controller, in chemical industries, and as a fungicide for the treatment of seeds in agricultural fields. It is absorbed into organisms via the alimentary tract, respiratory tract, and skin. The primary toxicity of HCB leads to damage to the liver, thyroid gland, reproductive and developmental endpoints, and

carcinogenesis. HCB causes weakness, pigmentation, hypertrichosis, porphyrinuria, and blisters on the skin exposed to the sun. It has been reported that milk obtained from mothers exposed and unexposed to HCB had average levels of 0.51 μg/g and 0.07 μg/g, respectively (Cripps et al. 1984; Gocmen et al. 1989). An association between the exposure of HCB and thyroid hormones was examined in a group of 341 males which revealed an inverse relationship between total triiodothyronine (T3) and HCB levels of serum (Meeker et al. 2007). Another study showed a tenfold increase in the level of HCB in pregnant women and the concentration was associated with a 51% decrease in total T4 and 8% decrease in free thyroxin (fT4) (Chevrier et al. 2008). Some studies conducted on humans and animals demonstrated that HCB crosses the placenta to accumulate in the fetal tissues and ultimately transfers to breast milk (Cripps et al. 1984; Gocmen et al. 1989). In a case study of Turkey (1955–1959), it was found that almost all children died within a year after the exhibition of weakness, convulsions, and skin lesions that were born to mothers who consumed HCB-contaminated bread during the period of pregnancy (Cam and Nigogosyan 1963; Gocmen et al. 1989). Many studies reported the association between the impact of HCB in the biological fluids of humans and various health problems occurred. On the other hand, the study also evaluated the correlation among the levels of HCB in the serum of the umbilical cord during decreased gestational length and at birth, and (Fenster et al. 2006) increased body mass index (BMI), body weight during childhood (Smink et al. 2008), and increased urinary coproporphyrins in childhood (Sunyer et al. 2008), and poor social competence (Ribas-Fitó et al. 2007). It has also been demonstrated that HCB reduces neonatal viability and growth by impairing reproductive efficiency. A number of associations between the exposure of HCB and cancer have been evaluated especially breast cancer, non-Hodgkin's lymphoma, prostate, and testicular germ cell carcinoma (Starek-Świechowicz et al. 2017).

DDT (Dichlorodiphenyltrichloroethane) and PCBs (Polychlorinated biphenyls) were highly toxic organochlorine compounds which can lead to the thinning of egg shells and abnormal gonadal growth in birds (WHO 2002). Similarly, exposure to PAHs (Polycyclic aromatic hydrocarbons) impairs the health of children. Furthermore, the most common disorder found in children due to PAH exposure and toxicity is attention-deficit hyperactivity disorder (ADHD). Failures in focus on a task, obsessional imperturbable behavior, and quiescent in attention are some common symptoms of ADHD which causes the destruction of family, school, and social life (Barkley 2002). Due to entry via blood–brain barrier, PAHs are responsible for synaptic plasticity and neuronal activity (Chepelev et al. 2015; Dutta et al. 2010). Pentachlorophenol (PCP) also causes a serious threat to human health which includes headaches, contact dermatitis, mucosal problems, respiratory disorders, and asthma (Kelman 2004; Ahmad et al. 2019).

Endosulfan is a compound that also comes in the category of pesticide that can persist in the environment for a long time period and hence bioaccumulates in plants and animals and leads to the contamination of food (Briz et al. 2011). It is also responsible to damage the central nervous system and exhibits higher acute inhalation toxicity than dermal toxicity. Dioxins in human ovarian follicular fluid have

been reported in some studies which may lead to the development of endometriosis. Several autoimmune diseases, including multiple sclerosis and eczema, were reported to occur due to exposure to dioxins (Sinaii et al. 2002). Prenatal exposure to HCB, β-BHC, DDT, and mirex causes a decrease in the birth weight of infants which was demonstrated in a study conducted in China (Guo et al. 2014; Jayaraj et al. 2016).

10.5 Effect on Aquatic Life

Due to high anthropogenic activities such as generating municipal waste, industrial waste, hospital waste, and agricultural runoff increased the load of organic and inorganic contaminants in river ecosystems. Of these, POPs such as PCBs, polychlorinated dibenzodioxin (PCDD), polychlorinated dibenzofuran (PCDF), organochlorine pesticides (OCPs), and carcinogenic PAHs have been reported to adversely affect aquatic life due to their endocrine disruption, mutagenic, carcinogenic, and immunotoxic properties (UNEP 2001). Most of the persistent organic contaminants were resistant to easy biological and photochemical degradation therefore transported to long range in the atmosphere (Wong et al. 2005; Buccini 2003; Wania and Mackay 1993). Organic contaminants were introduced into the river ecosystem through various anthropogenic and natural actions from various contaminated environments thus accumulating in environmental compartments as well as in the organism's tissue due to their lipophilic nature. These accumulated contaminants adversely affect the reproduction and various metabolic activities of aquatic organisms and also subsequently transported to the next tropic level which is harmful to human health (Kumari et al. 2001b; Singh and Singh 2008; Aktar et al. 2009; Jiang et al. 2014; Gundersen et al. 2017).

Indian rivers serve as a home for hundreds of varieties of fishes and many other aquatic organisms. The rivers, directly and indirectly, contribute to the economic growth of the riparian public and to the national economy by supporting the fisheries resources. India comes in third place as a fish producer and second place in inland fish production (Feroz and Panikkar 2006). For the Indian population, fish and other aquatic organisms are the major sources of protein. It is also observed that more than 80% of POPs intake in humans passes through the food chain (Martínez et al. 1997). Indian rivers were reported to accommodate endemic and commercially valuable fish species (Vass et al. 2010). According to previous studies, a significant decrease in commercially valuable fish was reported (Sarkar et al. 2012). Rivers with increasing load of pollution and continuous decline in water level have drastically affected the fishes and other aquatic life. Due to the high load of pollution, several fish species including dolphin in the Ganges are under the threat (Dwivedi et al. 2018). The highly polluted stretch of the Ganges was reported to exhibit loosening of scales and damaging lepidonts of fish (Khanna et al. 2007). In few studies, some toxic metal accumulation in fish' organs such as muscles, brain, liver, skin, gills, and kidney have also been reported (Vaseem and Banerjee 2013).

The level of chlorinated pesticides in the Ganges river was reported to be 8.31 ∑-HCH, 13.23 ∑ − DDT, 0.22 ∑-Aldrin, 2.95 ∑ − Endosulfan, and 5.02 ∑-Dimethoate per kg weight of fish muscles (Dwivedi et al. 2018). HCH and DDT were reported in all the fish tissue from the rivers (rain-fed and snow-fed) in Kumaon Himalayas, Uttrakhand. In a comparative analysis of the concentration of DDT in fish tissue, a lower concentration was detected in rain-fed river samples (ranging 6–9 ng/g) in comparison to snow-fed river samples (ranging 13–55 ng/g) (Sarkar et al. 2003). Another study on bioaccumulation of HCH and DDT levels in fish tissue (Cat and Carp fish) from the Varanasi site of Ganges in comparison to the unpolluted control pond site showed a significantly higher level of DDT and HCH in Ganges fish samples. The level of DDT and HCHs in Ganges fish samples was many folds higher from the USEPA permissible limits (Singh et al. 2008). The level of DDT and HCH in Catfish liver tissue was 4688.69 and 2952.33 ng/g in the Ganges river samples and 67.7 and 96.68 ng/g in the control pond sample. Whereas, in Carp fish liver tissue DDT and HCH amounts to 134.81 and 1181.13 ng/g in the Ganges river samples and 20.9 and 20.71 ng/g in the control pond sample. The concentration of DDT and HCH in catfish was observed to be comparatively very high with respect to Carp fish (Singh et al. 2008).

10.6 Regulation, Prevention, and Mitigation

Since 1970, many international and regional environmental agreements have been signed by India and various sanctions were also set in order to control the risk of chemical contamination, protect the environment and human health. India, in spite of being a democratic industrialized country with an articulated chemical sector, can lead to a rapid change in the environment with many conflicting positions as accessed by different stakeholders. Both management and legal aspects pertaining to the protection from chemical risk were under the responsibilities of the state ministries, central government, and a range of government central agencies in India. Since the assumption of the constitution, the Government of India has approved about 35 pieces of regulations in order to protect the quality of the environment and public health. These existing legal tools deal with persistent organic pollutants and other hazardous chemicals at different stages (registration, classification, identification, production, preservation, packaging, and permits for inspections, operation, discharge, import–export, trade, and transportation) under different authorities (NIP 2011). To secure the safe circulation, use, and disposal of chemicals, and permit the individual responsible authority in order to conceive a ban or restriction are the goal of all these pieces of governance. To tackle the measures in order to continue, restrict, or prohibit the use of pesticides, a Pesticide Management Act, 2008 is also followed. The Ministry of Chemicals & Fertilizers and Central Insecticides Board & Registration Committee (CIBRC) are the chief gazettes to decide whether a contaminant has to be controlled or not. The manufacture, Import and Storage of Hazardous Chemical (1989); The Merchant Shipping Act (1958); The Indian Ports Act (1908), and the Customs Act (1962) regulate the illegal availability of banned chemicals.

Since 1981, India has acquired a number of international conventions, viz., the Stockholm, Rotterdam, and Basel conventions on the management of chemicals followed by the protection of the environment and health safety.

Moreover, since 2005, on the Prior Informed Consent (PIC) procedures India has got the Rotterdam Convention for certain chemicals and pesticides in international trade (Ministry of Environment and Forest, Govt. of India 2006). The first crucial step at the international level to reduce the production and development of toxic wastes and to establish an advanced consent and notification system for trans-boundary movement of wastes is the convention on the control of trans-boundary movement and disposal of hazardous wastes (Sharma et al. 2014b). The government plays a lead role in the regulation of pesticide application because producers and consumers were careless in limiting their sales and use of pesticides (Abhilash and Singh 2009). A meticulous pesticide and chemical registration strategy which ensures the testing in four different climatic conditions and substantiates the availability of toxicological data in Indian conditions tends to confirm the quality control of pesticides. Under a comprehensive statute (The insecticides act 1968) and the rules; the import, transport, manufacture, sale, and use of pesticides are being modulated to verify the availability of quality, potent, and safe pesticides to the farming group and also comprehensively regulate that no portion of the pesticide industry functions outside its attention. Not only the pesticides which are produced, imported, or used in India were needed to get registered with the Central Insecticides Board but also individuals who are involved in stocking, distributing, or selling pesticides also require a license. The act also permits the board to restrict or ban the use of pesticides. Furthermore, the use of more than 30 pesticides has been banned; the use of 7 pesticides including DDT has been restricted; 18 pesticides have been refused for registration by the government. For the application of pesticide equipment, India has a Bureau of Indian Standards (BIS) to prevent the misuse and inappropriate use of pesticides with equipment; implementation of legislations and standards at the field level requires to be strengthened. Time to time reviews of pesticide is done by the Registration Committee and the recommendations were considered by the Ministry of Agriculture. Under the provision of insecticides, Act/Rules, viz., Appellate Authority, Licensing Officers, Insecticide Analysts, and Insecticide Inspectors four important functionaries were notified to enforce the quality of the pesticides. Including the National Information Centre (NIC), India has four poison information centers, a specialized unit that provides information about poisoning treatment, prevention, and hazard management at All India Institute of Medical Science in New Delhi. Since 1985, the integrated pest management approach is being encouraged which explores the role of natural agents in harmony with other schemes of pest management and thus offers an eco-friendly strategy of pest containment and also with the objective to create a little bit disturbance to the environment. In an integrated pest management strategy, the use of plant resistance, natural enemies, and cultural regulation are compatible and supportive approaches (Abhilash and Singh 2009).

To control pollution in the river systems the Government of India has taken some actions such as “Ganga Action Plan” (GAP) which was launched for rapid

minimization of pollution in the river Ganges. In 1984, the Central Pollution Control Board carried out a survey on the Ganga basin, on the basis of which GAP was launched by the Ministry of Environment & Forests (Department of Environment) in December, 1984. In April 1985, the scheme approved by the government had two objectives which are (Abad et al. 2005) to minimize the load of pollution in the river Ganga and (Abhilash and Singh 2009) to set up a sewage treatment system in 25 Class I cities situated at the border of the river. To lay down the policies and programs and also to supervise the performance of the Ganga Action Plan (GAP), the Central Ganga Authority (CGA) under the chairmanship of the Prime Minister was established by the Government of India in February 1985. To carry out the projects under the guidance and supervision of the CGA, in September 1995, National River Conservation Authority (NRCA) was renamed as a wing of the Department of Environment. Although GAP I could not cover the load of pollution in the Ganges completely, GAP II (the Ganga Action Plan Phase II) was launched between 1993 and 1996 in stages. During the period of April 1993 to October 1996, the GAP II was approved by the Cabinet Committee on Economic Affairs (CCEA) at different stages. The states which applied the GAP II by the treatment of 1912 MLD of sewage include Uttar Pradesh, Bihar, Uttarakhand, Haryana, Delhi, and West Bengal. The first important law for the protection of environmental resources with the constitution of a National Committee on Environmental Planning and Coordination, and the approval of the Wildlife Protection Act, 1972 occurred during 1970s. Since then, three major texts, viz., the Prevention and Control of Pollution Act (Water Act), 1974; the Environment Protection Act, 1986; and the Water Prevention and Control of Pollution (Water Cess Act 1977) were preceded at the central level. The Pollution Control Boards at the central and state levels were organized by the Water Act 1974. However, The Pollution Control Boards along with the funding tools were furnished by The Water Cess Act, 1977, which permits them to charge the water user with access thus providing the financial support required for the board's activities. The Environment Protection Act, 1986, was an umbrella legislation which was assigned to plug the loopholes of previous environment-related legislations and pays attention towards the protection of the environment. The impact of informal regulation of pollution on the water quality of Indian rivers was evaluated by Goldar and Banerjee (Goldar and Banerjee 2004). In Indian rivers, an econometric analysis of determinants of water quality is carried out for this purpose during 1995–1999 from 10 important rivers by using the data for 106 monitoring points. The results revealed a significant and positive impact of pollution regulation on water quality in Indian rivers (Agrawal and Sharma 2010).

Microbial remediation of pollutants is an eco-friendly approach which leads to the conversion of organochlorine compounds into CO_2 and H_2O as end products which poses no toxicity. This is considered as a cost-effective technology than the physical and chemical methods used to remove the contaminants (Nwankwegu and Onwosi 2017; Rajiv et al. 2010). Organochlorine pollutants degrading microbes can be isolated from contaminated sites or marine sediments which serve as the reservoir for such microbial diversity (Ferreira et al. 2016). Interestingly, the production of enzymes from microorganisms which have the ability to degrade organic pollutants

is an advanced technique to remediate contaminated sites. The bioaugmentation strategy is an alternative technology for the bioremediation of contaminants. The use of vegetation or phytoremediation is another promising and innovative way to remove pesticides from contaminated site. Moreover, phytoremediation is an economically effective and alternative approach which involves the ability of plants to remove the contaminants from various environmental matrices, viz., soil sediment, surface water, groundwater, and even the atmosphere. To remove the pollutants from the environment through phytoremediation, plants can also be used in combination with rhizospheric microorganisms which promotes the removal and/or degradation of organic pollutants from the soil ecosystem (Susarla et al. 2002; Gerhardt et al. 2009; Flocco et al. 2004). Furthermore, genetically modified (GM) plants also contribute to the remediation and prevention of organochlorine pollution. It has been reported that the genetically modified plants of the first generation were resistant to certain pesticides. However, *Bacillus thuringiensis* (Bt) in combination with plants such as Bt-cotton and Bt-corn plays a vital role in suppressing the use of pesticides. The constitution of wetlands is another path for the bioremediation of pollutants coming through the runoff and drainage from agricultural contaminated sites. This technique can lead to a synergistic association among the phytoremediation, its rhizosphere, and bioaugmentation. Vymazal and Březinová (2015) broadly reviewed the examples of these constructed wetlands. In recent decades, the use of fungi as bio resources has been considered as a powerful and beneficial tool for in situ degradation and remediation purposes. Moreover, the characterization and application of a number of fungi have been done to carry out the bioremediation and biodegradation of organic pollutants. Many studies proposed that the application of fungal strains is an effective approach to degrade various pesticides such as lindane, atrazine, methamidophos, cypermethrin, dieldrin, endosulfan, heptachlor, chlorpyrifos, and methyl parathion. A number of biochemical processes, viz., oxidation, hydroxylation, deoxygenation, demethylation, esterification, dechlorination, and dehydrochlorination play a crucial role in the activities of fungal isolates during the biodegradation of various pesticides residues. Moreover, different enzymes such as hydrolases, peroxidases, lactases, esterases, dehydrogenases, manganese peroxidases, and lignin peroxidases support to carry out the biodegradation of a broad range of pesticides (Maqbool et al. 2016). The physical and chemical features of some environmental factors like the characteristics of substrate, pH, temperature, nutrients, inoculum density, pesticide concentration, and others may lead to the failure of bioremediation or biodegradation strategies of the pollutants (Anwar et al. 2009; Chishti et al. 2013). Therefore, on the basis of studies it has been evaluated that the bioremediation technique is an eco-friendly and cost-effective tool as compared to the physical and chemical technologies. Furthermore, it can also be considered as an advanced approach for natural resource management by the decontamination of soil and water bodies which can be achieved through the development of processes, strategies, and regulations (Marican and Durán-Lara 2018).

10.7 Conclusion

Rivers in India play a crucial role in people's lives from the beginning of the civilization until the current urbanization and industrialization. There are 12 major river systems in India, and three of the river systems shared neighboring countries, i.e., Bangladesh, Bhutan, China, Nepal, and Pakistan. Amazingly, 42% of the geographical area in India was drained by these three river systems alone, and serve as the lifeline of millions in India. Globally, the continued industrial, agricultural, religious, and human development activities are expected to release large qualities of contaminants into the major rivers of India. These contaminants are expected to persist, accumulate, and undergo trophic transfer in the food chain. Already several aquatic and terrestrial species have been severely affected and have become extinct, highly impacting the biodiversity. In order to find ways and methods to rehabilitate disturbed water bodies such as lakes, rivers, and streams, it is necessary to find multiple sustainable and reliable solutions. Unless the global communities undertake remedial measures on a war footing, the public health management costs will escalate as already human populations have been affected. This chapter emphasizes the implementation of innovative solution through the bioremediation process as one of the option that offers to rehabilitate and restore the contaminated sites. Although the challenge lies in finding methods for individual group of contaminants, viz., metals, dyes, pesticides, PCBs, and emerging pollutants like PPCPs, the complementing effort from environmental engineers, analytical chemists, geochemists, and biologists together may address the issues of contamination. To find a long-term sustainable solutions for pollution abatement, institutional mechanisms and coordination of regulatory agencies will play a crucial role in safeguarding the mother earth making it a livable place for all.

Acknowledgments This manuscript bears IITR communication number 3650. VKG is thankful to Council of Scientific and Industrial Research, New Delhi, for providing Senior Research Fellowship.

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Is Marine Waste a Boon or Bane? An Insight on Its Source, Production, Disposal Consequences, and Utilization

11

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Abbreviations

ASC	Acid soluble collagen
BOD	Biological oxygen demand
EPA	Environment protection authority
FPC	Fish protein concentrate
FPH	Fish protein hydrolysate
PSC	Pepsin soluble collagen
TSE	Transmissible spongiform encephalopathy
TTX	Tetrodotoxin

11.1 Introduction

Fish is consumed as a good source of protein and other vital nutrients by millions of people around the globe. Consumption of fish over the years has increased dramatically such that more than 3.2 billion people relied on fish for over 20% of their protein requirement during the year 2016 (Zhou 2017). According to past 20 years data, the maximum production of fisheries is found from coastal regions like China, Indonesia, and India (as mentioned in Fig. 11.1). India has seen a massive jump in the amount of seafood being consumed especially between the years 1990 and 2010. To cope with the increasing demand for fish and other seafood, the farming of

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_11

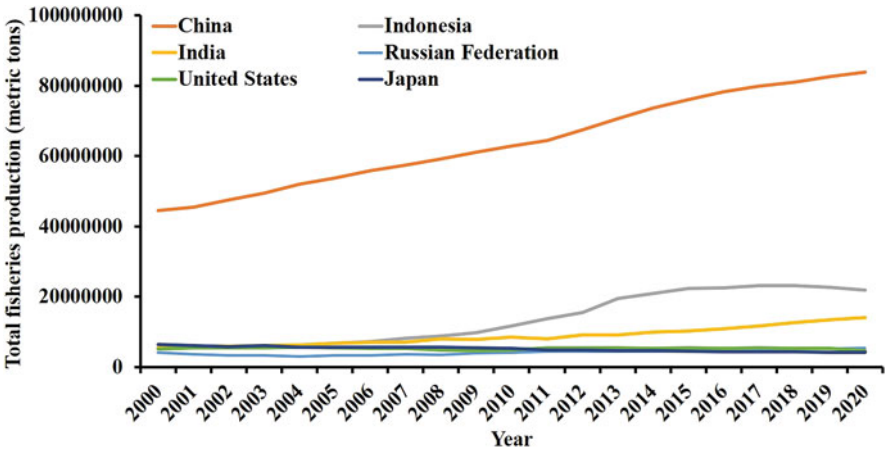

Fig. 11.1 (Source: https://data.worldbank.org/indicator/ER.FSH.PROD.MT)

marine organisms has boomed over the past few decades (Rao 2010). The increase in the consumption of seafood has resulted in the generation of high amounts of waste; more than 27% of the captured fish are either thrown away or rot before they can be sold (Ryder 2018; Kruijssen et al. 2020). Whole fish and shellfish are rarely consumed; most people only consume the flesh (40%), while the discard bones, shells is roughly 60% of total weight (Agustin et al. 2021). India being one of the largest fish-producing nations, generates 2 metric million tonnes of fish waste annually according to a survey conducted. The data on fish waste generation in the major coastal cities of India showed that Mumbai (22.60%) is the largest contributor followed by Chennai (15.10%) and Goa (12.50%) (Ahmad and Bhuimbar 2019). The improper discard of these wastes causes pollution to the surrounding environment and disrupts the coastal ecosystem. The waste management process can be best optimized by proper disposal or finding a specific utility for it. For finding an economically valuable purpose of the byproducts, it is very important to know the source of fish waste production (Boziaris 2014; Munari et al. 2016).

11.2 Sources of Fish Waste Production

11.2.1 Aquaculture and Fisheries

The fisheries industry is dependent on two major forms of fishing: (i) capture fisheries and (ii) aquaculture. Capture fisheries consist of the traditional form of catching fish in the wild while aquaculture is an advanced technique which involves building enclosures for the fish, crustaceans, mollusks, and aqueous plants in the water bodies. The fish in these enclosures are given high protein and other nutrient feeds which enhances their growth rate (Ryder 2018). However, this comes with a

consequence of impact on its surrounding environment, when an attempt to produce a higher number of fish per unit area is made. The attempt for increasing profits must be made in accordance with the amount of waste that can be managed to make the process sustainable. Thus, the fish must be fed according to its size, digestibility, excretion, and other health and physiological factors to ensure an optimal growth process (Bowyer et al. 2012).

The fish which are unable to survive the culture process accumulate as solid waste or suspended solid waste. This accumulation could alter the physiochemical balance of the aquaculture including biological oxygen demand (BOD) and chemical oxygen demand, which would further degrade the habitability of the aquaculture. Due to the high BOD of the fish waste, it is imperative to perform timely rectification to avoid health hazards in the aquacultures and oceans (Dauda et al. 2019). The environment protection authority (EPA) in Victoria, Seychelles has tested fish wastes and has declared them safe for biological decomposition under controlled conditions. They can be used for land applications after being subjected to appropriate treatments, irrespective of their shape and size as they fall under prescribed industrial waste. Thus, these waste materials can be processed in a designated composting facility after acquiring EPA Works Approval and License. The composting facility is built in proximity to the aquaculture if it has optimal leachate, odor, moisture, and surface run-off conditions (Venugopal 2021).

11.2.2 Seafood Industry

The seafood industry usually discards inedible components like skin, fins, bones, and scales as waste. These components however can be used for the production of value-added products. Organs like liver, gills, and kidney are highly susceptible to contamination by toxins like tetrodotoxin (TTX) and conotoxin, a powerful neurotoxin, which makes them unsuitable for consumption and are thus usually removed from seafood to avoid seafood poisoning (Saravanan et al. 2009; Lago et al. 2015; Schneider et al. 2018). Consumption of crustaceans such as crab, shrimp, and lobster gives rise to an enormous amount of shell waste (Joshi et al. 2020). It has been estimated that annually about 6–8 million tons of shell waste are being produced in the world out of which, Southeast Asia itself accounts for about 1.5 million tonnes, which is an alarming amount of waste (Yan and Chen 2015). Despite having high amounts of calcium, carbohydrates, and proteins, they are not considered to be of much value and are sold. Currently, these wastes are being utilized for the production of microparticles used for various applications including anti-inflammatory drugs, chitin production, and auxiliary fertilizer or as a component of animal feed. A large amount of research is being carried out to value chitosan as a natural biopolymer and its potential to be part of high-quality bioplastic (Hamed et al. 2016).

11.2.3 Fishing Harbors

Fishing harbors are one of the highest accumulators of fish waste for two main reasons: (i) during transportation of fish from harbor to market, fish and other marine organisms are accidentally dropped, resulting in random displacement of fish and fish remnants all around the harbor; (ii) the damaged fish are thrown either back to the ocean or in the harbor shore (Balde et al. 2020). The vendors are also known to sell only the edible part of the fish to the customers, and throw the inedible parts in their vicinity which can lead to the accumulation of fish offal and blood water waste (Fig. 11.2). This can produce an unpleasant sight and odor, degrading the esthetic value of the place. Mostly, the damaged fish are collected for animal feed. But, due to high yield per day, fish waste continues to be dumped in the harbor area, though disposal of that high level of waste is a difficult task (Read and Fernandes 2003).

Fig. 11.2 Images of fish waste dumped in fish harbor in Royapuram Coast, Tamil Nadu

11.2.4 Industrial Fish-Based Processes

Many industries using fish and other marine organisms generate huge amounts of semi-processed marine waste. This includes fish and shrimp processing units subjected to chemical treatments like calcium hypochlorite, or cleaning using soaps and detergents (Mathew et al. 2021). Each part is processed separately according to its specific needs for the products they are capable of synthesizing. The carcass of the fish is rich in meat, processing of which is first started by the removal of swim bladder, which has no significant contribution to human diet. Other parts like skin, bones, etc., are separated from the edible parts and are processed separately for production of leather and other valuable products. As a result, a huge amount of waste is generated when only the essential parts of the fish and other marine organisms are used (Sasidharan and Mathew 2011; Willis et al. 2018). The fish and shrimp processing units generate wastes which are released in nearby water bodies creating an imbalance in various parameters like BOD, suspended solids, etc., and thus polluting it and making it unfit for use (Dauda et al. 2019).

11.3 Classification of Fish Waste

Marine wastes can be categorized as solid and liquid waste; both solid and liquid wastes have their respective problems and disposal issues. The following flowchart (Fig. 11.3) explains the classification of marine waste along with their method of treatment/disposal. Aquaculture waste is categorized into three categories 1, 2, and 3 ranging from the highest to lowest risk, respectively. Waste disposal is carried out under strict regulations to prevent any health hazard. Category 3 consists of the fish that are caught for the sole purpose of being converted to fish meal or other by-products (Sharp and Mariojouls 2012). These are of least harm to the environment, but still require licensed facilities containing the proper apparatus for disposal. Mortalities that are not caused by intentional slaughter for human consumption are classified as Category 2, these should be disposed of through legal disposal routes. Fish and other marine organisms affected by communicable diseases belong to Category 1, posing the highest risk and require appropriate disposal methods (Dauda et al. 2019; Miller and Semmens 2002). Table 11.1 describes all the categories of aquaculture/fishing waste:

11.4 Waste Treatment

11.4.1 Primary Waste Treatment

The main objective of primary treatment is to remove the large sized solid particles that either float on the surface or settle at the bottom. It involves treatment of the wastewater using three basic physical processes. (i) Screening: Screening involves passing the liquid through a sieve-like structure, through which solid particles are

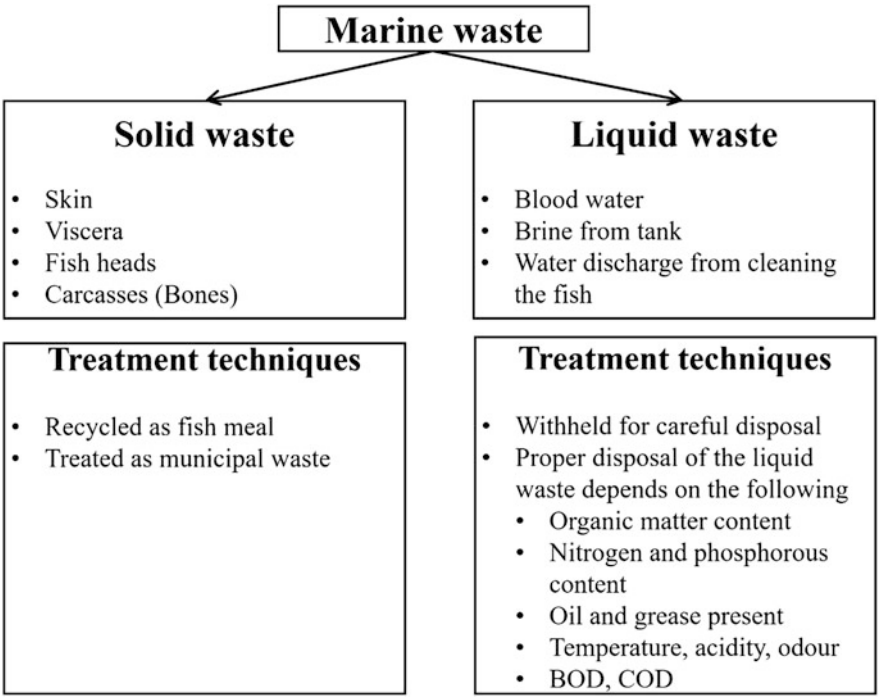

Fig. 11.3 Classification of marine waste

trapped in the sieve, while the liquid flows out. The size of the sieve can be changed depending on the size of particles present in the liquid. (ii) Sedimentation: Sedimentation is the tendency of particles to settle in a solution. Particles settle due to the difference between their density and that of the solution. Higher density particles settle while lower density ones remain afloat. This concept is very useful in the removal of solid particles present in a liquid solution. (iii) Flotation: As opposed to sedimentation, during flotation particles float over the liquid surface (Fig. 11.4). These suspended or lower density particles can be collected and removed (Cristóvão et al. 2014).

11.4.2 Secondary Waste Treatment

It involves the use of microbes for the degradation of wastewater. This is done through the usage of aerobic as well as anaerobic microbes for efficient treatment of the waste. Aerobic microbes digest the organic matter in the presence of oxygen to release carbon dioxide along with biomass, while anaerobic microbes produce methane, carbon dioxide, and water. Following this, the physicochemical process (Coagulation-flocculation) utilizes substances known as flocculants/coagulants to

Table 11.1 Categories of fish waste and their disposal

Category	Raw material	Disposal
1	All body parts affected by TSE (transmissible spongiform encephalopathy) Containing traces of environmental contaminants Suspected of being infected with diseases communicable to human or other animals	Incineration processing in approved category 1 processing plants buried in approved landfill sites
2	Fish farming mortalities (routine mortalities or catastrophic mortalities) Mortalities where the fish are dead on arrival mortalities where the fish show clinical signs of disease On-farm mortalities where the fish have died due to either a harmful algal bloom, jellyfish attack, adverse weather condition	Incineration processing in category 2 processing plants. The raw material can be ensiled or composted and used as feed for other animals
3	Fish or other sea animals caught in the open sea are meant for the purpose of reduction to fish meal Fresh fish by-products from plants manufacturing fish products for human consumption	Incineration used as raw material in pet food ensiled or composted

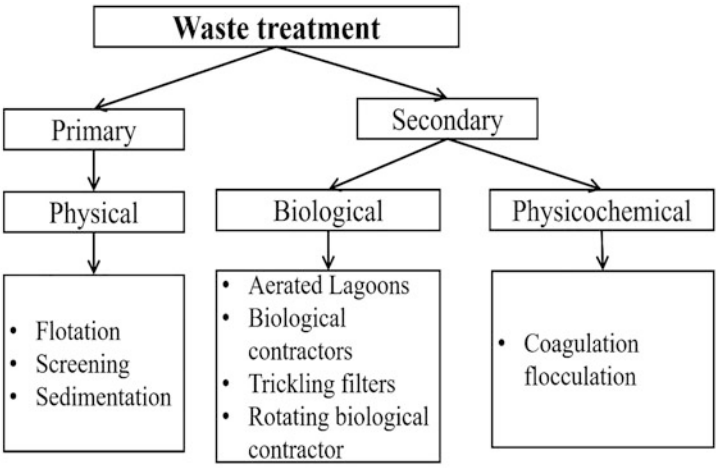

Fig. 11.4 Waste Treatment Processes

cause aggregation of the particulate matter to form "flocs" which then settle down (Mack et al. 2004).

11.5 Fish Waste Disposal

The importance of organized fish waste management is increasing due to concern over health and sanitation issues. Fish waste which is disposed and circulated improperly can lower the oxygen levels in water bodies and as water decomposes it leads to foul odor. Small-scale fish industries produce around 20–80% of fish waste (Islam et al. 2004; Arvanitoyannis and Tserkezou 2014).

11.5.1 Fish Disposal Treatment Methods

They are classified mainly into two different types: traditional fish waste disposal and anaerobic digestion as illustrated in Table 11.2. Traditional methods are the conventional methods which are used to dispose of fish waste (Arvanitoyannis and Kassaveti 2008; Green and Mattick 1977). These methods have major drawbacks and are not commonly used.

11.5.2 Anaerobic Treatment

Fish canning industries produce a large amount of anaerobically digested solid waste. Most of this solid waste can be used to produce methane in reasonable amounts. Research has shown that anaerobic digestion and co-digestion of fish waste has significant potential for bio-methane production (van Rijn et al. 1995; Nges et al. 2012). Fish waste has seen very limited use as substrate in anaerobic digestion due to two major issues: (i) variable composition (up to 60% protein, 20%

Table 11.2 Traditional/conventional fish waste treatment

Technique name	Description
Landfill	Technique employed for disposal of waste by burying under soil
Ocean dumping	Dumping of waste into the ocean. An easy and ineffective disposal method
Direct feeding	It involves feeding the animals dried and ground fish waste to increase the nutritional value of the feed
Minced-based products	Mince products are prepared using either whole fish or gutted fish. Fish mince finds application in several foods like fish finger, cutlets, etc.
Incineration	The process of incineration involves combustion of the sample containing organic material. It converts the sample to ash, flue gas, and heat
Ensilage and land injection	Liquefied products made from either whole fish or gutted fish by the action of the natural enzymes present in the fish along with added acid. The ensiled fish can then be injected into the land

fat, calcium and hydroxyapatite from bones and scale, palmitic acid, mono-saturated acids); (ii) release of high quantities of ammonia as a result of fish waste digestion inhibits digestion of the substrate and can also result in the accumulation of volatile fatty acids. In contrast, co-digestion has been found to be a suitable option to overcome these issues by increasing the organic content of the substrate through the addition of another substrate, such as cow manure, water hyacinth, and sisal pulp (Boziaris 2014; Mshandete et al. 2004).

11.6 Utilization of Marine Waste

As previously stated, the incorrect disposal of marine waste leads to many disagreeable circumstances. Thus, there is a need for novel techniques for the utilization of waste to produce useful products (Fig. 11.5). Utilization of marine waste can be of two broad categories: (i) for the production of nutritional supplements or consumable products, and (ii) non-nutritional uses (Plazzotta and Manzocco 2019; Radziemska et al. 2019).

11.6.1 Nutritional Supplements or Consumable Products

11.6.1.1 Fishmeal and Fish Oils

Fishmeal is commercially used nutritive feed for aquaculture and farm animals. It is a powdered product produced from fish waste or whole fish of any species through drying and grinding which are generally not used for human consumption. When combined with fish oil, it is a rich source of protein, vitamins, and essential oils (Han 2015). The production of fishmeal is initiated by pressing cooked fish waste or fish, yielding a cake and the removed liquid. This cake along with any remaining solids, which can be separated from the removed liquid, is dried to produce the fishmeal. This liquid is then subjected to centrifugation to separate the oil from water (Fig. 11.6). The oil thus obtained can be further processed and sold commercially as fish oil (Das et al. 2011; Mo et al. 2018).

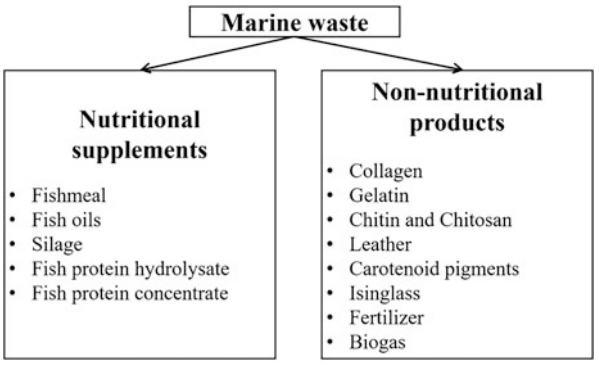

Fig. 11.5 Utilization of marine waste

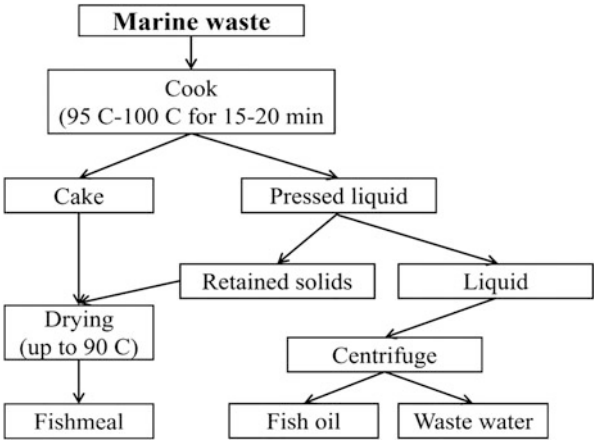

Fig. 11.6 Production of fishmeal

Fish oils are of two types: Oil present in the muscle of the fish and the oil present in the visceral mass of the fish including the liver. It is also possible to extract fish oils from the heads, fins, and scales in fish waste (Aidos et al. 2001). During fishmeal production, the crude oil obtained consists of both muscle and visceral oil components which are then purified and used in aquacultures or for bulk production of products such as margarine, ink, and rubber. They have also been used in the treatment of hide in leather production. Products such as cod liver or shark liver oils are most commonly used due to their high content of omega-3 fatty acids, Vitamins D and A (Bockisch 2015). Crude oil obtained during fishmeal production is washed and centrifuged for usage in animal feed followed by purification if it is intended for human consumption. The process begins with alkaline refining to prevent the rancidity of the oil during storage, followed by the removal of the clear layer formed upon settling of the mixture. Following this, bleaching of the oil is carried out to remove undesirable fatty acids and pigments. Hydrogenation is then carried out using hydrogen in the presence of a catalyst (Fig. 11.7). In succession, vacuum steam distillation is carried out to remove more volatile compounds and stabilize the flavor of the oil (Archer 2001).

11.6.1.2 Silage

Fish silage is a liquid product used in animal feed similar to fishmeal due to its high nutritious value. It can be produced from any type of fish or from fish waste through low value, relatively inexpensive fish that are generally used for the production of silage. Silage can be produced through the enzymatic digestion of fish waste or through the addition of acid for degradation (Ke et al. 2017). Production of fish silage by acid digestion consists of four main stages. The first stage involves the rupture of cells to release intracellular enzymes through mincing of the fish. This is followed by acidification of the sample using 3.5% Formic acid, though mineral acids can be used as cheaper alternatives. This addition of acid initiates the production of the silage which is enhanced by intermittent mixing (Fig. 11.8). The last two

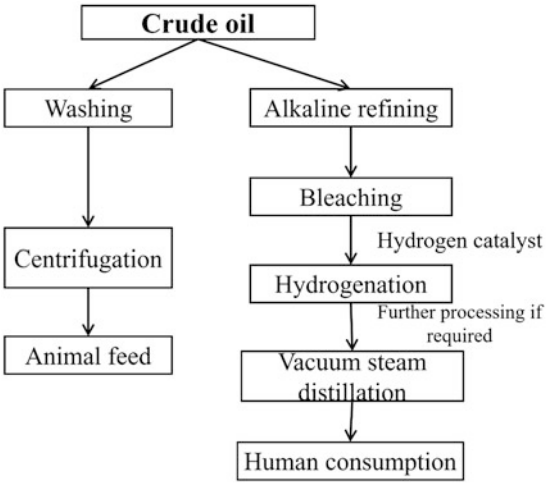

Fig. 11.7 Production of fish oil

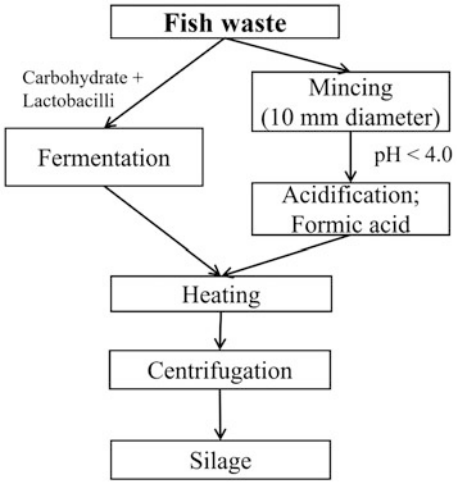

Fig. 11.8 Production of silage through acid digestion and enzymatic degradation

stages of the process are removal of oil from the silage through heating followed by centrifugation (Arruda et al. 2007).

Silage production through enzymatic digestion involves the addition of an inexpensive carbohydrate source and lactobacilli to the fish waste. The bacteria cause acidification of the system by converting the sugars to lactic acid while simultaneously producing additional products associated with the deterrence of spoilage. This mixture is then incubated at 25–30 °C and the oil is removed through the same processes as silage produced by acid digestion. The major drawback of silage is the inconvenience faced during its transport (Ghaly et al. 2013; Olsen and Toppe 2017).

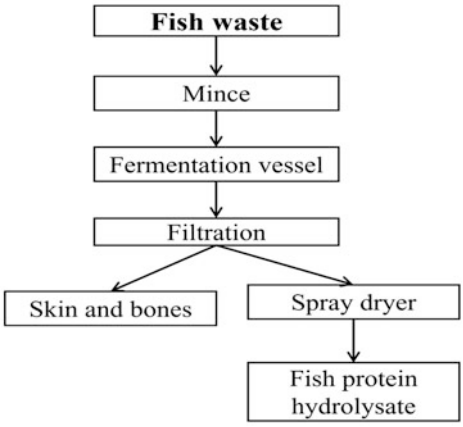

Fig. 11.9 Production of Fish Protein Hydrolysate

11.6.1.3 Fish Protein Hydrolysate (FPH)

FPH is a powder produced by proteolytic digestion of fish to yield amino acids of numerous lengths as mentioned in Fig. 11.9. It is used in animal feed or as an emulsifying agent due to its high gelling and whipping properties (Kristinsson and Rasco 2000). The production of FPH is either through an autolytic process or through the addition of external proteolytic enzymes, i.e., accelerated hydrolysis (Balde et al. 2021). While the autolytic process depends on the enzymes of the fish itself, accelerated hydrolysis is generally preferred as there is no interfering enzyme activity. The autolytic process is time-consuming and involves the breakdown of peptides over a period of time ranging between a few days to a few months. Preservative agents such as organic acids or salts are added to prevent spoilage (Rustad and Hayes 2012; Yoshida et al. 1999, 2003). FPH production using external enzymes involves the mincing of the fish waste followed by agitation with the addition of a commercial protease (Rustad et al. 2011). Optimum conditions for the protease activity are then maintained through the addition of an acid or alkali and maintenance of temperature. The resultant broth is then filtered and spray-dried for commercialization (Anal et al. 2013). FPH may also be utilized further for the production of bioactive peptides and proteins showing various biological activities (Narayanasamy et al. 2020).

11.6.1.4 Fish Protein Concentrate (FPC)

FPC is a powder similar to FPH which is intended for human consumption. It is often used to increase the protein content of food or even as a food product in itself (Ahmad et al. 2019). The FPC is of three grades: Type A, B, and C. Type A FPC is used in a variety of edible products such as biscuits, bread, and soups while Type B is used as a nutritional supplement in some Asian countries. Both are manufactured through a multistage solvent extraction method followed by the removal of fats and odor components. Type C FPC is produced through a more hygienic fishmeal process (Archer 2001).

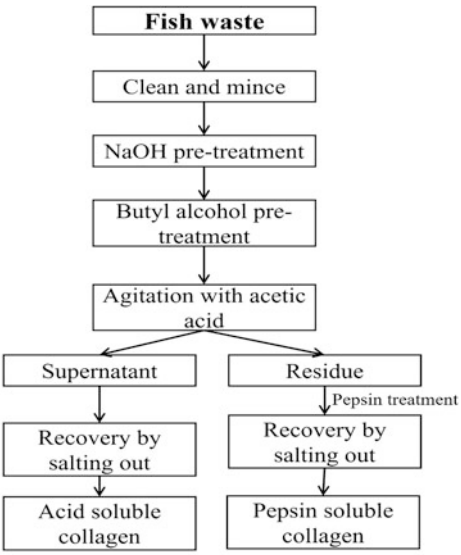

Fig. 11.10 Production of collagen

11.6.2 Non-Nutritional Uses

11.6.2.1 Collagen

Collagen is the major structural protein in the extracellular matrix found abundantly in skin, bones, and fins of fish. It is used by the food, cosmetics, biomedical, and pharmaceutical industries as well as being considered for a novel wound dressing material in the form of films (Govindharaj et al. 2019). Isolation of collagen is in three general stages: Sample preparation, Extraction, and Recovery. The fish waste is prepared by cleaning and pretreatment using an alkali-like sodium hydroxide as described in Fig. 11.10. Extraction is achieved by agitation with an acid to give acid-soluble collagen (ASC). Pepsin is also used to improve the yield of collagen; this is pepsin soluble collagen (PSC) (Sampath Kumar and Nazeer 2013). Recovery of the extracted collagen is through salt precipitation or salting out of the protein (Kumar et al. 2012; Kim and Mendis 2006).

11.6.2.2 Gelatin

Gelatin is a voraciously used substance in the food industry for the formation of gels, for the improvement of elasticity, and stability of food. Fish gelatin has found application in food, formation of coatings, and photograph processing (Kouhdasht et al. 2018). It is produced by hydrolysis of collagen. Fish skin is soaked in an alkali solution to remove pigments and undesirable proteins after which it is washed and soaked. The process is accompanied by continuous gentle stirring in acetic acid followed by distilled water upon swelling of the skins. The product is filtered and the filtrate is lyophilized to give gelatin powder (Nazeer and Deepthi 2013; Nazeer and Suganya 2014).

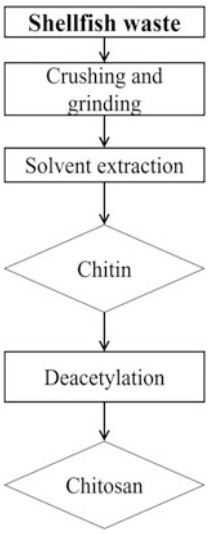

Fig. 11.11 Production of chitin and chitosan

11.6.2.3 Chitin and Chitosan

Chitin is a biopolymer composed of repeating N-acetylgluoseamine units that are primarily manufactured from shellfish waste. It is converted to the more stable chitosan through deacetylation by alkali treatment which is used in effluent treatment, paper, food, and cosmetic industries (Morin-Crini et al. 2019). Extraction of chitin is initiated by crushing the waste shells followed by the extraction of pigments from the shell waste using a suitable solvent to produce chitin (Fig. 11.11). Chitosan is produced by deacetylation of chitin using a strong alkali (Sudhakar et al. 2020).

11.6.2.4 Leather, Carotenoid Pigments, and Isinglass

Leather is made from fish skin similar to animal hide to make clothing, bags, shoes, etc. The skins of larger fish such as cod, shark, and salmon are generally preferred for the leather-making process. Fish leather is often lighter, strong, and attractively patterned and is thus becoming more prevalent in the fashion industry through prominent designer clothing brands (Duraisamy et al. 2016).

Carotenoids are pigments which contribute to the characteristic pink-red color of fish and shellfish. They are extracted from shellfish waste through solvent extraction during the extraction of chitin and are essential to aquacultures as they cannot be synthesized by the fish and thus must be taken up through food. Astaxanthin is a commercially marketed carotenoid used in aquacultures and as a nutraceutical product for the treatment of a variety of conditions including Alzheimer's disease, Parkinson's, and sunburns (Stepnowski et al. 2004).

Isinglass is a tough leather-like product sold commercially as a powder or paste. It is produced from the swim bladder of fish and is used as a common additive to beverages such as beer and in the food industry as a replacement for gelatin. Production of isinglass begins with the cleaning of the swim bladders of the fish

followed by salting for 1–3 months (Asty et al. 2018). After slating, the swim bladders are washed thoroughly and the fat-rich tissue is removed. They are then stretched out and dried in sunlight to remove all water. The innermost collagenous layer is separated from the other two layers and sundried. The product at this stage is called fish maw. This maw is moisturized in water and flattened by pressing between iron rollers to form thin sheets that is the final isinglass (Shahidi et al. 2019).

11.6.2.5 Biodiesel and Fertilizer Production

Fish and other marine organisms are usually rich in fats and oil. This is the primary requirement for the synthesis of biodiesel and is a suitable alternative fuel for the current conventional sources, which is currently being rapidly depleted. Fish oil can be converted to biodiesel by suitable preliminary treatments (Ahmad and Bhuimbar 2019). Fish oils are less dense than commercially used vegetable oils, which lessens the carbon deposition and viscosity, making it a better source for biodiesel production. Ozone pretreatment is one of the most essential steps in biodiesel production. It aids in structural modifications of various types of biomasses, which would result in release of carbohydrates and other substrates for hydrolytic processes, resulting in production of biodiesel. The filtration processes are carried out in high-grade kaolin filters. The biodiesel produced is tested for various parameters like flash point, density, percentage of sulfur, etc. (Travaini et al. 2016).

The harmful side effects of synthetic fertilizers has encouraged the development of new innovative organic fertilizers. A mixture of agricultural waste and fish waste has proven to have very good nutritional properties and rapid degradability, which makes it a good alternative component of fertilizers (Radziemska et al. 2019). Though this application is only recently put into research spotlight, it has been traditionally used as fertilizers by Native Americans, using Herring fish. This technique can be proven to be very useful, especially in islands and remote coastal areas, where access to commercial fertilizers is bleak. Any fertilizer must essentially have NPK elements, i.e., nitrogen, phosphorus, and potassium, respectively. Apart from this, they should enable the soil to regenerate other minerals in the required proper ratio. Amalgamation of fish waste and seaweed was proven to be a good fertilizer after being tried in North-west Spain coast plantations (López-Mosquera et al. 2011). This way, we can get rid of seaweed and also improve crop yield. The fertilizer was synthesized by composting for months together, to derive the required nutrient ratio, enzyme degradability, moisture, and various other parameters. Fish waste has also proven to improve the C to N ratio of soil. As the proteins in the fish remains start degrading on burial, the essential nutrients, nitrogen and carbon get released into the soil. It is important that before being brought to use, it must be subjected to phytotoxicity tests (like root growth inhibition) and derive clearance. However, it must be noted that fish fertilizers are notorious for attracting animals like bear and raccoons. Thus, it should be buried deeper in the soil, or completely avoided if there is any risk of these animals (López-Mosquera et al. 2011).

11.7 Conclusion

Fish consumption has rapidly increased over the years, which has led to a complementary increase in fish waste accumulation as a large percentage of fish is inedible. Various sources of fish wastes have been identified including poorly maintained aquaculture, fisheries, and inedible fish remnants from seafood industries. Harbors are also a site of fish waste accumulation, degrading their esthetic value. Industries release untreated or partially treated fish wastes in water bodies leading to a decline in its quality index. Waste segregation practices are gradually being adapted based on the treatment and disposal methods used. The most commonly practiced disposal techniques include acid hydrolysis and anaerobic digestion. Various traditional methods of waste disposal have been put into practice including landfills, ocean disposal, incineration, direct feeding, and its usage in silage and mince-based products. Currently, this waste is used for the production of commercially valuable products. Mostly, nutritional values are tried to be exploited by making fishmeal for aquaculture and farm animals, extract vitamins and omega-rich oil, preparing silage, fish protein hydrolysates for commercial purposes, and fish protein concentrates which are used in edible products. Non-nutritional products like collagen, gelatin, chitin and chitosan, leather, fertilizer, biodiesel, isinglass, and carotenoids are also extracted from fish waste and can solve various environmental issues.

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A Waste-to-Wealth Prospective Through Biotechnological Advancements

12

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12.1 Introduction

Agricultural wastes are end-products or by-products of the production of agricultural commodities and the indecorous management of these wastes may contribute to various environmental hazards. Usually, the agro-wastes are discharged into the environment without any proper treatment or are burnt off, which leads to municipal landfilling and environmental load along with potential contamination and transmission of hazardous materials to the environment (Chia et al. 2018). Also, the incineration of such wastes produces greenhouse gases that are dangerous to the environment and human health (Bosio et al. 2013). Amounting to 350 million tonnes annually, India produces agricultural wastes whose appropriate handling is still in its primary stage (Saikia et al. 2020a, b). The majority of these leftovers are utilized as wood fuels, and also they might serve as the raw materials for a variety of commercial goods. The valorization of waste materials including agro-wastes is an appealing economic approach, due to the existence of cellulose backbone (Ren et al. 2009; Saikia et al. 2020c; Rathankumar et al. 2020a). Yet, the current research gap around essential scientific investigations makes large-scale management becomes difficult. The absence of appropriate treatment and downstream technologies and the viability of different integrated waste treatment procedures serve as typical examples of this gap. Moreover, the improper classification of the agro-wastes poses another obstacle

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_12

in the development of recycling and valorization procedures, which have immensely affected the conversion of agro-industrial activity into a closed loop biorefinery model and the access to sustainable raw materials. Even though there have been several scientific studies on the viability and desirability of valorization technologies, the majority of these technology have been merely developed as theoretical models and have yet to be implemented in the industry. This chapter discusses an summary of the processing and agro-wastes application as a veritable resource to produce industrially important products like biofuels, enzymes, adsorbents and organic acids, for commercialization and environmental applications with their simultaneous management.

12.2 Types of Agro-Wastes

12.2.1 Crop Residues

The agricultural residues obtained after the harvesting of crops represent the most abundant, economic, and easily available source of organic waste that can be bio-transformed. This class of agro-wastes includes husk, bagasse, straw, peelings, cobs, and other lignocellulosic residues (Mtui 2009). These residues are biodegradable and can be subjected to various processes like anaerobic digestion and solid-state fermentation (Ren et al. 2009) to produce biofuels and several other industrially important biological macromolecules.

12.2.2 Animal Manure-Livestock Wastes

The production of animal manure is more than 1500 annually, out of which cattle manure corresponds up to 1284 million tons and pig manure corresponds to 295 million tons (Mtui 2009). The unused manure when not managed or treated poses a great threat to the air and water systems. Moreover, animal manure releases up to 18% CO_2 equivalent and 37% methane, which directly contribute to the greenhouse effect (Ren et al. 2009). In the past few years, extensive work has been done in the anaerobic digestion of animal manure which can be subsequently used as fertilizer in agriculture. Moreover, this manure can also be co-digested with agro-waste for the production of methane and biohydrogen, etc.

12.2.3 Food Wastes

Food wastes constitutes up to 75–80% moisture and 85–90% of volatile solids which favors the growth of microorganisms with high energy content (Li et al. 2008). In general, these wastes are mostly landfilled which create foul odors and leachates which potentially pollute the groundwater table and nearby water bodies. Over the last few years, food wastes have been studied extensively as potential feedstock for

the production of biofuels and other value-added commercial products (Li et al. 2008).

12.3 Agro-Waste Utilization Routes

12.3.1 Conventional Methods of Agro-Waste Management

12.3.1.1 Direct Combustion

Direct combustion of agro-waste as fuel is the oldest method of biomass conversion. The complete combustion of agro-waste involves the rapid oxidation of biomass with oxygen and the subsequent release of energy. However, this method is environmentally not friendly due to the release of CO2 during combustion, which adds to the greenhouse gases (Obi et al. 2016). Despite the adverse effect of combustion on the environment, it is the most widely used method for addressing agro-waste and accounts for up to 95% of the total biomass energy.

12.3.1.2 Pyrolysis

Pyrolysis is a thermochemical process where agricultural waste is heated at 400–600 °C in the absence of an oxidizing agent to produce char and bio-oil. Pyrolysis of agro-wates has garnered great attention in Europe and America in recent time and many researchers have utilized various lignocellulosic wastes for bio-oil production by pyrolysis (Aravind et al. 2020). Bio-oil has a high calorific value, can be easily stored or transferred, and can be converted to other useful chemicals due to the low content of sulfur and nitrogen. The maximum yield of 70%, w/w, bio-oil from rice husk was obtained at 450 °C by Guedes et al. 2018. The valorization of agro-wastes by pyrolysis is shown Fig. 12.1.

12.3.1.3 Vermicomposting

Vermicomposting is the solid phase decomposition of the organic residues by combined action of microorganisms and earthworms in an aerobic environment. Agro-waste, which is a by-product or end product of agricultural materials, can serve

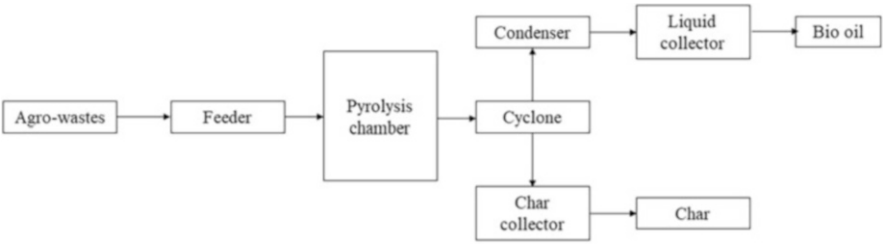

Fig. 12.1 Schematic flow for pyrolysis of agro-wastes to produce biochar and bio-oil. Pyrolysis becomes a thermal process which is performed in the absence of an oxidizing agent at high temperatures generally for the production of biofuels

Table 12.1 Different agro-wastes tested for vermicomposting

Agro-waste	Species of earthworm	References
Crop residues of post harvest	*Eudrilus eugeniae*	Suthar (2008)
Bagasse	*Eudrilus eugeniae*	Sen and Chandra (2007)
Wood waste	*Eisenia fetida*	Maboeta and Van Rensburg (2003)
Olive pomace	*Eisenia Andrei*	Plaza et al. (2008)
Rubber leaf litter	*Eudrilus eugeniae, Eisenia fetida*	Chaudhuri et al. (2003)
Vegetable wastes	*Eudrilus eugeniae, Perionyx excavates, Eisenia foetida, Pheretima elongate*	Pattnaik and Reddy (2010)
Sugar cane bagasse	*Drawida willsi*	Tambe (2011)
Organic matter, dried yard waste and crushed leaves	*Eisenia foetida*, *Lumbricus rubellis*	Pattnaik and Reddy (2010)
Wheat straw	*Eisenia foetida*	Suthar (2008)

Table 12.2 Chemical composition of vermicompost

Parameters	Values
Total carbon (%)	9.1–17.8
Total nitrogen (%)	0.5–0.9
Phosphorus (%)	0.1–0.2
Sodium (%)	0.05–0.3
Potassium (%)	0.15–0.25
Copper (mg/kg)	2.0–9.5
Sulfur (mg/kg)	128.0–548.0
Zinc (mg/kg)	5.7–9.3

as potential substrates for earthworms (Pattnaik and Reddy 2010). Presently, these wastes are not utilized completely due to in situ land disposal or burning. Thus, these wastes could be selected for resource recovery through vermicomposting for agricultural land restoration (Tambe 2011). Table 12.1 shows various agricultural wastes that have been explored for vermicomposting.

The vermicompost obtained after the composting process has high humus content and exhibits nominal phytotoxicity. It consists of most of the nutrients required for plant growth, such as nitrates, phosphates, and calcium (Table 12.2). Thus, the vermicompost can be utilized as a fertilizer for the restoration of land applications (Pattnaik and Reddy 2010). The major benefits of vermicomposting are as follows:

- Increases soil fertility
- Improves the holding capacity of water in soil
- Mediates the restoration of soil microbial population

12.3.2 Valorization of Agro-Wastes

Agro-wastes generated from different activities which can be valorized in various ways to produce many value-added products as shown in Fig. 12.2.

12.3.2.1 Production of Biofuels

Energy is the backbone of global economic growth and the rapid economic growth over the past decades has urged energy consumption considerably, which has anticipated unprecedented pressure to save energy (Srivastava et al. 2020). The change in lifestyles along with industrialization and globalization are the key drivers for the rise in energy demands and the 2010 World Energy Outlook predicted that the global energy demand will rise by 36% by 2035 (Birol 2008). In this context, a global energy transition from fossil fuels to low-carbon solutions is essential, which could be addressed by technological innovations in renewable energy. Bioenergy can substitute heat, electricity, or transport fuels and accounts for 11–14% of the world's present total energy supply (Kumar et al. 2019). Renewable energy can constitute the largest low-cost substitute for energy security and reduce the dependency on limited energy sources.

Biofuels, compared to the other sources of renewable energy, constitute the most popular source as they can be transported and stored, and can be used for power generation on demand (Srivastava et al. 2017). The initial copious views on the production of biofuels were challenged due to the dawdled pace of development and the varied understanding of the impacts of this technology on sustainability.

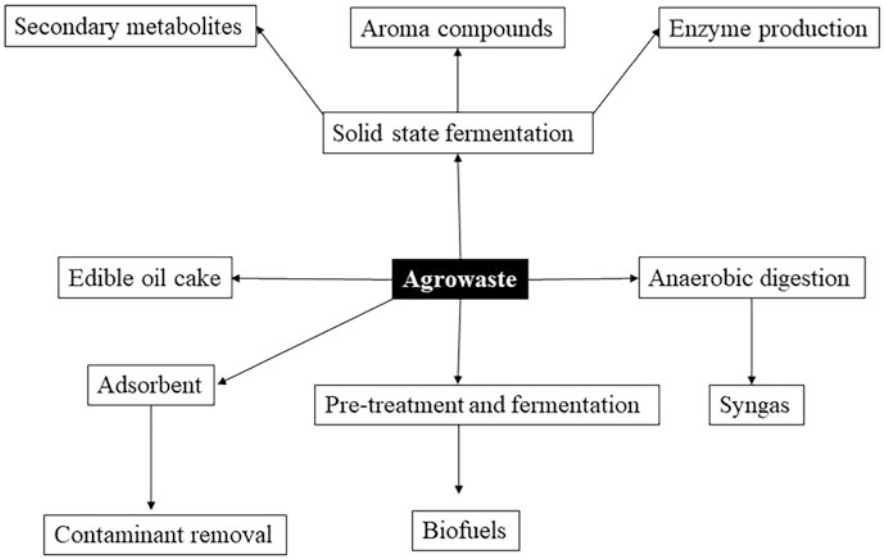

Fig. 12.2 Schematic pathway to convert the agro-wastes into industrial bioproducts and biofuels through biotechnological processes like aerobic and anaerobic fermentation

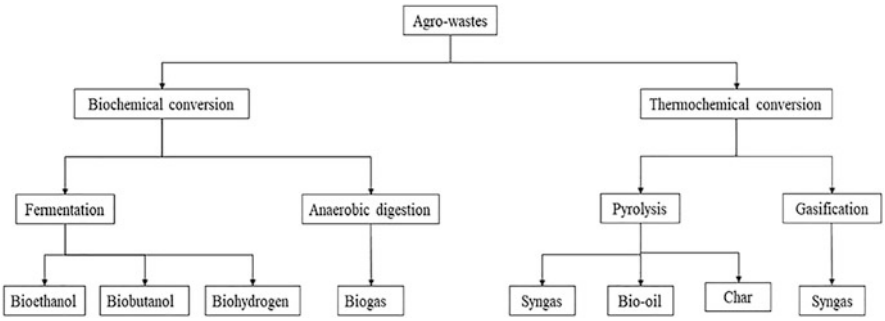

Fig. 12.3 Potential biochemical and thermal treatment routes to convert the agro-wastes towards the production of biofuel. The biochemical route mostly involves the utilization of aerobic and anaerobic fermentation processes to produce bioethanol, biobutanol, etc. Pyrolysis and gasification are the commonly used thermochemical treatments for the production of syngas, bio-oil, etc.

Table 12.3 Biofuel potential of various agro-wastes

Agro-waste	Yield of bioethanol	Yield of biomethane	Yield of biohydrogen	References
Rice straw	12–29[a]	302[b]	3.40[c]	Akhtar et al. (2017)
Wheat straw	11–79[a]	290[b]	21.40[d]	Yuan et al. (2018)
Rice bran	–	–	2.75[c]	Tandon et al. (2018)
Barley straw	11–46[a]	–	47.20[d]	Qureshi et al. (2014)
Corn stover	40–55[a]	338[b]	50.10[d]	Qureshi et al. (2014)
Sugarcane bagasse	23–59[a]	278[b]	14.10[c]	Hu et al. (2018)
Apple pomace	8.44[e]	–	12.92[d]	Wang et al. (2010)
Orange Peel waste	6[e]	217[b]	–	Joshi et al. (2015)

[a]L/kg of dry mass
[b]L/kg of volatile solids
[c]g/L acetone-butanol-ethanol
[d]g/L
[e]%

However, biofuel production reached 143 billion liters in 2017; the five major countries in the area of biofuel production include the United States, China, Germany, Argentina, and Brazil (Kumar et al. 2019).

Using agricultural wastes like rice bran, rice straw, and sugar cane bagasse are regarded as a common method of producing biofuel feedstock (Fig. 12.3). The

biofuel potential of various agro-wastes is shown in Table 12.3. When compared to grain crops, the main benefit of employing agricultural wastes is that no extra land is needed for cultivation, minimizing land competition and reducing direct influence on commercial farming. Moreover, removing agricultural waste helps some crops indirectly and perhaps reducing insect attacks (Kumar et al. 2019; Yuan et al. 2018).

Biotechnological processes to convert the and residues to produce biofuels are efficient in decreasing greenhouse gas and hazardous by-product emissions, which will help in solving the crisis of fuels.

12.3.2.2 Production of Organic Acids

Organic acids are soluble, hygroscopic, and chelating in nature that makes them suitable for various formulations at 37°C. These advantages of organic acids have established their importance in the food and beverage industries. Various scientists have widely evaluated the production of organic acids from agricultural residues through solid-state fermentation (Table 12.4). The use of agro-wastes provides a cheaper and easily available raw material which is produced in large quantities.

Table 12.4 Solid state fermentation utilizing various agro-wastes to produce the organic acids

Agro-waste	Microorganism	Organic acid	Acid production (g/kg)	References
Sugarcane bagasse	*Lactobacillus sp.*	Lactic acid	249	John et al. (2007)
Pineapple waste	*Aspergillus niger, Aspergillus Foetidus*	Citric acid	132	Lima et al. (1995)
White grape pomace	*Aspergillus niger*	Citric acid	85	Papadaki et al. (2019)
Bagasse	*Aspergillus niger*	Citric acid	–	Vaishnavi et al. (2012)
Sugarcane bagasse	*Rhizopus oryzae*	Lactic acid	–	Pandey et al. (2001)
Corn husk	*Aspergillus niger*	Citric acid	259	Hang and Woodams (1985)
Cassava peel	*Aspergillus niger*	Citric acid	88.73	Adeoye et al. (2015)
Cassava bagasse	*Streptococcus thermophilus*	Furmaric acid	–	Pandey et al. (2001)
Coffee husk	*Aspergillus niger*	Citric acid	150	Hang and Woodams (1985)
Fig waste	*Aspergillus niger*	Gluconic acid	490	Singh et al. (2003)
Sweet potato waste	*Rhizopus sp.*	Oxalic acid	26.4	Leangon et al. (1999)

12.3.2.3 Production of Enzymes

During any fermentation process, substrate selection is an significant factors that determine the success of the process. The economic perspective of the process completely depends on the availability and cost of the substrate. In this context, the use of agro-wastes represents a possible low-cost materilas for the synthesis of microbial enzymes (Robinson and Nigam 2003). Lignocellulosic wastes contribute majorly to the agro-wastes available worldwide and represent the most abundant renewable biomass source (Kumar et al. 2019). The various agro-wastes utilized as substrates for the microbial production of enzymes are listed in Table 12.5.

12.3.2.4 Production of Protein-Enriched Feed

Agricultural residues have found signification applications for the production of energy; but their animal feed usage is greatly constrained due to the low content of protein, vitamins, and other nutritional components. However, after protein enrichment by using various microorganisms through solid-state fermentation, they could be utilized for animal nutrition (Robinson and Nigam 2003). A number of researches are available in the literature on the use of agro-waste as animal feed after protein enrichment which is listed in Table 12.6. The choice of microorganisms used for the fermentation process depends on the substrate used. Although these wastes are cheap sources of raw materials to produce protein-rich feed, the scale-up of these processes is constrained mostly due to logistic costs.

12.3.2.5 Production of Aroma Compounds

The growing interest in the utilization of natural products in the food industry has definitely stimulated in developing the biotechnological processes to produce various aroma compounds. These compounds also find their application in the manufacture of perfumes and cosmetics among many (Medeiros et al. 2001). On this front, the development of biotechnological processes to produce these metabolites by microbial bioconversion or fermentation constitutes an economical alternative to the higher cost extraction processes involved with raw materials like plants. In recent years, constant efforts have been undertaken in utilizing agricultural wastes like coffee husk, cassava bagasse, and sugarcane bagasse as substrates to produce of food aromas through solid-state fermentation. Even though numerous microorganisms are employed for the synthesis of potentially valuable aromas, the yields are very low which restricts their industrial application (Christen et al. 2000). The common agro-wastes utilized for the production of aroma compounds are listed in Table 12.7.

12.3.2.6 Production of Secondary Metabolites

The production of econdary metabolites are microbial secretions produced at the log phase and in the stationary phase. They constitute a class of industrially important microbial products and majorly include antibiotics, steroids, and alkaloids. In recent years, various agricultural wastes, like rice husk, rice bran, corncobs, wheat straw, etc., have been globally considered as cheaper and easily available raw materials for

Table 12.5 Utilization of various agro-wastes as substrates for enzymes production

Enzymes	Agro-waste support	Microorganisms	Productivity	References
Cellulase	Wheat bran	*Rhizopus oryzae*	437 U/g, 5 days	Pandey et al. (2016)
	Corn stover	*Aspergillus fumigatus*	526 U/g, 4 days	Liu et al. (2011)
	Rice husk	*Aspergillus niger*	401 U/g, 96 h	Dhillon et al. (2012)
	Apple pomace	*Aspergillus niger*	134 U/g, 48 h	Dhillon et al. (2012)
	Wheat bran	*Aspergillus niger*	395 U/g, 96 h	Bansal et al. (2012)
Amylase	Date waste	*Bacillus licheniformis*	209 U/g, 7 days	Afrisham et al. (2016)
	Wheat straw	*Bacillus sp.*	6900 U/g, 5 days	Qureshi et al. (2016)
	Wheat bran	*Aspergillus oryzae*	1491 U/g, 3 days	Kaur et al. (2012)
Laccase	Sugarcane bagasse	*Pleurotus ostreatus*	167 U/g, 5 days	Karp et al. (2012)
	Rice straw	*Pyrenophora phaeocomes*	10,859 U/g, 4 days	Rastogi et al. (2016)
	Wheat bran	*Coriolus sp.*	2661 U/g, 10 days	Mathur et al. (2013)
Lipase	Castor bean waste	*Penicillium simplicissimum*	155 U/g, 96 h	Godoy et al. (2011)
	Jatropha seed cake	*Pseudomonas aeruginosa*	932 U/g, 9 days	Joshi et al. (2011)
	Sugarcane bagasse	*Burkholderia cenocepacia*	72.3 U/g, 96 h	Liu et al. (2013)
	Sugarcane bagasse and soybean oil	*Thermomucor indicae seudaticae*	15 U/g, 72 h	Ferrarezi et al. (2014)
Pectinase	Sugarcane bagasse	*Aspergillus oryzae*	40 U/g, 18–24 h	Biz et al. (2016)
	Citrus peel	*Aspergillus niger*	265 U/g, 96 h	Sethi et al. (2016)
Protease	Wheat bran	*Aspergillus niger*	262.78 U, 48 h	de Castro et al. (2015)
	Jatropha seed cake	*Aspergillus versicolor*	3366 U/g, 96 h	Veerabhadrappa et al. (2014)
	Wheat and rice bran	*Pleurotus sajor-caju*	85 U/g, 192 h	Ravikumar et al. (2012)
Xylanase	Wheat bran	*Bacillus aerophilus*	45.9 U/g, 24 h	Gowdhaman et al. (2014)
	Wheat bran	*Aspergillus oryzae*	2830 U/g, 24 h	Pirota et al. (2013)
	Rice straw	*Promicromonospora sp*	85 IU/g, 96 h	Kumar et al. (2011)

Table 12.6 Valorization of agro-waste to produce the protein-rich feed

Substrate	Microorganism used	Product	References
Cassava bagasse	*Lactobacillus sp., Saccharomyces cerevisae, Rhizopus oryzae, Brevibacterium divaricatus., Cephalosporium eichhorniae, Pleurotus sp., Lentinus sp., Aspergillus sp., Geotricum fragrans*	Animal feed and food; protein-enriched biomass, single cell protein	Ubalua (2007), Oboh and Elusiyan (2007), Oboh (2006), Obadina et al. (2006), Fagbemi and Ijah (2006), Sriroth et al. (2000), Jyothi et al. (2005), Damasceno et al. (2003)
Apple pulp and waste, grape waste, pineapple waste	*Rhizopus oligosporus, Penicillium funiculosum,*	Protein-rich feed	Villas-Bôas et al. (2003)
Waste fiber of cactus	*Myrothecium verrucaria, Aspergillus niger, Saccharomyces sp. Saccharomyces cerevisae*	Protein-rich feed	Araujo et al. (2005)
Rice bran/ straw; paddy straw; sawdust; lignocellulosic waste	*Trichoderma viridae; Aspergillus niger; Pleurotus ostreatus Trichoderma reesei,*	Protein-rich feed/ biomass	Bonatti et al. (2004), Yang et al. (2003), Banik and Nandi (2004)
Cane bagasse	*Trichoderma viride; Trichoderma reesei*	Protein-rich feed	Valino et al. (2002)

Table 12.7 Aroma compounds production from agro-wastes

Substrate	Microorganism used	Aroma compounds	References
Coffee husk	*Ceratocystis fimbriata*	Pineapple aroma	Sugawara et al. (1994)
Cassava bagasse	*Kluyveromyces marxianus*	Fruity aroma	Medeiros et al. (2000)
Palm bran	*Kluyveromyces marxianus*	Fruity aroma	Medeiros et al. (2000)
Apple pomace	*Ceratocystis fimbriate*	Fruity aroma	Medeiros et al. (2000)
Rice waste	*Neurospora sp.*	Fruity aroma	Bramorski et al. (1998)
Tropical agro-waste	*Rhizopus oryzae*	Volatile compounds	Christen et al. (2000)

the production of secondary metabolites at a commercial level. In this context, the culturing of microorganisms on agro-wastes for the generation of secondary metabolites is an ideal approach. This is mainly done by solid-state fermentation with a lower moisture content which allows the microbial transformation of biological molecules. Apart from other microbes, the majority of fungal species

Table 12.8 Production of secondary metabolites from agro-wastes

Substrate	Microorganism used	Secondary metabolite	Yield (mg/g)	Application	References
Cottonseed oil cake	Streptomyces clavuligerus	Cephamycin	15	Antibiotic	Kota and Sridhar (1999)
Rice husk/ bran	Aspergillus oryzae	Aflatoxin, Ochratoxin	–	Mycotoxin	Pandey et al. (2001)
Wheat straw	Acremonium chrysogenum	Cephalosporin C	22.28	Antibiotic	Adinarayana et al. (2003a)
Wheat waste	Streptomyces marinensis	Neomycin	17.15	Antibiotic	Adinarayana et al. 2003b
Wheat bran	Tolypocladium infautum	Cyclosporin A	–	Immuno suppressive	Pandey et al. (2001)
Wheat bran	Bacillus subtilis	Iturin	3.66	Antibiotic	Ohno et al. (1992)
Peanut shells	Streptomyces sp.	Tetracyclin	13.18	Antibiotic	Asagbra et al. (2005)
Sugarcane bagasse	Penicillium chrysogenum	Penicillin	10.55	Antibiotic	Barrios-González et al. (1993)
Wheat bran	Bacillus licheniformis	Bacitracin	4.82 iu/g	Peptide	Farzana et al. (2005)
Soyabean residues	Bacillus subtilis	Surfactin	–	Antibiotic	Pandey et al. (2001)

Table 12.9 Types of oil cakes and their composition

Oil cake	Protein (%)	Fat (%)
Coconut	21	8
Cotton seed	40	8
Groundnut	51	1
Linseed	29	8
Mustard	35	8
Sesame	37	8

are used in solid state fermentation to produce secondary metabolites is shown in Table 12.8.

12.3.2.7 Edible Oil Cakes

Oil cakes could be a the solid waste which are generated after the extraction of oil from the plants by solvent extraction or pressing. Because to their excellent nutritional properties, these cakes are typically utilized to meet the nutritional needs of both livestock feed and human consumption (Table 12.9). The major edible oil cakes that dominate the global oil cakes market are soybean, rapeseed, cotton-seed, linseed, groundnut, sunflower, and copra cake. Out of them, soybean cake

represents up to 54% of the total production, followed by 10% cottonseed and 10% rapeseed (Gangadharan and Sivaramakrishnan 2009).

12.3.2.8 Agro-Waste as Adsorbents for Contaminant Removal

Due to rapid industrialization and urbanization, there is an excessive discharge of organic and inorganic contaminants into the environment which affects human health (Akpomie and Conradie 2020, Rathankumar et al. 2020b). Due to various disadvantages of the already available treatment processes, like low metal removal, high energy requirements, generation of toxic by-products, etc., Many studies have been conducted recently with the agricultural wastes as biomass which acts as adsorbents to facilitate the removal of pollutants (Kulshreshtha 2019). Numerous works have been published on the heavy metals adsorption on agro-waste and a number of studies showed the immobilization of heavy metals on agro-waste (Najam and Andrabi 2016). Further, the adsorption of contaminants, mainly dyes and organic pollutants, in various studies have established agro-waste as an excellent environmentally friendly and economical adsorbent for the removal of contaminants from the ecosystem (Dupont et al. 2005; Sahmoune 2019). The various agro-wastes utilized for the contaminants removal are shown in Table 12.10.

12.4 Conclusion

The global discernment for agro-waste generation and management is rapidly shifting towards sustainable utilization due to the necessity for environmental conservation and world's food security. Due to this elevated requirement for sustainability, different techniques were developed for the effective utilization and reprocessing of these wastes. Biotechnological approaches, such as solid-state fermentation, have laid down efficient platforms with low substrate cost and low energy requirements for the utilization of agro-wastes to produce various value-added commercial compounds. Further, the protein-enriched feed produced from agro-wastes offers renewable opportunities for animal nutrition. Thus, future research on biotechnological approaches and technologies will improve the deployment of improved products from waste thereby addressing the management of the surplus agro-wastes produced annually.

Table 12.10 Utilization of various agro-wastes as adsorbent for contaminant removal

Contaminants	Agricultural waste	Adsorption capacity (mg/g)	References
Organic pollutants			
Tetracycline	Rice straw	14.16	Wang et al. (2017)
Tetracycline	Sugarcane bagasse	48.35	Wang et al. (2017)
Fluoroquinolone	Rice husk	63.5	Ashrafi et al. (2016)
2,4-dichlorophenoxyacetic acid	Bagasse	7.14	Deokar et al. (2016)
Phenol	*Typha orientalis*	7.23	Feng et al. (2015)
Dyes			
Synolon black HWF-FS	Linseed oil cake	6.89	Safa (2016)
Congo red	*Stipa tenassicima* fibers	7.93	Chebli et al. (2015)
Malachite green	Solanum tuberosum	27	Gupta et al. (2016)
Cationic dye	Coconut coir waste	29.5	Etim et al. (2016)
Crystal violet	Coconut coir waste	33.22	Etim et al. (2016)
Heavy metals			
Cd (II)	Sugarcane straw	8	Farasati et al. (2016)
	Walnut shell	7.29	Najam and Andrabi (2016)
Cu (II)	Watermelon shell	9.54	Mohammed and Ibrahim (2016)
	Banana waste	6.49	Mokkapati et al. (2016)
	Walnut shell	14.54	Najam and Andrabi (2016)
Ni (II)	Apple pomace	83.33	Chand and Pakade (2015)
	Hemp fiber	206	Kyzas et al. (2015)
Zn (II)	Walnut shell	7.48	Najam and Andrabi (2016)
Cr (VI)	Teff straw	3.51	Tadesse et al. (2015)
	Rice husk	18.2	Ding et al. (2016)
Hg (II)	Rice straw	91.74	Song et al. (2015)
	Rice husk	98.33	Song et al. (2015)
	Peanut shell		Bai et al. (2015)

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Industrial Perspectives of the Three Major Generations of Liquid and Gaseous-based Biofuel Production

13

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13.1 Introduction

Bioenergy options triggered the interest globally as a potential alternative for fossil-based fuels in order to overcome certain limitations such as rise in oil price, elevated import costs, local energy security, and more importantly environmental pollution concerns with the excess usage of petroleum-based transportation fuels. By definition, biofuel is termed as a fuel derived from renewable resources like plant biomass that can replace fossil fuels. It can be classified as a liquid which includes ethanol, methanol, Fischer-Tropsch liquids, and diesel, whereas gaseous biofuels like methane, hydrogen, and dimethyl ether are produced from energy crops or from waste resources. Based on the resources utilized it has been classified as first-generation of biofuels from food-based crops such as corn, sugarcane, sugar beets, and vegetable oils, second-generation biofuels from lignocellulosic biomass, third generation involves microalgae and fourth generation, i.e., genetically modified organisms and carbon sequestration options. Among these, first-generation biofuels is produced in countries like USA, Mexico, and Brazil. Whereas, second-generation fuels are not yet produced commercially in any country. There is a promising demand in modernizing the use of biomass resources so that fuel demand can be met to a greater extent.

Most of the developing countries are gaining attraction towards biofuel production as biomass production is labor intensive which in turn offers new employment

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_13

opportunities for the rural population. In addition to that, energy production from biomass supports restoring degraded lands and aids in preserving biodiversity. At the same time, the growth of feedstock utilized for biofuel production demands an enormous amount of water which is a major concern. This chapter provides articulated information about the industrial aspects of biofuels for application in aid to understanding technology related bottlenecks of biofuels development. It seeks to (a) provide a better understanding of the major limitations concerning first-generation biofuels production; (b) provide deep insights on second-generation biofuels technologies for beginners, and (c) present detailed aspects on bioreactors currently employed for third-generation biofuels production.

13.2 First-Generation Liquid Biofuels

At commercial scale, liquid biofuels like bioethanol and biodiesel are being conventionally produced from wide variety of sugar, starch, and oilseed crops that act as a potent first-generation feedstock in bioenergy market. Sugar containing feedstock includes sugarcane (*Saccharumo fficinarum L.*), sugar beet (*Beta vulgaris L.*), and sweet sorghum (*Sorghum bicolor (L.)*Moench) whereas maize *(Zea mays L.)*, oat *(Avena sativa L.*), barley (*Hordeum vulgare L.),* and wheat *(Triticum aestivum L.)* belong to starchy crops. Similarly, Jatropha (*Jatropha curcas*), sunflower seeds (*Helianthus annuus*), soybean (*Glycine max (L.) Merr.*), rapeseed (*Brassica napus L.),* and oil palm (*Elaeis guineensis*) are the potential oilseed crops that were typically utilized in the biodiesel production. (Charusiri and Vitidsant 2018; Lu et al. 2011; Ziebell et al. 2013; Panella and Kaffka 2010; Propheter et al. 2010; Hameed et al. 2009; Hansen et al. 2013; Mitchell et al. 2012)

13.2.1 Sugarcane, Sweet Sorghum, and Sugar Beet as Energy Crops

Brazil is the leading producer of sugarcane with a production of 768, 678, 382 tons that constitute about 40.8% of the world total production followed by India with 18.5% production, China (6.2%), and Thailand (4.8%) as of 2017 report by Food and Agriculture Organization of the United Nations (FAO), 2017. Currently, around 107 countries become the major producer of sugarcane that fetch more than 40% of the total ethanol fuels besides involved in rum, molasses, and alimentary sugar production (WWI 2006). According to India scenario, 330 distilleries have been established with 4.0 billion liters of ethanol production capacity per annum from sugarcane and sugarcane molasses as India is one among the predominant sugar producing countries. Primarily, it has been estimated that 85 L ethanol is being produced by the fermentation of 40 kg of molasses that was obtained while one ton of sugarcane is being processed for 85–100 kg of sugar production. Currently, one-fourth of the ethanol produced is being utilized in industries which accounts for about 30–35% of beverages industries and 3–4% for other practices as well the leftover ethanol is being blended to support the transportation fuels needs (Raju et al. 2012).

Sweet sorghum comprising 16–18% of the sugar is widely cultivated in the United States, India, Mexico, Argentina, Brazil, and several other countries for its efficient utilization as a sweetening agent in the form of sorghum syrup. The extraction of sugar-rich juice from sweet sorghum constitutes about 78% of the total solid biomass consisting of 70% of sucrose, 20% glucose, and 10% of fructose. Its sole drought tolerance property with a productivity of 49–56 tons/ha in the United States and 60–100 tons/ha in Mexico could serve as feedstock for ethanol production (Cifuentes et al. 2014; Regassa and Wortmann 2014).

A total of 51,366,830 tons of sugar beet are being produced from the Russian federation, the leading producer accounting for 18.5% of all as compared to France and the United States (12%) as well as Germany (9.2%). Sugar beet is grown commercially in industrialized countries constituting 16% of the sugar production globally, i.e., 45 million ha could lead to 277 million tons of sugar production (Food and Agriculture Organization of the United Nations (FAO) 2017; Ballesteros and Manzanares 2019). Sugar beet, a hardy biennial crop comprises an average of 14–16% sugar content rendering yield from 6.6 to 7.8 tons/ha with 80% of sugar recovery under ideal conditions (Punda 2009). As of now, US is one of the dominant manufacturer contributing to almost 80% of the global ethanol and consumer of ethanol is not stated for their ethanol production from sugar beet whereas European countries like Germany and France conquered the major producer of ethanol from sugar beet. Ninety to hundred liters of ethanol are produced from a ton of sugar beet where molasses and sugar beet pulp are used as animal feed (EurObserv'er Biofuels Barometer 2017; TEREOS 2017).

13.2.2 Sugary Crops Conversion to Bioethanol

The simplest well-established technique for the production of bioethanol from the feedstock containing sucrose is acted upon by the conventional fermenting organism like Bakers' yeast (*S. cerevisiae)* that directly converts them into ethanol without any pre-hydrolysis process. Badger (2002) stated that the theoretical yield of ethanol is 51.4 g from 100 g of glucose obtained from the biomass with 48.8 g of carbon dioxide released. Various alternatives like primary juice, molasses, secondary syrups, and even a mixture of all were used to obtain ethanol from sugary crops. Manufacturing of ethanol in distilleries involves preprocessing of feedstock, i.e., juice extraction and treatment followed by yeast fermentation, distillation, and finally dehydration of the product ethanol (Clifford 2019).

At first, the feedstock is harvested by mechanical means for chopping down into smaller pieces of 20–25 cm and then it was subjected to the crushing system within 24 h of harvesting. The juice obtained after crushing was subjected to chemical treatment using lime and sulfur followed by evaporation steps in order to remove the impurities and can be utilized either for sugar as well as ethanol production by yeast fermentation followed by distillation under one roof based on the demand. While, leftover bagasse obtained only from the sugarcane fiber was burned out and utilized for the production of electricity in the ethanol industry that makes the sugarcane

ethanol producer as a pioneer in bioenergy sector. Whereas, in the case of sugar beet the sustainability of the ethanol production is lagged because they do not generate any byproducts like bagasse (Ballesteros and Manzanares 2019).

13.2.3 Maize and Other Cereal Grains as Energy Crops

Maize holds the second position in the worldwide crop production sector providing food for human consumption with a production of 1,060 million tons from 188 million ha which is 80% of worldwide grains production and also plays as a major contributor to ethanol production. According to the total world production of maize, US is the leading producing region with 36.3% that was followed by China—21.9% and Brazil—6.0% whereas European countries accounting for about 117 million tons of maize sort them as the third major maize producing region (France—10.2%) in the world (Food and Agriculture Organization of the United Nations (FAO) 2017). From a report by Renewable Fuels Association USA, 2017 is the largest producer of bioethanol from maize worldwide with 15,250 million gallons of fuel ethanol. While maize contributes 95% of ethanol production in the US besides only 3% from other cereal grains like wheat and barley. Similarly, European Union is the major producer of ethanol from wheat where only 10.3 million tons, i.e., only 2% of wheat supply are utilized for ethanol production as the food crops being used for bioenergy purpose derived a dispute at global level (European Renewable Ethanol Association Belgium 2016). In case of potato, the fourth largest crop produced after rice, maize, and wheat widely exploited as a bioenergy feedstock in Oy Shaman Spirits Ltd in Tyrnava, Finland, which utilizes 1.5 million kg of waste potatoes/year for the production of bioethanol (Liimatainen et al. 2004).

13.2.4 Starchy Crops Conversion to Bioethanol

Conventionally, the starch in cereal grains is being subjected to enzymatic hydrolysis, i.e., saccharification in order to break them down into glucose followed by yeast fermentation. Basically, cereal grains-based bioethanol production involves two main routes of milling process either dry or wet. In wet milling process, initially, the compounds like germ oil, starch, and fiber present in cereal grains are separated by steeping process by soaking them in aqueous sulfur dioxide, then the starch is subjected to the enzymatic hydrolysis (α-amylases) by heating at 120 °C–150 °C followed by yeast fermentation. Further, the cooled mixture is acted upon by glucoamlyase in order to convert the liquefied starch into simple glucose followed by yeast fermentation. Distillation is carried out to separate ethanol from the solids and water after fermentation and then it was dehydrated using a molecular sieve. The leftover solid biomass is provided as animal feedstock as it is rich in protein. Whereas in dry milling process which requires low capital cost investment makes them more preferable among most of the commercial ethanol producers. In this method, the processing of starchy biomass is the same as above aforementioned wet

milling process, but the separation of non-fermentable solid components was not performed (Ballesteros and Manzanares 2019). In 2017, a report by Renewables Fuels Association, USA states that 200 ethanol plants were adapting 90% of dry milling and 10% of wet milling process to produce 58 million m^3 of ethanol and 42 million tons of protein-rich animal feed (Renewable Fuels Association USA 2017).

13.2.5 Jatropha and other Oilseed Energy Crops

In recent decades, oil crop sector played the dominant role in the world's agriculture with 465.5 million tons of production contributing 79% to the food sector and only 13% is attributed to biofuels. Overall, biodiesel production from jatropha has been well-known as commercial feedstock in India, where 64 million hectors of waste land or uncultivable land are present (WWI 2006). The biodiesel production from oilseed crops like soybean is practiced in US, Brazil, and Argentina, followed by sunflower and soybean in European Union, and palm oil in Malaysia and Indonesia were the major driving biofuel sector worldwide (FAO 2015). Rapeseed containing 43% of oil, a dominant feedstock in Europe accounts for 85% of biodiesel production by planting 1.4 million hectares of land (WWI 2006; Food and Agriculture Organization of the United Nations (FAO) 2017). Whereas, soybean is generally used as rotation crops in US and Brazil with corn and sugarcane respectively for biodiesel production. A report by Green palm states that palm oil is the prime feedstock for biodiesel production in Indonesia followed by Malaysia and several other countries of south east Asia (Green Palm: Supporting Sustainable Palm Oil 2016).

13.2.6 Oilseed Crops Conversion to Biodiesel

In industry, biodiesel from oilseed crops is made through an alkaline catalyzed transesterification reaction using NaOH and KOH but however alkali catalyzed process could be applicable only for feedstock with lower than 0.5% of free fatty acid concentration and less than 0.06% of alcohol as well as anhydrous glycerides in order to avoid saponification reaction (Ballesteros and Manzanares 2019). In case of catalysts, homogenous catalysts are readily dissolved in a liquid reaction mixture which implies an increase in the cost of the purification process like washing and drying to meet the standard quality of biodiesel. On the other hand, Semwala et al. (2011) stated that a reduction in capital and operating costs could be obtained by utilizing heterogeneous catalysts like immobilized lipase, mixed oxides, and earth alkaloids as it cuts down the purification step. In addition to that, glycerol obtained after the transesterification process could be reused again as biodiesel feedstock or raw material for other products including soap, food additives, and other cosmetics.

13.3 Overview of Second-Generation Biofuels Production

As an alternative to expensive feedstock, a wide variety of second-generation lignocellulosic feedstock either as a whole plant biomass or any production/processing waste that is rich in cellulose and hemicellulose are being utilized for the production of biofuels as they could solve the dispute of food versus energy crisis. Lignocellulosic biomass is mainly comprised of three major components like cellulose, hemicellulose, and lignin. Cellulose is a highly organized crystalline structure made up of 1,400–10,000 units of monomers, i.e., polysaccharides whereas hemicellulose comprised of C6 and C5 sugars that are amorphous in nature. Both cellulose and hemicellulose are entrapped by the complex lignin, i.e., phenyl propane polymer that forms a cross-link between polysaccharides thereby providing structural strength to the plant cell wall. However, the presence of lignin on the outer layer of the cell wall prerequisite an efficient pretreatment process in order to breakdown the cellulose into fermentable sugars for liquid biofuel production (Ballesteros and Manzanares 2019; Ricardo-Soccol et al. 2011)

13.3.1 Second-Generation Feedstock

Currently, agro-industrial wastes like rice straw, sugarcane bagasse, and corn stover are being either completely burnt or utilized as soil organic matter in order to enhance the soil structure and also protect the soil from erosion. Availability of a surplus amount of agro-industrial waste has the greatest potential to be used as a second-generation feedstock for bioethanol production because of its low-cost production and high product yield (Saini et al. 2015). For example, rice straw, the leftover rice, is the staple food in Asia that ranks 90% of the global rice straw production per annum, i.e., 731 million tons. The United States, the largest corn cultivator led to the production of corn stover which is the leftovers like leaves, cobs, stalks, and husk resulting in 7.6 tons/ha currently maintaining the organic carbon level in the soil (Tan et al. 2012). Other studies (Regis et al. 2013; Leal et al. 2013) stated that green cane management where mechanical harvesting of sugarcane would lead to the maintenance of sugarcane trash, i.e., the top leaves and straw part of the sugarcane in the cultivation ground than the manual method of cane harvesting which is usually subjected to burning. Sugarcane is the primary source of sugar producer in Brazil where sugarcane bagasse is subjected to various pretreatment methods followed by enzymatic hydrolysis and yeast fermentation to produce 180–190 tons of bioethanol/ton of sugarcane bagasse. Similarly, forest areas which are highly dense lead to the production of wood waste residues and several other small trees after harvesting that form a local feedstock for biofuel production however, difficulty in the collection and very expensive to transport are the major limitations.

On the other hand, in North America, perennial grasses such as reed canary grass (*Phalaris arundinaceace*), switchgrass (*Panicum virgatum*), and miscanthus (*Miscanthus sinensis*) whereas in Europe, eucalyptus (*Eucalyptus sp.*), poplar

(*populus sp.),* and willow (*salix sp.)* are predominantly grown as a dedicate short rotation energy crops are currently being mainly focused for second-generation biofuel production (Ballesteros and Manzanares 2019).

13.3.2 Second-Generation Bioethanol Production

Lignocellulosic biomass is biological waste; cheap, potential carbon source, renewable in nature, and easily available on the earth in huge quantities (Srivastava et al. 2017). The major steps involved in the commercial production of bioethanol from second-generation feedstock involve: (i) Pretreatment and detoxification; (ii) Saccharification; and (iii) Fermentation.

13.3.2.1 Pretreatment and Detoxification

The first and foremost step in the second-generation biofuels includes the pretreatment of the lignocellulosic biomass that breakdown the complex lignin thereby enhancing the accessibility of holocellulosic components by cellulase. Alvira et al. (2010) reported the different pretreatment strategies such as physical which included extrusion and milling, chemical pretreatment method involving acid, alkaline, or ionic liquids, and biological using ligninolytic enzymes.

In case of detoxification, the lignin-degraded intermediatory by-products like phenolic compounds, furfural and 5-hydroxymethyl furfural formed during acid or steam explosion pretreatment method are being removed as they could act as an inhibitor for the subsequent saccharification and fermentation process (Palmqvist and Hahn-Hagerdal 2000). In addition to that aliphatic acids like formic, acetic, and levulinic acid are also formed through deacetylation of hemicellulose and 5-hydroxymethyl furfural degradation that could further affect cellulase activity in saccharification process (Parawira and Tekere 2011). Improper pretreatment of lignocellulosic biomass is a major reason for the excess in the cost of production (Srivastava et al. 2017). In order to overcome the above problem various detoxification approaches have been carried out using various chemicals such as sodium hydroxide, calcium hydroxide, and ammonium hydroxide, different extraction techniques like liquid-liquid and liquid-solid methods, whole microorganism (*Trichoderma reesei*, *S. cerevisiae, etc.*), and enzymatic pretreatment method using laccase or peroxidase but however cost-effective detoxification is essential to increase the product yield (Jonsson et al. 2013).

13.3.2.2 Saccharification

After depolymerization of lignocellulosic biomass, cellulase which is a cocktail of endoglucanases, exoglucanases, and β glucosidase has been commercially used to convert the polysaccharides into monosugars. Primarily, the insoluble crystalline cellulose is breakdown by the synergistic mechanism of endoglucanases and exoglucanases into soluble amorphous intermediates like cellubiose and cellulo-oligosaccharides by cleaving the β-1-4-glycosidic linkage between the polymers followed by the action of β glucosidase in order to produce fermentable sugars

(Andric et al. 2010). In addition to the initial adsorption of cellulase to cellulose surface, conversion of polysaccharides to monosugars, i.e., cellulose to glucose followed by final desorption of cellulase from the biomass are three main steps involved in the enzymatic hydrolysis of cellulose (Han and Chen 2010). However, the initial adsorption of cellulase to cellulosic biomass is considered as rate-limiting step because of the presence of non-cellulosic components like lignin together with the cellulose at a high degree of crystallinity, and hemicellulose. Taherzadeh and Karimi (2007) reported that the requirement of mild operation conditions in cellulase hydrolysis for pretreated lignocellulosic biomass makes them mostly preferred strategy as it resulted into the formation of intermediate degradation components and leads to the corrosion of the reactor which generally occurs during acid hydrolysis of lignocellulosic biomass. So far it has been reported that cellulase are being produced from both bacteria (*Clostridium, Streptomyces, Cellulomonas, and Bacillus)* and fungi *(Penicillium, Aspergillus species, Fusarium, and Trichoderma*) however the utility cost of cellulase hydrolysis is high as compared to the acid or alkaline hydrolysis as it requires the mild operating conditions (Duff and Murray 1996). The source and efficiency of cellulase as well as cell wall composition, cellulose crystallinity, porosity, and size of the biomass are the significant factors overlay the enzymatic hydrolysis process of lignocellulosic biomass (Pattanaik et al. 2019). Additionally, Sukumaran and Pandey 2009 stated that the overall economic process feasibility of high ethanol yield from pretreated lignocellulosic feedstock largely relies on the cellulase loading and duration of cellulase hydrolysis.

13.3.2.3 Fermentation

Followed by enzymatic hydrolysis, fermentable sugars are acted upon by the most commercially utilized *S. cerevisiae* to produce ethanol that are known for its ethanol tolerance and high yield. On the other hand, wild type *S. cerevisiae* was unable to ferment hemicellulose-derived xylose. Apart from *S. cerevisiae* many microorganisms like *E. coli* and *Zymomonas mobilis* has been genetically engineered in order to produce ethanol that would ferment both cellulose and hemicellulose-derived monomers such as glucose and xylose by overcoming the drawback of the wild type strain. Some of the industries like Poet-DSM, Beta Renewables, Raizen, and DuPont are at early commercial phase in the production of cellulosic bioethanol. The final step involves the distillation and dehydration of bioethanol to achieve the fuel quality standard specifications (Ballesteros and Manzanares 2019)

13.3.3 Second-Generation Biobutanol Production

Biobutanol is one among the promising renewable liquid biofuels produced from second-generation lignocellulosic feedstock through biological routes, i.e., Acetone–Butanol–Ethanol fermentation. Flexible blending with other fuels, higher octane number, lubricity, viscosity, lower vapor pressure and volatility are the potent properties of biobutanol (da Silva Trindade and dos Santos 2017; Nanda et al. 2017; Qureshi and Blaschek 2001). At first, biobutanol was produced from the juice of

energy crops like sugar corn since it is the fastest-growing crop. Whereas, the compositional analysis of sugar corn juice shows the presence of 80% of the total sugars comprising 145 g/L of carbohydrates that were acted upon by the *Clostridium beijerinckii* to yield 8.3 g/L of biobutanol (Gomez-Flores et al. 2018). Industrial processing of peas leads to the generation of an enormous amount of peapod waste which constitutes 53.22% of holo cellulosic components that are subjected to the ABE fermentation by *Clostridium acetobutylicum B 527* using the peapod hydrolysate obtained after detoxification which resulted in 5.94 g/L of biobutanol production (Nimbalkar et al. 2018). The cost of raw material holds 60% of the total biobutanol production cost that finds a notable position in the industry at commercial level along with its surplus availability of lignocellulosic biomass disposed of as waste thereby playing a prominent role in waste management, reducing the release of toxic gasses into the environment via Acetone–Butanol–Ethanol fermentation at the same time as it increases the revenue of the industry. Recently, a wide variety of research are being carried out in second-generation biobutanol production by adopting the various genetic, metabolic strategy, and metabolic flux analysis of fermentation in order to improve the yield, fermentation rate, and productivity that could be efficiently tuned in the near future to make them in real at commercial scale (Zheng et al. 2015).

13.3.4 Anaerobic Digestion

Biogas constitutes 50–80% of the methane and the rest of the carbon dioxide are being produced through anaerobic digestion of feedstock by breaking down the organic matter which primarily depends on the nature and carbon to nitrogen content of the feedstock. Four major steps are involved in biogas production i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Sindhu et al. 2019). Among the second-generation feedstock, corn stover, rice straw and wheat straw has high methane potential of about 338 L/kg, 302 L/kg, and 290 L/kg respectively however, the presence of recalcitrant lignin requires the pretreatment process. On the other hand, co-digesting animal manure with low nitrogen content crop residue could be preferred for improving the biogas production (Pattanaik et al. 2019; Viswanath et al. 1992). As lignin-degraded by-products are formed during pretreatment of lignocellulosic biomass that inhibits the digestion process by reducing the carbon to nitrogen ratio. Currently, anaerobic digestion for biogas production is being separated by combining the two stages like hydrolysis and acidogenesis in one step and acetogenesis and methanogenesis in another step in order to maintain the temperature, conversion rate of the organic substrate, as well as for better maintenance of organic loading (Achinas et al. 2017). Effectiveness in the conversion of lignin biomass into biogas by anaerobic digestion is associated with lignin, cellulose, and hemicellulose content, which are the distinctive characteristic polymeric fractions of lignocellulosic biomass (Pérez-Rodríguez et al. 2018)

13.3.5 Global Producers of Second-Generation Biofuels

Ever since 2015, the commercialization of second-generation biofuels has been started and presently one-third, i.e., 24 of 67 second-generation biofuel facilities around the world are operating at full commercial scale. Among other countries, US contributes about 35% of the second-generation ethanol plant in 2015 as the potential availability of first-generation feedstock like corn biomass around 1.1–1.6 billion tons utilized to meet the 5% of the US transportation fuels need is far greater than the bioenergy production levels. Additionally, North America accounts for 5 Pilot, 6 Demonstration, and 9 Commercial, and a total of 20 operating second-generation biorefineries producing ethanol, biodiesel, and aviation biofuels as of 2015 whereas, Europe has 7 pilot, 7 demonstration, and 5 commercial operating plants. Majorly, Canada and most parts of European countries are utilizing grains like wheat and barley to generate ethanol by fermentation process. In 2013, Beta renewables in Crescentino, Italy was the world's first cellulosic ethanol producer on a commercial basis which utilizes rice and wheat straw as well as giant reed like *Arundodonax* with 75 million liters per year capacity.

13.3.6 Limitation of Cellulosic Biofuels

As the global demand for biofuels is constantly increasing for replacing the depleting fossil fuels, various technologies has been adapted so far however, they are more complex and challenging. At first, the selection of lignocellulosic feedstock should be done appropriately in order to avoid the dispute between food versus fodder versus fuels though we have surplus availability of several feedstocks. Secondly, the biomass that has been utilized for biofuel production is cheap but the biomass conversion process to fuel is relatively expensive. Because of the variations in the composition of lignocellulosic components in different biomass like hardwood, softwood, and herbaceous plant to which developing an ideal depolymerization process is very difficult. An efficient eco-friendly integrated biorefinery approach as shown in Fig. 13.1 could be implemented in the biofuel industry to provide a balance in both economical and ecological sector through the adaptation of sustainable zero-waste processing techniques.

13.4 Third-Generation Biofuels

Compared to the first- and second-generation biofuels, third-generation biofuels derived from microorganisms (microalgae, microbes, etc.) are considered as an promising alternative as they can avoid major disadvantages of food competition and non-biodegradability (Chang et al. 2020; Zhu et al. 2018). The third-generation directly use organisms such as algae for the production of biofuels. It is high-yielding and also cost-effective. The diversity offered and quantity produced by algae makes them as a promising candidate under this category. Extensive research

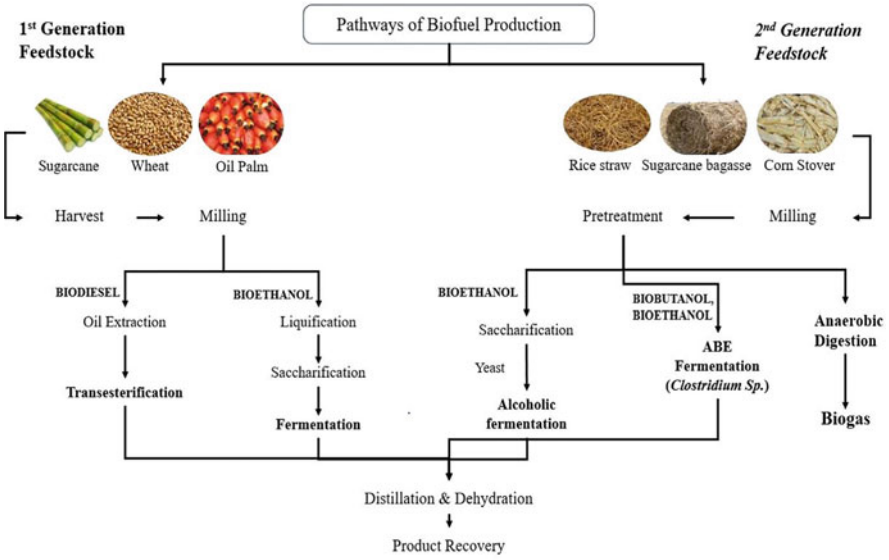

Fig. 13.1 Pathways of biofuel production from first- and second-generation feedstock. Sugarcane, wheat, and oil palms are harvested and after milling oil is extracted and followed by transesterification for biodiesel production. Similarly, another pathway after milling, undergoes liquefaction, saccharification, and fermentation for the production of bioethanol. Rice straw, sugarcane bagasse, and corn stover are examples of second-generation feedstock. After milling and pretreatment, alcohol fermentation and acetone-butanol-ethanol fermentation for bioethanol and biobutanol production, respectively. Biogas is produced by anaerobic digestion of the pretreated second-generation feedstock. **ABE* acetone-Butanol-Ethanol

is going on to expand and fully utilize biofuels from these sources. Given the potential of microorganisms, it should not be difficult to replace fossil fuels with biofuels from microorganisms.

13.4.1 Algae

Algae are a group of photosynthetic eukaryotic organisms. They consist of simple unicellular organisms as well as comparatively complex multicellular organisms. One of the generally accepted definition of algae is that they have photosynthetic chlorophyll pigment which aids them to produce their own food and they lack a covering or wall which separates other cells. Another definition is that algae are aquatic organisms with the potential to perform photosynthesis. Photosynthesis is the process of producing carbohydrates by utilizing energy from the sun. Algae have adapted themselves to thrive in various environmental conditions. They have the ability to sustain in freshwater as well as salt water oceans. They have the potential to withstand a range of temperatures, carbon dioxide, and oxygen concentrations.

Planktonic algae are mostly unicellular which are free floating in aquatic conditions. The ones which adhere to surfaces have been termed as benthic algae. These can be found growing on plants, animals, stones, especially mud.

13.4.1.1 Functions of Algae

Algae have an important function in the ecosystem as they let out oxygen during their process of photosynthesis. This tells us that they play a crucial role in maintaining the oxygen cycle. Algae utilize more of the energy from the sun and produce more oxygen than all of the plants combined. They form the foundation of the marine food chain and support the continuation of the food chain. If utilized properly algae can become the major source of food and fuel for future generations.

Algae can be classified into two major categories based on the size as microalgae and macroalgae. It is essential to categorize a group of organisms as diverse as algae, as they have many different types of organisms under a common name. Thus, categorizing algae makes it easy for us to study and understand them better.

13.4.2 Microalgae

Microalgae or microscopic algae are found in marine and freshwater systems. They are unicellular organisms which can exist in groups, chains, or individually. As they are microscopic species, their range varies within micro-meter ranges only. They have simpler cell structures and their growth requires water, light, nutrients, and carbon dioxide. Lipids, carbohydrates, and proteins are the major chemical constituents found in microalgae (Becker 2007).

Especially the high lipid content in microalgae makes them an alternate source of feedstock for biodiesel. Also, they use very less arable land when compared to food crops. Given their potential to synthesize lipids, microalgae play a crucial role in third-generation biofuels (Ragauskas 2006). Studies and research are going on to make microalgae as a food supplement as they can produce essential compounds which are necessary for human survival.

Microalgae are being used in so many different ways given their characteristics (Chisti 2007). They are being used in metallurgy sites for the removal of metal ions from the effluents. Industrial dyes can be treated and their toxic levels can be brought down.

13.4.3 Macroalgae

Macroalgae are multicellular algae, which mostly are visible to the naked eye. As they belong to the group of algae, they are capable of performing photosynthesis just like any plant species, but they differ from them as they lack the stems, roots, or leaves to perform the same function. Classification of macroalgae cultivation can be divided into two subtopics, wild seaweed and aqua-cultured seaweed method (Aravind et al. 2020). Macroalgae are a major part of the marine ecosystem

Table 13.1 List of widely used microalgae for biofuel production along with their targeted biofuel production and the type of cultivations preferred

Algal species	Cultivation/reaction conditions	Biofuel produced	Reference
Chlorella vulgaris	Autotrophic batch cultivation	Biodiesel	Converti et al. (2009)
Chlorella protothecoides	Heterotrophy	Biodiesel	Xu et al. (2006)
S. obliquus	Semi-continuous cultivation	Biodiesel	Abomohra et al. (2014)
Chlamydomonas reinhardtii	Sulfur deprived conditions	Biohydrogen	Kosourov et al. (2002)
Nannochloropsis oculata	Batch cultivation	Bioethanol	Reyimu and Özçimen (2017)
Spirulina sp	Anaerobic digestion	Biomethanol	Rodionova et al. (2017)
Microbial consortia	High rate algal ponds	Biogas	Passos et al. (2013)

(Heimann and Huerlimann 2015). They are a part of the food chain as well help in oxygen production. They take different forms like simple crusts, filamentous, and leafy forms to much more complex ones with specialized structures for reproduction, support, light capture, and flotation. The different colors of algae are a result of different chlorophyll or photosynthetic pigments present in them. Macroalgae are favorable for biofuel production as the pretreatment techniques are easier due to the relative size of the algae. The harvesting of macroalgae is easier and economical than that of microalgae. However, it is labor intensive. Also, harvesting cannot be done on a regular basis (Aravind et al. 2020).

Selection of algae plays an important role in producing economical biofuel. Important algal species for biofuel production are represented in Table 13.1.

13.4.4 Systems for Microalgae Cultivation

Primarily used microalgae cultivation systems are open raceway ponds and closed photo bioreactors.

13.4.4.1 Raceway Open Ponds

Raceway ponds are widely used technology for the cultivation of algae. This is one of the traditional systems for culturing microalgae. These raceways open ponds have been in use for almost 60 years. It is generally a closed-loop recirculation channel that has a depth of 0.1–0.3 (James and Boriah 2010). The configuration of these raceway ponds is shallow and this helps in preventing light limitation inside the microalgae culture. Increasing the depth of the raceway pond reduces the sparger efficiency which is due to the less gas-liquid time of contact. In open ponds, CO_2 efficiency ranges from 10% to 30%. Figure 13.2 shows the flow diagram of the growth media and biomass being performed efficiently by a paddlewheel. This

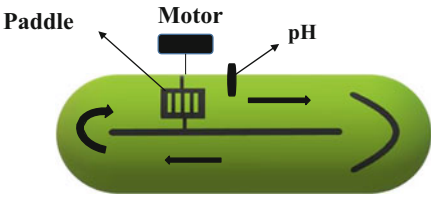

Fig. 13.2 Open raceway pond. The flow of microalgae in a raceway pond is operated by a paddle powered by a motor. Various parameters like pH and temperature can be monitored with the help of sensors. The movement of the flow is unidirectional with a divider in the middle of the pond to avoid mixing

method is economically possible to produce algal biomass as it allows the use of algae to produce biofuel while treating wastewater simultaneously. The high rate algal ponds (HRAP) are referred to in other words as open raceway ponds, and HRAPs require various operational parameters for analysis and optimizations like temperature, pH, CO_2, depth flow characteristics, geometry, and nutrient availability, etc. (Pavithra et al. 2020; James and Boriah 2010)

13.4.4.2 Closed Photobioreactors

A number of closed photobioreactors have been developed considering the geometry of the photobioreactors. Closed photobioreactors can be classified into tubular, flat, and column.

13.4.4.3 Tubular Photobioreactors

Tubular reactors are often used for outdoor mass cultivation of microalgae because it has a large surface area available for illumination. It requires glass or plastic for the construction of these photobioreactors (Huang et al. 2017). The material should be transparent and it can arrange in different orientations like vertical, inclined, and horizontal in order to capitalize the sunlight to the maximum (Brennan and Owende 2010). Tubular photobioreactors shown in Fig. 13.3 have a diameter of 10 mm to a maximum of 60 mm. In order to produce high cell concentrations less than 10 mm diameter tubes can be used. The disadvantages are capital and operating cost, high temperature, and photo limitations, etc. (Huang et al. 2017).

13.4.4.4 Column Airlift Photobioreactors

In this type of photobioreactor, the mixing is efficient and has a better gas-liquid mass transfer rate because of its defined flow in a circular pattern. The design of this column photobioreactor is vertical which helps in the movement of the gases from the bottom to the top. Column photobioreactor requires low power and low shear stress. The major obstacles faced by this type of photobioreactor are high capital cost and cleaning cost. This photobioreactor has the configuration potential for the industrial production of microalgae (Huang et al. 2017).

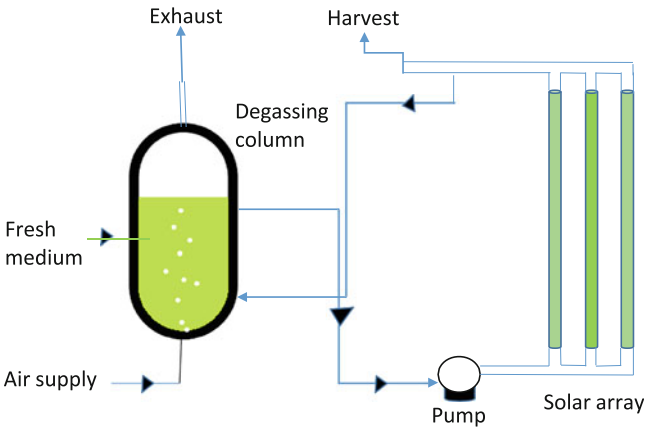

Fig. 13.3 Tubular photobioreactor representation was adapted from Singh and Sharma (2012). Air supply and fresh medium are supplied to the reactor vessel. Later, the microalgae is transferred to the horizontal tubes with the help of a pump and further leads to the harvesting of microalgae cells for biofuel production. The horizontal tubes which are transparent are subjected to sunlight where the growth of microalgae takes place

13.4.4.5 Flat Plate Photobioreactors

This type of photobioreactor is generally placed horizontally or vertically on the ground and this has a high surface area for illumination which results in high cell density. This flat plate has a lower accumulation of dissolved oxygen and maximum photosynthetic efficiency when compared to tubular photobioreactor. This photobioreactor is manufactured with transparent material for obtaining maximum light exposure in the reactor (Tan et al. 2018). When the path of the light is 1.2–12.3, a biomass yield of 0.25–3.64 g is produced. (Lee 2001) This is the most suitable method for the mass cultivation of microalgae. The major disadvantage is the difficulty in uniform sterilization of these photobioreactors by heat because of the large surface area to volume ratio (Huang et al. 2017).

13.4.5 Hybrid System

There are pros and cons in both open as well as closed methods. Open Raceway ponds are cheap but very prone to contamination. On the other hand, closed photobioreactors help in maintaining the cultures but it is very expensive and the capital cost of setting up the reactor is also too high. So, a hybrid system, i.e., combining both open and closed systems will help in increasing biomass productivity as well as reducing the cost. This type of hybrid system is called as Two Stage Hybrid system (Tan et al. 2018).

The cultivation first occurs in photobioreactors and the rapidly growing cells are transferred to the open raceway pond system. Studies suggest that hybrid systems are very much useful for lipid production and lipid-rich microorganisms (Narala et al.

2016). A large space is required, the cost is moderate and the operation is continuous.

13.4.6 Heterotrophic and Mixotrophic Cultivation

The growth characteristics and composition of microalgae are known to significantly depend on the cultivation conditions. Each type of cultivation is discussed in detail in the following sections.

13.4.6.1 Heterotrophic Cultivation

Heterotrophic cultivation use organic carbon like glucose, acetate, wastewater, and others as a substrate to reproduce microalgae. The growth of the microalgae is independent of solar or light energy, which permits scale-up possibility due to the small surface-to-volume ratio of the reactor. Heterotrophic growth is an aerobic process where the assimilation of organic substrates produces energy via oxidative phosphorylation accompanied by oxygen consumption as the final electron acceptor. There is also another metabolism used by microorganisms for aerobic glycolysis such as the Embden-Meyerhof pathway and the pentose phosphate pathway. Under the condition of darkness in the heterotrophic system, glucose is mainly metabolized through the pentose phosphate pathway. This process has high cell production and is easy to harvest due to higher cell density. But, care should be taken as heterotrophic cultivation might utilize more energy than autotrophic cultivation due to the requirement of organic carbon source. Miao and Wu (2006) reported that the lipid content of *C. protothecoides* was four times higher when cultivated under heterotrophic environment. Nevertheless, the main disadvantages of heterotrophic cultivation are: (Abdel-Raouf et al. 2012) only limited types of microalgae strains can grow heterotrophically, (Abomohra et al. 2014) it is expensive due to the addition of organic substrates like glucose, nitrogen, phosphorus, and trace elements, (Abreu et al. 2012) easily contaminated by other microorganism and, (Achinas et al. 2017) unable to generate light-induced metabolite. It was reported that *Chlorella sp*., cultivated under heterotrophic conditions in a conventional stirred-tank fermentor by using glucose as the organic substrate could attain 45 g L^{-1} of cell concentration and 20 g L^{-1} d^{-1} of biomass productivity (Tan et al. 2018).

13.4.6.2 Mixotrophic Cultivation

The mixotrophic cultivation is a process in which microbes could reproduce their cells under both autotrophic and heterotrophic conditions. This indicates that light energy and organic carbon are not the limiting factors for the cell to reproduce as the microbes could utilize both energy sources to sustain their growth. Abreu et al. (2012) reported, *Chlorella* was successfully cultivated under the mixotrophic condition. The ratio of carbon between the microalgae biomass and glucose is 138 kcal C mol^{-1} and 114.3 kcal C mol^{-1}, respectively. These energy ratios are not sufficient to support the process of conversion of organic substrate to all carbon. Therefore, under the condition of heterotrophic, extra carbon is converted into

carbon dioxide and the microalgae could further fix the carbon dioxide into glucose via photosynthesis. It was reported that the biomass productivity of *Chlorella* was around 127 g m^{-2} d^{-1} during daylight and 68.7 g m^{-2} d^{-1} at night under heterotrophic conditions. Also, a recent study compared *Spirulina* sp. growth under photoautotrophic, heterotrophic, and mixotrophic conditions. The study showed that mixotrophic culture reduced the effect of photoinhibition and improved the cell reproduction rate in comparison with other cultivation. Although the mixotrophic method attained higher biomass and lipid yields than phototrophic cultivation, the cost of the organic carbon substrate is appraised to be around 80 % of the total cultivation medium cost. Thus, low-cost organic sources needed to be intensively explored to reduce the overall processing cost under mixotrophic culture conditions (Tan et al. 2018).

13.4.7 Cultivation Methods

There are three methods to cultivate microalgae, which are batch, semi-continuous, and continuous.

13.4.7.1 Batch Cultivation

In batch cultivation, the microalgae are cultivated in a closed environment or a container. In this method, all the requirements like the microalgae, nutrients, and water are added at the beginning of the reaction and the additional nutrients are not added during cultivation. Therefore, the concentration of nutrients will be reduced with cultivation time. There are several microalgae species cultivated via batch systems, such as *Chlorella vulgaris*, *Scenedesmus sp*., and others. Factors such as temperature, pH, and dissolved oxygen are kept constant throughout the process. In Fig. 13.4, the operational flow of batch cultivation is represented. The main disadvantages of batch cultivation are inconsistent irradiance due to cell self-shading effect and nutrient is continuously consumed by microalgae.

13.4.7.2 Continuous Cultivation

In continuous cultivation, the nutrients are added continuously to the reactor while the products and effluents are discharged continuously. The continuous cultivation system is similar to batch cultivation, in which the microalgae growth pattern follows the ordinary growth cycle in the beginning. After this, new mediums or nutrients are introduced during the exponential growth phase as shown in Fig. 13.5 and this allows the microalgae to reproduce continuously at an indeterminate rate. Therefore, the volume of microalgae biomass will be greatly increased. Also, the biomass yield produced is low at steady-state operation. The advantages are the nutrient concentration and pH can be easily manipulated (Reichert et al. 2006).

13.4.7.3 Semi-continuous Cultivation

Semi-continuous cultivation is a process in which part of the cultivation medium is regularly discharged and the remaining culture is utilized as the seed to continue the

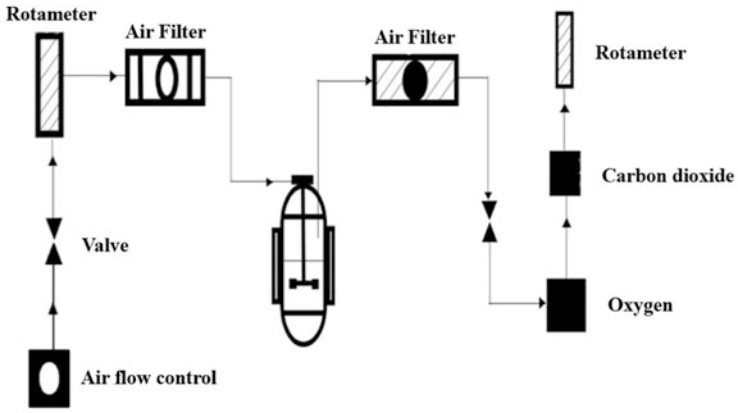

Fig. 13.4 Microalgae batch cultivation adapted form Lim and Shin (2013). This figure majorly focuses on the air flow in and out of the reactor as the microalgae is not supplied with nutrients once the process begins. The air flow rate adjusts the air flow which reaches the rotameter through the value and passes through an air filter to reach the culture vessel. Similarly, the exhaust air goes through the air filter and O_2 and CO_2 gases are separated and the remains reach the rotameter

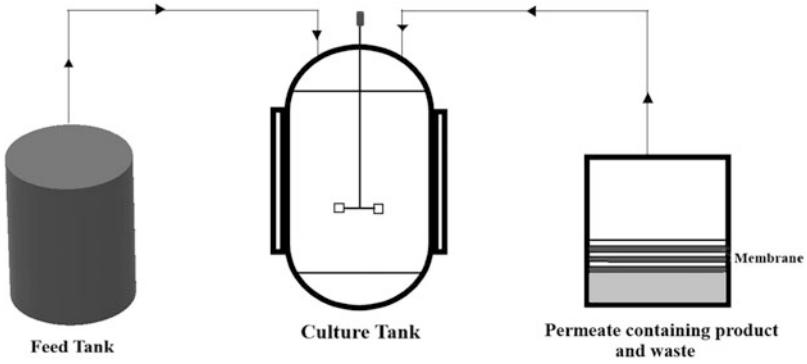

Fig. 13.5 Microalgae continuous cultivation adapted form Lim and Shin (2013). In this cultivation, a continuous supply of feed is transferred to the culture tank. This type of cultivation is slightly prone to contamination

cultivation. Also, a high inoculum ratio must be maintained at the moment of introducing a new cultivation cycle. The amount of fresh culture added to the cultivation is known as "renewal rate" and the biomass concentration is known as "blend concentration" (Reichert et al. 2006). Semi-continuous cultivation can be operated for multiple cycles, depending on the microalgae reproducibility. This will help to increase the overall biomass productivity due to the elimination of lag-phase, resulting in high biomass yield. The advantage of using a semi-continuous cultivation process is it will maintain the quantity of inoculum in the cultivation as they

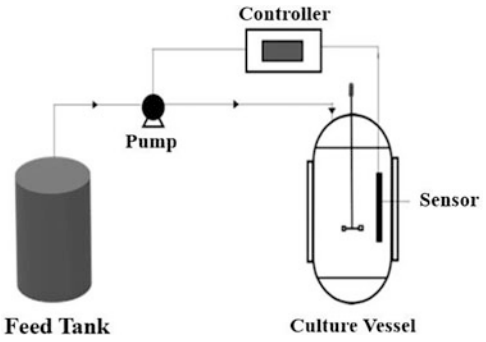

Fig. 13.6 Microalgae semi-continuous cultivation adapted form Lim and Shin (2013). In this cultivation type, the feed is pumped into the culture vessel by a pump from the feed tank which is connected to a controller. The culture vessel has a sensor attached to the controller and based on the parameters, the controller allows the feed to enter the vessel

have a controller as shown in Fig. 13.6 and this ensures that the microalgae are always remained at a high specific growth rate (McNeil and Harvey 2008).

13.5 Industrial Liquid and Gaseous-Based Biofuel Production from Algae

Microalgae or microphytes are unicellular species which are found in freshwater and marine systems. They usually exist individually or in chains. Different types of macroalgae and microalgae have different sizes ranging from a few micrometers to a few hundreds of micrometers. One of the vital characteristics of microalgae is that they can perform photosynthesis. This process occurs in small organelles called chloroplasts inside the cells. For example, in the case of *cyanobacteria*, each cell is assumed as a small ethanol factory. Algae absorbs sunlight and CO_2 and produces biomass and O_2. This gives rise to pyruvate which in the presence of a controller gives rise to ethanol.

The use of microalgae for biofuel production has many advantages over first- and second-generation methods. They contain high oil content, produce 20–300 times more oil for biodiesel production than traditional crop methods which is time-consuming, and need more manpower. Their harvesting cycle is very short ranging from 1 to 10 days and allows multiple or continuous harvests with increasing yields. They also produce biomass rapidly. In recent years, their use as an alternative feedstock for biodiesel, bioethanol, and other biofuels has been very significant. Different end products can be obtained from the harvested biomass. Figure 13.7 provides the different treatment processes involved in the production of liquid and gaseous-based biofuel.

Although some microalgae are fast-growing and have high oil content, the best strain needs to be selected for biofuel production in such a way that biomass productivity and lipid content are higher and this can be done via genetic

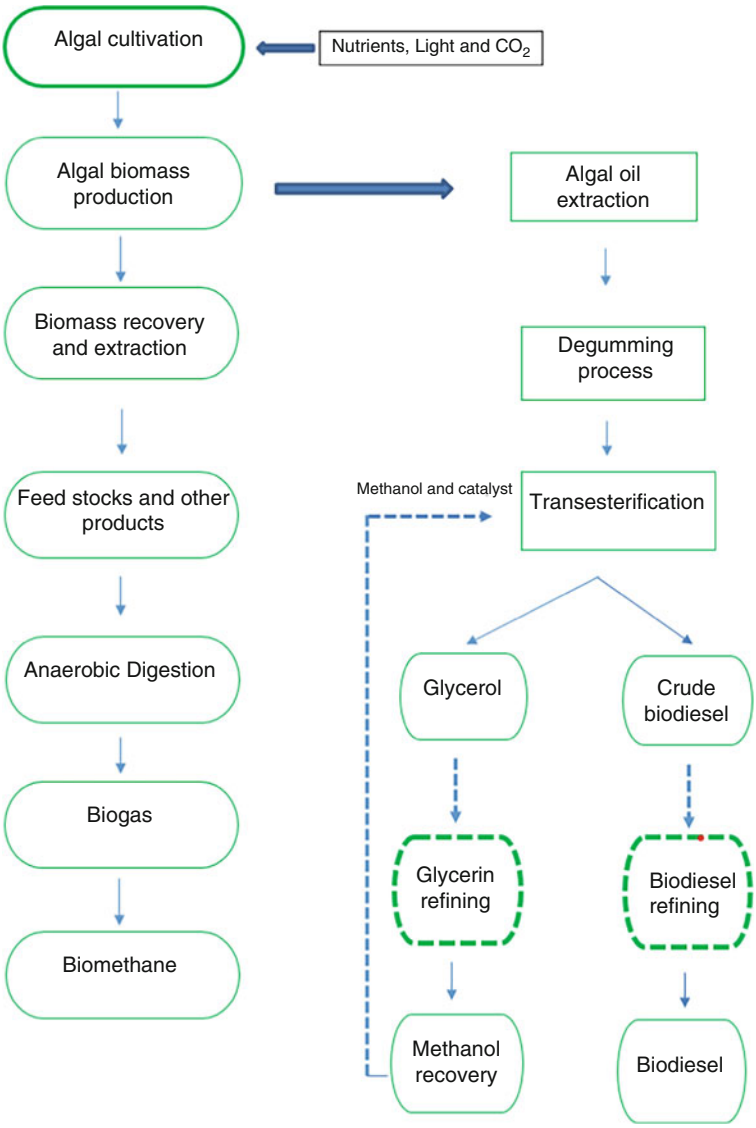

Fig. 13.7 Pathways of third-generation biofuel production. The biomass generated as a result of microalgal cultivation can be further used for the production of biomethane as a result of anaerobic digestion, biomethanol, and biodiesel production by transesterification. In the figure, there are two paths, one path follows biomass recovery and anaerobic digestion leading to biomethane production. The second path follows oil extraction and transesterification leading to the production of glycerol and crude biodiesel which on further purification produces methanol and biodiesel, respectively

engineering. For example, several knockout genes involved in nitrogen metabolism were used in the genetic transformation of the marine algae, *Nanochloropsis* sp., for efficient biofuel production (Peng et al. 2020; Kilian et al. 2011).

13.5.1 Industrial Liquid-Based Biodiesel Production

Biodiesel is derived from renewable biological resources like vegetable oils and animal fats by reacting oil or fat chemically with an alcohol in the presence of a catalyst. It is a vegetable oil-based fatty acid methyl ester. The final product contains a mixture of biodiesel as well as glycerol. As microalgae produce rapid biomass and more oil content, they are widely used for the production of biodiesel (Demirbas and Demirbas 2011). Generally, green algae are used as they can produce twice the amount of biomass produced in less than 1 day.

Several strains of microalgae contain different oil content. *Botryococcus braunii* contains 25–75% oil content in terms of dry weight. *Chlorella sp.*, and *Crypthecodinium cohnii* contain 20–35% of oil content (Cenciani et al. 2011). Oil is extracted from microalgae through the extraction process. There are various methods of extraction which include microwave-assisted extraction, supercritical fluid extraction, ultrasonic-assisted extraction, solvent extraction, etc. Solvent extraction is usually carried out using suitable solvents such as methanol, ethanol, acetone, and ethyl acetate. Sometimes, supercritical fluid extraction is used as an alternative for the extraction of oil since solvent extraction's efficiency is low (Lee et al. 2015). The degumming process is carried out which converts phospholipids into lysophospholipids. Basically, it removes sericin, a natural macromolecular protein from the substance. This is followed by transesterification process. In this process, triglycerides react with alcohol and form alkyl ester mixtures and a high co-value product (glycerol). This reversible process is carried out by either acid, alkali or lipase catalyst. Two types of catalyst can be used—homogeneous and heterogeneous catalyst. In homogeneous catalysts, reactants, products, and the catalyst are in the same phase making the reaction highly selective. In heterogeneous catalysts, reactants and products are in one phase and the catalyst is in another phase making it less selective. Homogeneous catalysts include base catalysts such as NaOH and KOH and acid catalysts such as H_2SO_4, H_3PO_4, and $CaCO_3$. Typical base concentrations of NaOH and KOH are in the range of 0.3–1.5%. Although acid-catalyzed transesterification is an efficient method due to its high conversion, base-catalyzed transesterification proceeds faster with a higher reaction rate. This is due to the fact that bases are less corrosive than acid catalysts and do not cause environmental problems. Heterogeneous catalysts are widely preferred over homogeneous catalysts because of their catalyst activity, catalyst life, and oil flexibility. This results in high tolerance to free fatty acids and moisture content in various types of oils and maximum ability to perform esterification and transesterification processes simultaneously. Biodiesel and glycerol are produced and this glycerol is refined and used for methanol recovery. The other factors that need to be considered for microalgal-based biodiesel production are the fuel properties which include

viscosity, density, flashpoint, cold filter plugging point, and heating value. If the cold filter plugging point is relatively low (−11 °C), it can be a suitable fuel for aviation compared to petroleum (Lee et al. 2015). Since several strains of microalgae have different fatty acid compositions, the selection of microalgal strain and its genetic improvement is very important. *Chlorella protothecoides* is a perfect feedstock for biodiesel production since they can accumulate 55% of lipid under heterotrophic conditions with nitrogen limitation (Xu et al. 2006). Studies have shown that biodiesel can be extracted from the green microalgae *Chaetomorpha linum* using two methods—thermochemical liquefaction and superficial CO_2 (sc-CO_2) extraction method, and after transesterification and characterization of the oil content, it was observed that the liquefaction process turned to be more effective than sc-CO_2 extraction method because long chain fatty acids are broken down at high temperatures thereby giving rise to more oil content (Aresta et al. 2005).

13.5.2 Industrial Liquid-Based Bioethanol Production

Bioethanol is the most widely preferred biofuel used as a substitute for gasoline. It can serve as a feedstock for ethyl tertiary butyl ether which blends more easily with gasoline. It is derived from starch or sugar through fermentation. The worldwide production of bioethanol reached almost 41 billion liters and the largest producers in the world are Brazil (37%), the USA (33%), and Asia (14%). In Brazil, ethanol-powered and flexible-fuel vehicles are manufactured for operation with hydrated ethanol which is an azeotrope of ethanol containing 93% (v/v) and the remaining water (Nigam and Singh 2011). Brown algae is the largest seaweed source available that is used in the synthesis of carbohydrates such as laminarin, cellulose, mannitol, alginic acid, and fucoidan. It is the conversion rate of carbohydrates that is more important than the extraction energy of rich oils for biofuel production (Fasahati et al. 2015). Studies showed that bioethanol productivity was achieved using *Chlorococcum sp*., (38%) by fermentation of yeast (Peng et al. 2020; Singh and Gu 2010). It was also reported that wastewater effluent was utilized by the microalgal consortium and the resulting biomass produced solid biochar (45+5.9% dw) with the energy density of 8–10 MJ/kg using hydrothermal liquefaction (Peng et al. 2020; Roberts et al. 2013). It was found that Miscanthus (Sacchariflorus) cellulosic biomass on pretreatment and enzymatic saccharification with fermentation resulted in higher bioethanol productivity (Srivastava et al. 2020; Cerazy-Waliszewska et al. 2019).

The process starts with pretreatment of brown algal biomass followed by saccharification process. Pretreatment includes chemical and biological treatments. Chemical treatment is further divided into acid hydrolysis and alkali hydrolysis. In acid hydrolysis, concentrated H_2SO_4 and HCl are used for hydrolysis even though they are corrosive, hazardous, and must be recovered. As a result, they degrade hemicellulose and reduce the crystallinity of cellulose. Addition of water is done at low solid loading (5–10%) at a temperature of 120–160 °C for 15–60 min and at high solid loading (10–40%) at a temperature of 160–190 °C for 5–30 min. Alkali

hydrolysis involves the use of NaOH and lime. The neutralization process is generally vital to neutralize the acidic pH. Ammonia is preferred over lime because of its high solubility and no sugar loss. In this process, intracellular bonds cross-linking hemicellulose, cellulose, and lignin are broken. As a result, they increase the porosity and internal surface area, decrease crystallinity, disrupt lignin structure, and decrease the degree of polymerization. On the other hand, biological treatment includes brown-rot fungi which attack cellulose and white and soft-rot fungi which attack lignin and cellulose (Nigam and Singh 2011). This fungal treatment method is inexpensive but time-consuming. It has also been shown that acid and alkali hydrolysis result in higher saccharification yields followed by enzymatic hydrolysis. The enzyme cellulase is added at a dose range of 7–33 FPU/g substrate. It attacks the (Abdel-Raouf et al. 2012; Achinas et al. 2017) β-glycosidic bonds of cellulose and decreases the viscosity of cellulose solution. The products proceed to fermentation where different microorganisms are used. *S. cerevisiae* is used for hexose and *Candida sp.,* is used for lactose or pentose. The production of ethanol is growth-associated with *S. cerevisiae*. Medium requirements include carbon source from sugarcane, starch, or cellulosic materials, nitrogen, and phosphorous minerals. The process is carried out at a pH of 4–6 and a temperature of 30–35 °C. The fermented liquor (beer) may contain alcohol as well as low boiling point volatile compounds. The alcohol is obtained by the dehydration process which includes distillation and molecular sieving and condensation process. Ethanol has a higher octane rating than petroleum fuels enabling combustion engines to run at higher compression ratio and giving superior performance. It also exhibits higher vapor pressure and heat of vapourization than gasoline resulting in increased power outputs.

13.5.3 Industrial Liquid-Based Biobutanol Production

Butanol is a type of alcohol which is used to make butyl acrylates for coatings and adhesives. n-butanol and iso-butanol have very good fuel properties. Butanol production is associated with oxo-alcohol process in which propylene syngas gives n-butyraldehyde and iso-butyraldehyde. n-butyraldehyde and iso-butyraldehyde combine with hydrogen to form n-butanol and iso-butanol. Basically, biobutanol process involves utilization of biomass like algae, cellulose, hemicellulose, and lignin in Acetone-Butanol-Ethanol fermentation to produce butanol. Acetone-butanol-ethanol includes other by-products like acetic acid, lactic acid, isopropanol, etc. *Clostridium acetobutylicum* is used for the production of biobutanol. Temperature ranges from 30 to 40 °C and pH of 6.8–7 drops to 5 due to acidogenesis and increases to 7 due to solventogenesis. This process results in lower butanol yields and the toxicity level of final products is around 20 g/L. The fermentation process can be improved using *C. beijerinckii* where it undergoes hydrolysis and fermentation in one reactor and genetically modified E. coli which converts acetoacetic acids to biobutanol and biodiesel. It has been reported that the production of hydrogen and butyric acid was maximized with continuous immobilized cultures of *C. tyrobutyricum* and *C. acetobutylicum* using various techniques. It was also

reported that immobilized cells of *C. acetobutylicum* in fibrous support produced acetone of 4.6 g/L (Nigam and Singh 2011).

Basically, bioethanol and biobutanol can be produced by different processes such as separate hydrolysis and fermentation, separate saccharification and fermentation, saccharification and separate consolidated fermentation, and consolidated bioprocessing.

13.5.4 Industrial Gaseous-Based Biohydrogen Production from Algae

The greenhouse gas effect increases due to more amount of CO_2 from fossil fuels. One of the main effects of this is global warming. Hydrogen undergoes combustion to produce water which is not detrimental to nature. There are different methods of hydrogen production. These include steam reforming of methane in which methane is converted to hydrogen and carbon monoxide in reaction with steam using nickel as a catalyst and electrolysis where splitting of water into hydrogen and oxygen is done by electric current. But one major factor associated with hydrogen production is its cost. There are two types of microbial hydrogen production—fermentative and Photosynthetic (aerobic/anaerobic). Fermentative method includes *Clostridia sp., (Clostridia beijerincki)* used in a fuel cell that produced 15 mA over 20 days using waste from alcohol distillery, Methanogens (*Methanotrix soehngenii*), and *Archaebacteria* (*Pyrococcus furiosus*-hyperthermophile). Photosynthetic method includes purple sulfur bacteria (*Thiocapsa* and *Chromantium*), non-sulfur bacteria (*Rhodospirillum* and *Rhodopseudomonas*), and Green algae (*Chlamydomonas reinhardtii*). This method requires only light and water and produces more hydrogen than the fermentative method. Purple and non-sulfur bacteria utilize reversible hydrogenase to form hydrogen. The green algae forms hydrogen through hydrogenase and is induced under anaerobic conditions. *C.reinhardtii* is a eukaryotic green algae. It is unicellular, bi-flagellated, and photosynthetic in nature. It can undergo sexual or asexual reproduction under adverse conditions and has the highest hydrogen yield. It requires 5% of CO_2 and fluorescent light and grows at room temperature in water. The enzyme hydrogenase which is located in the chloroplast receives electrons from reduced ferrodoxin. In dark fermentation, hydrogenase is stimulated under anaerobic conditions. *C. reinhardtii* produces oxygen during photosynthesis which in turn inhibits hydrogenase. It has been reported that sequential dark and photo fermentation processes convert 1 mole of glucose to 12 moles of H_2 when acetic acid is the volatile fatty acid obtained (Jacob et al. 2015). Storage and transportation are some of the major limitations in biohydrogen synthesis. Consequently, biohydrogen has been reported with highly attractive and renewable characteristics as it can be synthesized by various biological routes (Srivastava et al. 2020).

13.5.5 Industrial Gaseous-Based Biomethane Production from Algae

Biomethane is a type of gaseous biofuel which is produced by anaerobic digestion. Anaerobic digestion is a biological process in which a biodegradable material is broken down by microorganisms in the absence of oxygen. One major product produced during this process is biogas which consists of 60% methane and 40% CO_2. Following biomethanation, the digested slurry is used as a biofertilizer in agricultural fields. Anaerobic digestion involves three stages—hydrolysis, acidogenesis and acetogenesis, and methanogenesis. The biomass or fresh organic matter undergoes hydrolysis using group 1 organisms like hydrolytic bacteria. This soluble organic matter further undergoes acidogenesis followed by acetogenesis using group 2 organisms (Acetogens) resulting in hydrogen and acetate. This is followed by methanogenesis using group 3 organisms to produce biogas. Biomethane reduces fuel import dependency, protects our climate, stimulates regional development, and stabilizes the energy system. Basically, CO_2 scrubbers are used in order to absorb CO_2 thereby increasing the production of biomethane. Different algal strains absorb CO_2 and have different absorption capacities, e.g., *Chlorella vulgaris* has a CO_2 fixation efficiency of 74%. Due to less space, greater compatibility, and higher productivity, photobioreactor systems are preferred over raceway pond system. They also have higher carbon capture efficiency than open system (Jacob et al. 2015). Residues of algal biomass produced in the first step of production can be used for co-digestion with organic waste to produce biogas. Microbial biomass has limited digestibility due to its cell wall resistance and this can be improved by pretreatment methods. High methane yield was possible to achieve in cells which lack cell wall. If the protein content in microalgae is high, it produces a high amount of free ammonia which is toxic to the microalgae responsible for the production of methane (Jankowska et al. 2019).

13.6 Upstream Processing Parameters for Algal Cultivation

Upstream process is a stage which involves the preparation of liquid medium, separation of particulate and inhibitory chemicals from the medium, sterilization, air purification, etc. The strain is selected and subjected to genetic improvements to maximize its ability in order to synthesize valuable products. When the cells of microalgae experience environmental stress, this leads to carbohydrate accumulation. The composition of lipids and carbohydrates varies among different algal species. There are several parameters affecting microalgae carbohydrate production which include temperature, irradiance, pH, CO_2 supplementation, etc.

13.6.1 Irradiance

It is one of the primary factors for algal cultivation as it measures the rate of solar energy that falls on the surface. Uniform and sufficient irradiance can be provided to

microalgal cells by designing proper algal cultivation systems like raceway ponds and photobioreactors. The availability and penetrating of light are affected by the depth of light supply and the diversity and uniformity of light are enhanced by agitation. The intensity of light (Range 30–400 μmol/m.s) plays a major role in carbohydrate accumulation. As intensity increases, there is more amount of carbohydrate accumulation (Chen et al. 2013).

13.6.2 Temperature

The temperature also has an impact on carbohydrate accumulation and depends on the type of strain used. As the water temperature increases, the rate of algal growth increases. Further increase in temperature leads to slow production of algal species or even termination due to algal respiration. This in turn results in variation of species composition. Optimum temperature is different for various algal species but is found to be 28–35 °C for most of them. Algal growth rates, chemical composition of cells, and nutrient uptake are affected by temperature (Singh and Singh 2015).

13.6.3 pH

pH is one of the major factors affecting microalgal cultivation. It also affects the carbohydrate composition of different algal strains. Optimum pH observed during this accumulation is in the range of 7.5–9. Like temperature and light, as pH increases, more amount of carbohydrate gets accumulated (Chen et al. 2013).

13.6.4 CO_2 Supplementation

CO_2 supplementation affects carbohydrate accumulation as well as photosynthetic efficiency. Microbial algal growth (Autotrophic) favors photosynthesis for which CO_2 is needed to provide more carbon sources. Therefore, an increase in the concentration of CO_2 increases the efficiency of photosynthesis and the level of protein content which can be used as a nitrogen source under appropriate conditions (Chen et al. 2013).

13.6.5 Cell Harvesting

Cell harvesting refers to the process of separating cells from its corresponding medium. The need for separation is that the cells may need to be treated or some product needs to be extracted, for this the separation of cells and medium would be the first step. In bioprocess, upstream process involves all the steps associated with inoculum preparation, media development, and culturing till harvest. Downstream

process refers to all the steps associated with product extraction and formulation. A complete bioprocess has both upstream and downstream processes. Performing both with accuracy and precision decides the quantity and quality of the product produced. Everything done before cell harvesting comes under upstream process and everything from harvesting is a part of downstream processing. Thus, cell harvesting plays a very crucial part in a bioprocess.Some of the factors which influence the choosing of harvesting technique are:

- Type of product
- Fragility of cells
- Cost
- Purity percentage required
- Type of cells
- Constituents in media

13.6.5.1 Type of Product

There are many types of cell harvesting techniques available. It is influenced by many factors such as whether the desired product is intracellular or extracellular. If it is the former, then the cells need to be lysed for the extraction; in the case of the latter, the cells are of no use to us anymore as the extraction needs to be done on the used-up medium. Similarly, there are various factors which influence the method of cell harvesting that is chosen. At the current period of time, we have many harvesting techniques to choose from, and choosing the correct one is essential for good yield.

13.6.5.2 Fragility of Cells

Some cells are more shear sensitive than others. They need to be handled with more care. High pressure and force exhibiting techniques should not be used for their harvesting. This can narrow down the list of available options in choosing harvesting techniques. Some cells may require higher rates of shear forces for separation. Shear forces at this range may not be given by all techniques. Thus, the fragility of the cell is very vital while choosing harvesting techniques.

13.6.5.3 Cost

For a second -tier process with less budget, it would not be possible to afford a very high-cost separation technique. They may require low-cost equipment and processes. When done commercially, the cost of the processes plays a very vital role in determining the process chosen.

13.6.5.4 Product Purity

The purity of the product to be produced is a main criterion for choosing different processes as, therapeutic products require a 90% purity or greater, whereas products such as ethanol require only 70% purity. Thus, the purity of the product required is essential in choosing different downstream processes. Also, the higher the rate of purity required more will be the number of steps to produce the product.

13.6.5.5 Type of Cells

Sensitivity of cells varies from one another. Some cells may be sensitive to light whereas some may not. Some cells will be able to sustain only in basic conditions while some may sustain only at acidic conditions. Animal cell cultures are more shear sensitive when compared to plant cells. As different cells have different needs, these all need to be considered before choosing a separation technique.

13.7 Downstream Processing

13.7.1 Extraction

Extraction is an energy-intensive process in microalgae, and this is caused by a combination of various factors which includes temperature and pressure conditions.

13.7.2 Cell Disruption Methods

The cell disruption methods can be classified into three types: Biological, chemical, and physical.

13.7.2.1 Biological Method

This refers to the methods that degrade cell's envelope by using enzymes. The cell envelope of microalgae has very resistant sporopollenin layers, but these can be degraded by a mixture of enzymes. If the enzymes are selected carefully, enzymatic extraction can be effective. The major downfall of this method is the cost of the enzymes used. But this can be reduced by immobilizing the enzymes or by recycling the enzymes (Kim et al. 2013).

13.7.2.2 Chemical Method

Extraction occurs by osmotic shock. This is done by a sudden increase in the salt concentration in the liquid media disturbing the balance in the osmotic pressure between the exterior and interior of the cell. Hypo-osmotic stress occurs when the salt concentration is low outside the cell, this allows the cells to swell and burst and when the salt concentration is higher outside the cell, the cell shrinks due to the movement of fluids outside the cell (Kim et al. 2013). Hyper-osmotic shock is a suitable procedure used in industries to disrupt algal cells when compared to hypo-osmotic as it requires a lot of water for media dilution which is not possible on industrial scale (Cheng et al., 2010). It requires inexpensive materials and it is a simple process.

13.7.2.3 Physical Method

Physical methods of extraction involve high pressure homogeniser. This cell disruption process utilizes hydraulic shear force generated when the slurry under high pressure is sprayed through a narrow tube. This method is commonly used for the

extraction of substances that are found internal to the cells. It has a number of advantages like low cooling cost, low heat formation and thermal degradation, and easy scale-up. This type of extraction exhibits the highest cell disruption efficiency compared to other existing extraction methods and it is the most widely used method in industries (Kim et al. 2013).

13.7.3 Transesterification

Biodiesel is derived from transesterification process of triglycerides. This process involves short-chain alcohol like methanol with a catalyst mostly alkaline base to achieve a high conversion of triglycerides to fatty acid methyl esters at short reaction time. Chloroform, methanol, and water mixture known as the Bligh and Dyer's method is generally used to extract microalgal oil (Gonçalves et al. 2013). The lipids extracted from algal cells cannot be directly used as biodiesel because it is highly viscous compared to that of diesel and gasoline. Thus, it is converted to a low molecular weight structure component like fatty acid methyl esters and this is achieved by transesterification. This process is a reversible reaction and it is carried out by fatty acid, an alkaline catalyst, and alcohol. Base catalysts like KOH and NaOH are used because acid catalysts have corrosive property and long reaction time. In order to increase the reaction rate by using acid catalyst, high pressure and temperature conditions are required which is not suitable for an economical large-scale production (Tan et al. 2018). Biodiesel with high purity is produced without purification or washing, so that conversion of oil into fatty acid methyl esters reached a high of 93.5%. The process will be more favorable when the reaction and separation are carried out simultaneously (Hajilary et al. 2019)

13.8 Conclusion

Even though different categories of biofuel production technologies have been developed, there is a requirement to prioritize one biofuel over the other for any national context shall be examined and executed by considering factors such as an overall sustainability goal achievement and a wide range of development in all levels of the society. In addition the other deciding criteria may include availability of fuels resources, reduction in import economy of fuels, dissemination of rural employment opportunities, boosting of micro- and small-scale industrial clusters for biofuel production with world-class industrial technology, promoting the export economy, reclamation of barren and unusable lands for bio-energy crops cultivation, and improving the quality of the environment by reducing the greenhouse gas emission.

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Metabolic Engineering Approaches for Bioenergy Production

14

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14.1 Introduction

In the current era, the developments accomplished using molecular biology have led to advancements in various fields. It has allowed scientists all over the world to manipulate the genomic structure of microbes to reach their maximum potential. For increased production of biofuels, a few techniques are involved such as mapping the

Reghunathan Sarath and Rajathirajan Siva Dharshini contributed equally to this work in all attributes. Reghunathan Sarath performed conceptualization and interrupted the draft. Rajathirajan Siva Dharshini drafted the manuscript and revised it. Mohandass Ramya designed sections and supervised the manuscript. Ramachandran Srinivasan and Mohandass Ramya critically reviewed and revised the manuscript.

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_14

trait from a wild-type species and replicating the mechanism in a well-known organism or improving the production by enhancing the native strain to perform better. These strategies are made possible using metabolic engineering techniques. These techniques allow the scientists to construct or engineer a balanced metabolic pathway synthetically using artificially designed setup. However, expression of these engineered pathways in the microbial host requires a significant effort to produce higher titers than the native strains. Clustered Regularly Interspaced Short Palindromic Repeat—Cas (CRISPR-Cas), antisense ribonucleic acid, intron-mediated targeting, and other specific mutagenesis technique allows the manipulation of the metabolic pathway as desired. A few other research that are being done involve the development of bacterial and fungal hosts which are devoid of nonessential genes hence making their genomic structure much more compact for efficient engineering. Engineering a strain that already possess a desired trait can potentially avoid challenges such as the metabolic burden of high expression of heterologous genes, cofactor imbalance, and genetic instability of imported genes or pathways, among others (Wang et al. 2017). Though the application of these techniques on an industrial scale demands high initial expenditure, the profit that is expected to be gained through this is highly exceptional.

14.2 Bioenergy

Biomass-derived fuel is abundant and it is stated as the fourth largest energy source following coal, oil, and natural gas. It implies the growing significant economic, societal, and environmental potential (Kurchania 2012; Li et al. 2017). Development of bioenergy technologies varies by time and it was restricted in the case of technical, environmental, economic, and social concerns.

14.2.1 Types of Bioenergy

Thermochemical conversion has limitations like deforestation, poor biodiversity, increased food prices, and water crisis. Bioconversion mechanisms will be highly efficient over thermochemical conversion. In bioconversion processes such as anaerobic digestion and dark fermentation, processes followed from acidogenesis to methanogenesis by restricted anaerobes (Imam and Capareda 2011).

Microbes are extensively used for specific metabolic pathways and catalytic systems. *S. cerevisiae* has the mechanism for direct decarboxylation of pyruvate pathway to produce ethanol and *E. coli* has a CoA group to activate acyl group during pyruvate decarboxylation, that will reduce to ethanol (Liao et al. 2016). Microbial engineering is used to manipulate the non-fuel-producing microbes to fuel producers by expression of genes. It will prevent the enzymes and precursors that interfere the fuel production by using knockout in the engineering of microbes. For example, improved fatty acid biosynthesis in *E. coli* was done by acyl carrier protein knockout. Even catalytic systems can be improved in the microbes by

employing enzyme engineering techniques. The artificial metabolic pathway was effective under research to characterize proteins and mRNA for significant fuel production. Advanced research and analytical experiments are needed to understand the processes within the microbes and to manipulate the pathways by artificial analogs.

Microbial fuel cells (MFC) and microbial electrolysis cells (MECs) are emerging technologies for bioelectricity and biohydrogen production (Logan et al. 2015; Dai et al. 2016). These microorganisms transfer electrons from the microbial outer membrane to the conductive platform (Kracke et al. 2015). The commercialization of MFC was challenging and stuck at primary level. Advanced research and experimentations are needed to exploit them for higher energy production (Logan et al. 2015). Redox proteins and molecules in exoelectrogens transfer electrons from outer surface to electrode (Kracke et al. 2015). The engineering techniques have promising adaptability to the microorganisms with redox reactions for bioenergy production. Some of the major molecular techniques involved in engineering the strain are discussed in the upcoming sections.

14.3 Metabolic Engineering

Every living organism survives by producing various metabolites which is a byproduct of a complex metabolic reaction in them. This reaction involves generation of energy, production of fundamental building blocks for structural organization, expenditure of energy, and synthesis of biomolecules for building blocks. These processes are together called as metabolism which is currently the topic of interest in numerous labs worldwide. Combining the knowledge of the metabolic networks and techniques borrowed from genetic engineering, this field turned out to be an efficient method to produce biomolecules at a higher amount than it usually does. Metabolic engineering is an approach in which the modification of the metabolic networks is carried out to produce the desired compounds in higher amounts. Few basic requirements for metabolic engineering are knowledge of the metabolic pathway, genes, regulatory mechanisms, suitable transfer systems, and expression of the desired gene in the host organism. Bacteria and yeast hold a major part in the achievements gained through metabolic engineering, but other microbes such as algae, fungi, and certain animal and plant cells are also used in metabolic engineering. Various strategies have been implemented in developing recombinant strains.

14.4 Methods for Metabolic Engineering

Metabolic engineering approaches have gained the attention of many researchers all over the world. Combining the techniques derived from genetic engineering with the data and knowledge obtained using metabolomics lead to the improvement of various fields. Mutation is the most widely known approach for strain improvement which was initially induced by spontaneous agents. These spontaneous agents are not target specific and the effects are not guaranteed to produce desired effects.

Hence, site-specific mutagenesis techniques have been developed such as CRISPR/Cas system, antisense RNA, transposon-mediated targeting, intron-mediated targeting, and so on. These techniques are used to repress or overexpress the target region to improve the efficiency of the microbe. Detailed information about these methodologies is covered in the upcoming sections.

14.4.1 Random Mutagenesis

The mutation is the change of single or several nucleotides in the DNA of a microorganism. Mutation may occur spontaneously or induced by mutagens. Spontaneous mutation will occur in low frequency and it is compatible with industrial purposes. Induced mutation-based strain improvement techniques are done by subjecting the DNA to various physical and chemical agents called mutagens. There are several methods available to induce mutations such as physical, chemical-based, and biological-mediated. Most of these agents cause random mutagenesis which is referred to as the random changes in the genome resulting in irregular replication and improper repair of DNA damage. Random mutagenesis is an important tool to gain mutant proteins with different functional properties from the native wild type (Tachioka et al. 2016).

14.4.1.1 Mechanism Involved in Random Mutagenesis

There are various approaches employed for creating random mutagenesis. Common approaches are irradiation, chemical mutagenesis, and error-prone PCR. Each method follows its own pattern in creating mutations.

Ultraviolet Irradiation

Ultraviolet (UV) radiation is a caliber mutagen and its mutation efficiency was extensively studied for its ubiquity nature and accessibility. Both long and short-wavelength UV radiation will cause damage to DNA; they will be distinct in different ways. UV-A, long wavelength is less damaging radiation and it will cause mutation through the production of reactive oxygen species. The dimerization of pyrimidines caused by the short wavelength UV-B and C light will block the replication of plasmid DNA, or induce mutations after faulty repair.

Chemical Mutagenesis

Various chemical agents like ethyl methanesulfonate, N-methyl-N′-nitro-N-nitrosoguanidine, 5-bromouracil, ethidium bromide, etc., are used to create random mutations in microbial systems. Several factors are involved namely base analogs, changing the specificity of hydrogen bonding, modification of nucleotides by alkylating agents, and intercalating agents (Kuśmierek and Singer 1982). A base analog is a chemical compound similar to one of the four bases of DNA. It can be incorporated into a growing polynucleotide chain during normal process of replication occurs. They replace the bases and cause stable mutation. A very common and widely used base analog is 5-bromouracil which is an analog of thymine.

There are many chemicals that after incorporation into DNA change the specificity of hydrogen bonding hence causing a stable mutation at a random site. Some examples are nitrous oxide (HNO_2), hydroxylamine, and ethyl methanesulfonate. Nitrous oxide converts the amino group of bases into keto group through oxidative deamination. The deamination of the nucleotides will change the pairing patterns. Hydroxylamine hydroxylates the C4 nitrogen of cytosine and converts it into a modified base via deamination which causes to base pair like thymine. Therefore, Guanine-Cytosine pairs are changed into Adenine-Thymine pairs. Another class of mutagens is alkylating agents, where they add an additional alkyl group to the hydrogen bonding of guanine and adenine. Hence, various pairing error possibilities are increased. A few widely used alkylating agents are dimethyl sulfate, ethyl ethane sulfonate, and ethyl ethane sulfonate.

There are certain dyes such as acridine orange, proflavine, and acriflavine which are three-ringed molecules of similar dimensions as those of purine pyrimidine pairs. In aqueous solution, these dyes can insert themselves in DNA between the bases in adjacent pairs by a process called intercalation.

Error-Prone Polymerase Chain Reaction

Error-Prone PCR is PCR amplification-based mutation technique where the low fidelity Taq DNA polymerase is used to create undesired base pairing in the DNA sequence. In normal PCR, replication of DNA by polymerase is specific, but in the case of error-prone PCR the specificity of the polymerase will get altered by the changes in the composition of the reaction buffers like increased polymerase concentration, extension time, changes in MgCl2 ions concentration, dNTPs concentration, supplementation with MnCl2 ions (Leung et al. 1995). By changing the buffer composition, the polymerase will create an error in the process of base pairing during DNA replication that will lead to errors in the newly replicated complementary DNA strand. The mutation site is located upstream or downstream of the primer binding site. On further extension and amplification of the DNA, the frequency of mutation is increased. Taq DNA polymerase is recommended for error-prone PCR because of its inability of proofreading. Proof reading will auto-correct the mismatched nucleotide sequence and mutations that are created will be lost.

14.4.1.2 Applications and Drawbacks

Random mutagenesis was widely applicable for productive bacterial genetics and protein engineering to alter the properties such as thermostability, optimum pH, functional activity, structural, and functional relationship of the target gene (Bloom et al. 2005). It is employed in the generation of enzymes, proteins, entire metabolic pathways, and entire genome with favorable properties for industrial purposes. Combined selection and random mutagenesis will provide unique means to isolate phenotypic variants and become the solution for the difficulties in protein engineering.

It was the primary tool for directed site-directed mutagenesis. Random mutagenesis-based strain improvement was widely used in the area of bioenergy. It has the ability to develop genetic diversity and is useful to create combinatorial libraries (Labrou 2009). A list of microbial species modified using mutagenesis approach is listed in Table 14.1.

Table 14.1 List of microbial species mutated using chemical and physical agents for bioenergy applications

Random mutagenesis approach	Organisms involved	Applications	Reference
Physical agents			
UV-C	*C. vulgaris*	Biodiesel production	Gomaa et al. (2019)
UV-A	*Ettlia* sp.	Lipid accumulation	Seo et al. (2019)
Ultrasound	*Scenedesmus sp*	Biomass and lipid production	Sivaramakrishnan and Incharoensakdi (2019)
Laser-UV	*S. obliquus*	Lipid production	Zhou et al. (2019)
UV irradiation	*R. toruloides AS 2.1389, Chlorella* sp. *FC2 IITG, T. suecica*	Lipid production	Lim et al. (2015); Guo et al. (2019); Muthuraj et al. (2019)
UV irradiation	*Bacillus PC-BC6*	Cellulase production	Abdullah et al. (2015)
	S. stipitis NRRL Y-7124	Ethanol production	Hughes et al. (2012)
UV and EtBr treatment	*Rhodobacter M 19 and E. aerogenes*	Biohydrogen production	Veeramalini et al. (2019)
Chemical agents			
Ethyl methanesulfonate (EMS)	*R.mucilaginosa RmTun15 (MR2), T. asahii TaTun15 (MT-2; MT-3), and Y. lipolytica YlTun15 (MY-2)*	Biodiesel production	Bessadok et al. (2019)
	S. cerevisiae DMKU 3-S087	High-temperature ethanol production	Pattanakittivorakul et al. (2019)
	Tetraselmis sp.	Lipid storage	Dinesh Kumar et al. (2018)
	Nannochloropsis sps.,	Fatty acid production	Doan and Obbard (2012)
	C. reinhardtii	Improvement of oxygen tolerance	Flynn et al. (2002)
Diethyl sulfite	*T. reesei strain RUT C30*	Cellulase production	He et al. (2016)
Nitric acid	*Bacillus PC-BC6*	Cellulase production	Abdullah et al. (2015)
PCR-based methods			
Multistep error-prone PCR (ep-PCR)	*T. neapolitana*	Improvement of cellulase	Basit et al. (2019)

(continued)

Table 14.1 (continued)

Random mutagenesis approach	Organisms involved	Applications	Reference
		Cel12A efficiency	
Random PCR mutagenesis	*T. thermophilus*	Cold adaptation of xylose isomerase	Lönn et al. (2002)

In contrast, ionizing radiation mutagenesis needs expensive instruments and specialized laboratory setup. Also, the mutations caused by the chemical mutagens are genetically less stable, and there are chances of reverting back the mutation to the original wild-type form (Tadege et al. 2009). In the case of PCR-based random mutagenesis, mutated DNA is hard to replicate, and the competent cell to be used is expensive. Screening, sequencing, and specific primers were required to the great extent. The major limitation of random mutagenesis for protein engineering was time-consuming mechanisms involved in the screening of a large number of clones in continuous mutagenesis cycles to gain the desired enzyme property (Turner 2009).

14.4.2 Transposon-Mediated Gene Targeting

Transposons are mobile/transposable genetic elements that can "jump" from one location in the genome to another and thereby induce mutations. They have been widely used in genetics to alter genome functions. Based on their mechanism of transposition, transposons are assigned to one of two classes, the copy-and-paste (class I transposable elements) or the cut-and-paste (class II transposable elements) (Wicker et al. 2007). Up to date, Class II DNA transposons were highly applicable to industrial strains like *Clostridia, Bacillus,* and *Saccharomyces* sp. (Jasni et al. 2010; Kuehne et al. 2010; Liu et al. 2013).

14.4.2.1 Mechanism

Transposons used to jump on their own without the help of RNA intermediates. The presence of transposase gene and terminal inverted repeats at the end of the sequence will catalyze the transposons to reach the destination. When the terminal inverted repeats are duplicated, it results in the site targeted duplication (Munoz-Lopez and Garcia-Perez 2010). By the mode of transfer, it was divided into conjugative and non-conjugative transposons. Unless regulation, integration, and excision, conjugative transposons perform the cell to cell transfer mechanism (Burrus et al. 2002). Multicopy plasmids or bacteriophages and effective electroporation are used for the non-conjugative transposition (Vizváryová and Valková 2004).

Transposomes are priorly arranged synaptic transposition complexes comprised of transposase and transposon DNA in in vitro and in vivo transiently formed during

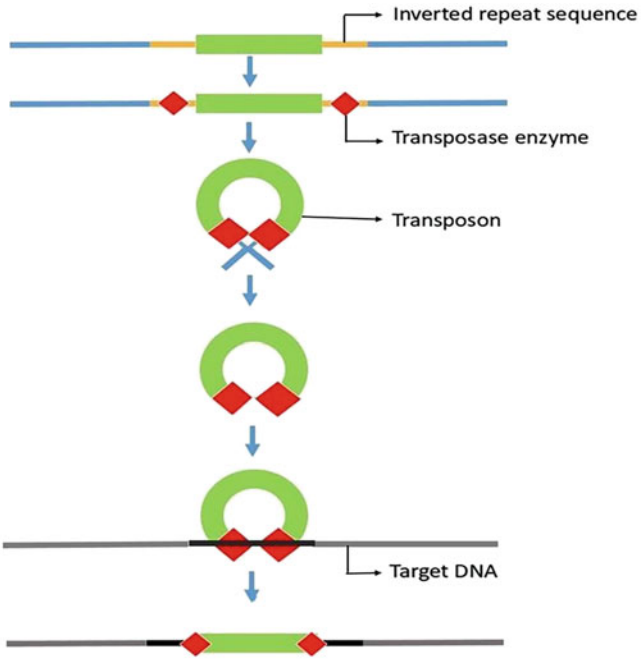

Fig. 14.1 The mechanism of transposon-mediated mutagenesis for strain improvement

transposition (Fig. 14.1). Transposase proteins recognize the terminal inverted repeats (IRs) and form a circular pre-excision synaptic complex from which the transposon is excised. This is as same as the cut-and-paste job, here transposon jumps from the chromosome and again it will reintegrate by the action of enzyme transposase.

In vivo, these complexes are formed transiently during transposition. But, in vitro, in the absence of divalent cations, such as Mg^{2+}, they can be formed and maintained stably. After their transfer into the target strain by electroporation, they become active due to the presence of divalent cations such as Mg^{2+}. This results in the integration of transposon DNA into the genome.

Typically, transposons contain a selectable marker for the rapid isolation of cells with transposon integrations. Conjugative transposons or integrative conjugative elements are typically integrated into the chromosome or endogenous host plasmid. However, they are able to excise themselves from it and, subsequently, form a covalently closed circular transposable intermediate that can either reintegrate into the chromosome of the same cell (intracellular transposition) or be transferred by conjugation to another cell of the same or a different species. In the recipient, they integrate into the genome or endogenous host plasmid (intercellular transposition) (Vizváryová and Valková 2004).Conjugative transposons vary widely in size (18–500 kbp) and contribute as much as plasmids to the distribution of antibiotic-resistance genes. The best characterized conjugative transposons in clostridia are Tn5397 and Tn916. Both are members of the large Tn916/Tn1545 family of

Table 14.2 List of bacterial species mutated using transposons for bioenergy applications

Species	Transposon	Applications	Reference
Nannochloropsis sps	Tn5	Accumulation of lipids	Osorio et al. (2019)
C. Ljungdahlii	*Himar1*	Production of acetone and isopropanol	Philipps et al. (2019)
S. cerevisiae	mTn3-lacZ/ LEU2	Production of ethanol and/or Thermotolerance	Kim et al. (2011)
Z. mobilis CP4	Tn5	Heat stress and malnutrition tolerance	Jia et al. (2013)
Z. mobilis	EZ-Tn5	Salt tolerance	Wang et al. (2016a)
Z. mobilis	Mini-Mu	Isolation of different auxotrophs	Pappas et al. (1997)

conjugative transposons, of which almost all members carry the tetracycline resistance gene tetM (Rizzotti et al. 2009). Despite high similarity, Tn5397 and Tn916 differ in their integration/excision system (Wang et al. 2006).

14.4.2.2 Advantages and Drawbacks

Transposon mutagenesis has several advantages because of its ability to yield large numbers of mutants in a single step and we can perform in equivalent strains with distinct mutations. It does not need prior knowledge and it can be applicable to non-model species, it will be highly unique in this way. It will prevent the low transformation efficiency in many species. It also prevents the fortuitous genome alterations (e.g., aneuploidies) that may incorporate the DNA transformation (Bouchonville et al. 2009). Transposon insertion is quite stable. It will cause the complete destruction of function in the interrupted gene, there are no leaky mutations.

In nonpathogenic Clostridia, the use of conjugative transposons has principally focused on acquiring mutants defective in solvent production or sporulation. The Tn916 transposon was initially used in two model organisms, *C. acetobutylicum* and *C. saccharobutylicum* P262 (formerly *C. acetobutylicum*) (Mattsson and Rogers 1994). However, the mutants obtained still remain largely uncharacterized to this day. List of microorganisms using transposon mutagenesis for the enhancement of biofuel production is given in Table 14.2.

The limitations of the transposons were not well-known but they may show low frequency of transposition and sometimes it will be inaccurate. Transposon-mediated mutagenesis has been used for modifying the metabolic pathway towards increasing feedstock utilization, solvents, biofuels, etc.

14.4.3 Targetron Technology

Targetron technology is a gene disruption technology which was initially described by (Karberg et al. 2001) that makes use of the mobile group II introns that can recognize their DNA target sites by base pairing RNA-DNA interactions with the aid

of site-specific binding reverse transcriptase. Group II introns are a class of self-catalytic ribozymes found within the genes of all life forms.

14.4.3.1 Mechanism of Targetron-Based Gene Knockout

The major mechanism involved in targetron technology is retrohoming. This method is mediated by a ribonucleoprotein that contains an intron-coded reverse transcriptase and an excised intron lariat RNA. In order to direct the intron RNA to specifically target the DNA, the RNA must be mutated at three regions namely IBS1, EBS1d, and EBS2 (Intron binding site 1, Exon binding site 1d, 2). The mutation is carried out using PCR with an oligonucleotide sequence generated by a computer algorithm for appropriate mutation which can be done with pertuka method (Perutka et al. 2004). The mutants are ligated into the targetron expression vector and it is transformed into the host cell. At this point, the intron RNA and the intron-encoded protein (IEP) are transcribed as a single transcript. The intron RNA is present at the 5′ end of the transcript and the IEP transcript is at the 3′ of the transcript. The mutants are present in between the intron RNA and the intron-encoded protein.

From the intron RNA precursor, the IEP is initially translated and the protein is formed. The IEP is a multifunctional protein with four different functions namely DNA maturase for the group II intron splicing, endonuclease, reverse transcriptase, and target site recognition. The IEP binds with the intron RNA precursor and the DNA maturase activity of the IEP assists with the splicing of intron RNA from the intron RNA precursor. Following the splicing, the lariat RNA forms a complex with IEP and forms an RNA protein complex, RNP. The RNP now scans throughout the DNA for the target DNA sequence by using the EBS sites assigned in the Intron RNA. As soon as the target site is identified, the RNP reverse splices the Intron RNA into the leading strand of the DNA sequence. Now the area of insertion will be a heteroduplex with a DNA in the bottom strand and the Intron RNA at the top strand. This heteroduplex form will be removed using the IEP by causing a nick at the bottom strand and by reverse transcribing the Intron RNA as depicted (Fig. 14.2). Now, through the host DNA replication function, a stable intron insertion will have been achieved.

14.4.3.2 Advantages and Drawback

Targetron technology has proved to be one of the most efficient methods because of its flexibility and feasibility even in organisms that are intractable to genetic manipulation with conventional techniques. It is highly efficient and the retrohoming frequencies can reach up to 100%. This method does not depend upon the host mechanism hence the range of bacteria where this methodology can be applied is vast (Zhuang et al. 2009). It has been widely used in the field of metabolic engineering for biofuel production. The most reported organism in targetron technology is the genus *Clostridium* for its outstanding capacity to produce solvents such as acetone, butanol, ethanol, and isopropanol. Various research have been conducted using intron-mediated targeting to accomplish the silencing of gene to bypass a metabolic network, some of which are enlisted in Table 14.3. Due to various factors like correct folding of lariat RNA (Michel and Ferat 1995), Mg^{2+} cations for proper

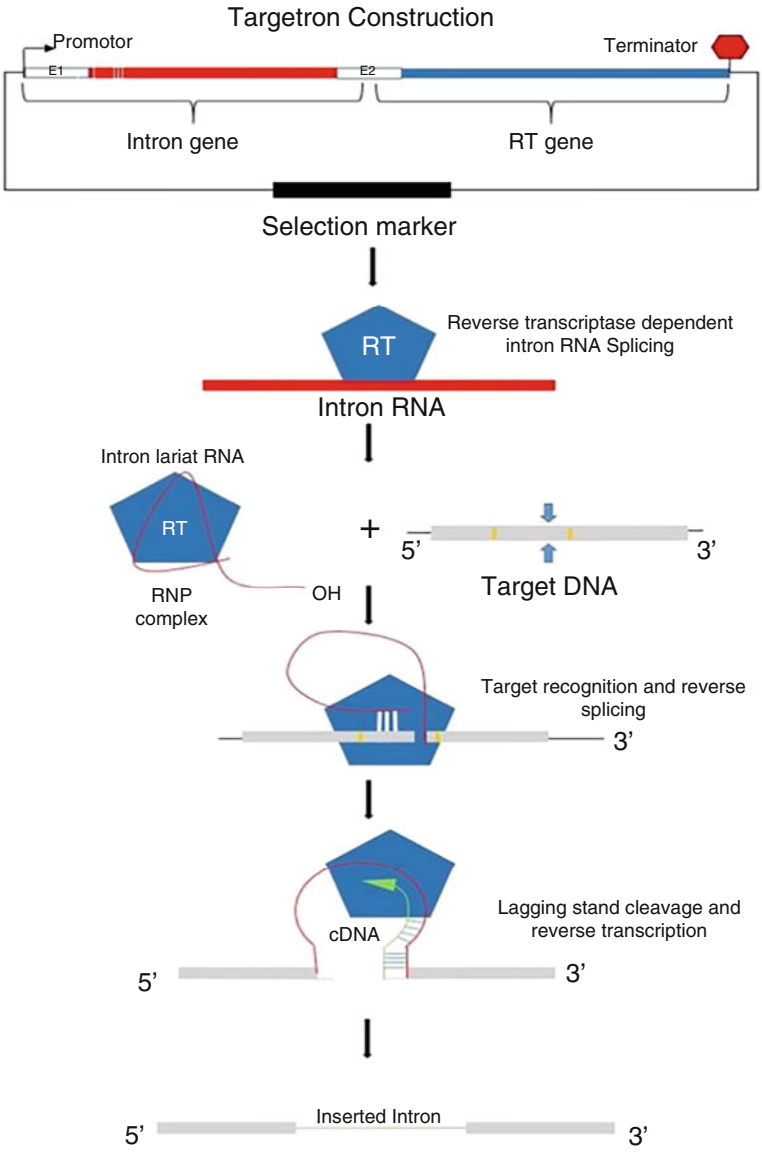

Fig. 14.2 A pictorial representation of the mechanism of intron-based targeting system. *RT* reverse transcriptase, *RNP* ribonucleoprotein complex, *cDNA* complementary DNA

folding and stabilizing the RNA (Qin and Pyle 1998). Only a few research have been done with the help of group II introns in eukaryotes (Guo et al. 2000). Concentration of $Mg^{2+}$is important, other than stabilization, it is also required for catalysis of group II introns and its concentration is important for self-splicing (Toor et al. 2008).

Table 14.3 Application of targetron technology in metabolic engineering

Organisms	Target gene	Outcomes	References
C. Acetobutylicum	Butyrate kinase (*Buk*)	Increase in butanol production	Shao et al. (2007)
	Acetoacetate decarboxylase (*Adc*)		Jiang et al. (2009)
	3-Hydroxybutyryl-CoA dehydrogenase (*Hbd*)	Increase in ethanol production and decrease in H_2 yield	Lehmann and Lütke-Eversloh (2011)
	Glucose phosphoenolpyruvate-dependent phosphotransferase system (*pep*)	Co-fermentation of glucose and xylose	Xiao et al. (2011)
C. Butyricum	β-hydroxybutyryl-CoA dehydrogenase (*Hbd*)	Increased ethanol production and decreased H_2 yield	Cai et al. (2011)
	Aldehyde-alcohol dehydrogenase (*Adh*)	Enhanced hydrogen production with the addition of sodium acetate	Cai et al. (2013)
C. Cellulolyticum	Lactate dehydrogenase (*Ldh*) and acetate kinase (*Ack*)	Enhanced ethanol production	Cui et al. (2014)
Rhodopseudomonas palustris	Adenosyl-hopene transferase (*hpnH*, *hpnG*, *hpnO*)	Increased hopanoid production	Welander et al. (2012)
E. coli (BL21) DE3	Fatty acyl-CoA synthetase (*fadD*)	Direct production of long-chain hydroxy fatty acids	Wang et al. (2012)
E. coli (BL21) DE3	Fatty acyl-CoA synthetase (*fadD* and *fadL*)	Increased production of free fatty acids	Meng et al. (2013)

Another drawback involves the invalid delivery systems to deliver the intron vehicle to the target host.

14.4.4 Antisense Technology

Antisense RNA technology is an innovative tool that is used for the inhibition/regulation of gene expression. The basic principle of the technique is that the antisense RNA molecule base pairs with the complementary mRNA which thereby prevents further translation to yield proteins. The complementary antisense RNA can either is synthetically synthesized, oligodeoxyribonucleotides (ODN), with around 30 base pair long nucleotide length or longer. One such example of sense and antisense RNA is -5'ACGU3'mRNA and 3' UGCA5'Antisense RNA (Bochot et al. 2000).

There are two modes to restrict the translation of RNA that takes place by binding of asRNA, 1) by making the ribosome binding site (RBS) unavailable for interactions with ribosomes and/or 2) Initiation of the target RNA degradation by production ribonucleases by altering its functional structure. The consideration of the association rate between antisense RNA and target mRNA was not sufficient enough for designing a stable antisense RNA. To resolve the implications of antisense RNA design, M-fold application was used for the structural-based design of antisense RNA in an effective way. M-fold is a computational algorithm that is used in predicting secondary structure, based on thermodynamics and structural information derived from the library of published data. In this method, free nucleotides and components (structural apparatuses that contain regions of intramolecular binding called duplex RNA) are identified as the factors for an antisense RNA.

14.4.4.1 Antisense RNA-Based Gene Silencing Mechanism

When an antisense strand binds to an mRNA sense strand, a cell will recognize the double helix as foreign to the cell and proceed to degrade the faculty mRNA molecule thus preventing the production of undesired protein. Although DNA is already a double-stranded molecule, antisense technology can be applied to it building a triplex formation. A DNA antisense molecule must be approximately 17 bases in order to function, and approximately 13 bases for RNA antisense strands can be either catalytic or non-catalytic. The catalytic antisense strands, also called ribozymes, will cleave the RNA molecule at specific sequences. A non-catalytic RNA antisense strand blocks further RNA processing, i.e., modifying the mRNA strand or transcription.

The exact mechanism of an antisense strand has not been determined. The current hypotheses includes "blocking RNA splicing, accelerating degradation of the RNA molecule, preventing introns from being spliced out of the mRNA, impeding the exportation of mRNA into the cytoplasm, hindering translation, and triplex formation in DNA." Figure 14.3 illustrate the fact that no consensus has been reached concerning how antisense accomplishes the reduction of protein synthesis.

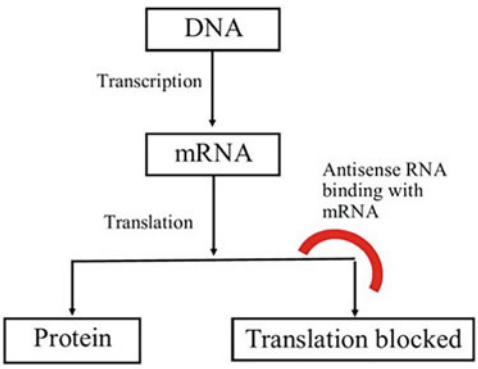

Fig. 14.3 A brief conceptual flow of Antisense RNA technology

14.4.4.2 Advantages and Drawbacks

The antisense technology is a flexible technique as the silencing occurs at the translational level of gene expression. In *C. acetobutylicum* ATCC 824, the downregulated expression of *adc, buk, ctfAB,* and *ptb* have been reported as successful cases of asRNA method for increased butanol production (Tummala et al. 2003). The major disadvantage of antisense technology is that it can be employed only to the genes whose sequence is already known. It is also found that off-target recognition is an undeniable risk factor when antisense technology is utilized. Another drawback is that the gene silencing efficiency of antisense RNA is low for a gene and if a gene is constitutively expressing, the suppression of that gene is highly unlikely.

14.4.5 CRISPR/Cas System

CRISPR/Cas system abbreviated as the Clustered Regularly Interspaced Short Palindromic Repeats was initially observed in *Streptococcus pyogenes*, further it was identified in 40% bacteria and 70% archaea. This system was originally used by the bacterium as an acquired immune system which allows them to specifically cleave and inactivate any foreign invading viral DNA by employing an RNA–Protein complex. The major process involved in CRISPR/Cas system is the introduction of double stranded breaks in the DNA specifically with the help of Cas protein and further these double stranded breaks are rejoined using two methods namely, non-homologous end-joining (NHEJ) and homologous recombination or homology-directed repair. In NHEJ, it is highly likely that it may lead to the formation of undesired indels in the target sequence which would disrupt the gene activity. Another method employed in the rejoining mechanism is the homology-directed repair where the template is inserted into the double stranded breaks using homologous template as illustrated in Fig. 14.4.

14.4.5.1 Mechanism

In the CRISPR/Cas system, small segments of invading DNA which are called as "spacers" 374 are inserted into the CRISPR loci and processed into short CRISPR RNA (crRNA). These 375 crRNAs anneal to trans-activating crRNAs (tracrRNAs) and proceed with sequence-specific 376 cleavage or silencing of pathogenic DNA with the help of Cas proteins. It was identified that a specialized sequence called the protospacer adjacent motif (PAM) which is present upstream of the crRNA binding region is required by the Cas protein for activation (Jinek et al. 2012). The CRISPR/Cas system can thereby be retargeted to cleave virtually any DNA sequence by redesigning the crRNA. Sequentially it happens in 8 steps, A) Bacteriophage infection and foreign DNA invasion. B) Cas genes are expressed and are bound to the fragment of the viral DNA(protospacer). C) The bound DNA is further inserted into the CRISPR locus and flanked by a repeat sequence. D) Secondary infection by the same phage occurs, CRISPR array is transcribed as a single transcript. E) Formation of CRISPR RNA (crRNA) by spacer processing using endonuclease. F)

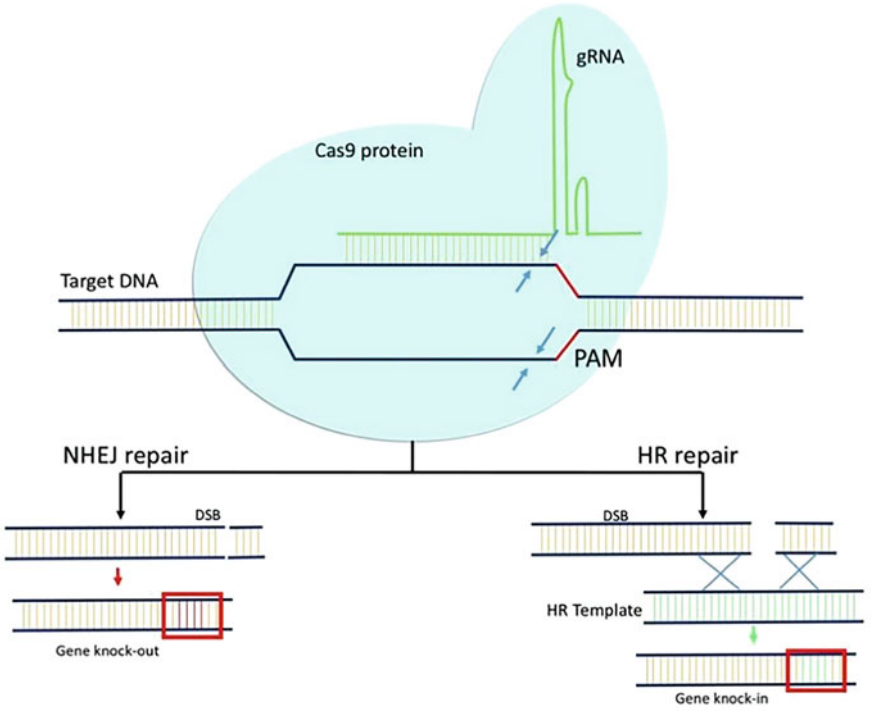

Fig. 14.4 The mechanism of CRISPR/Cas9 system in a bacterial system for Gene knockout and gene knock-in carried out using NHEJ-based repair and HR repair. Abbreviation: *gRNA* guide RNA, *NHEJ* Non-homologous end-joining, *HR* Homologous recombination, *DSB* Double strand break, *PAM* Protospacer adjacent motif

The crRNA is further bound by the Cas nuclease. G) Scanning the invading sequence for the PAM region for activation of Cas. H) The Cas Complex identifies the complementary sequence adjacent to the PAM. I) Cleavage of the invading DNA by Cas nuclease to prevent proliferation (Donohoue et al. 2018).

14.4.5.2 Advantages and Drawbacks

This powerful genetic tool has been utilized for genome engineering in two industrially important clostridial species namely *C. cellulolyticum* and *C. beijerinckii* (Wang et al. 2015; Xu et al. 2015) in butanol and ethanol production. Another similar system was established in *C. ljungdahlii*, a paradigm for clostridial acetogens, based on CRISPR/Cas9-based editing system in *E.coli.* (Huang et al. 2016). CRISPR/Cas system is used widely all over the world in bioenergy aspects to interfere in the metabolic pathway. It has also been used as CRISPRi technique which is exclusively used to knock down a gene sequence to improve productivity. A list of few microbial species modified using CRISPR/Cas9 for biofuel applications are listed in Table 14.4.

One of the critical challenges faced in using CRISPR-Cas9 editing technique is off-target recognition, where Cas9 enzymes cleave the undesignated genes.

Table 14.4 List of microbial species modified using CRISPR/Cas system for bioenergy applications

Organism	Outcomes	References
Bacteria		
C. Ljungdahlii	Ethanol production from gas	Huang et al. (2016)
C. Saccharoperbutylacetonicum	Butanol production	Wang et al. (2017)
C. Autoethanogenum	Ethanol production	Nagaraju et al. (2016)
C. Tyrobutyricum	Butanol production	Zhang et al. (2018)
E. coli	Butanol production	Kim et al. (2017)
Yeast		
Z. mobilis	Ethanol production	Cao et al. (2017)
S. cerevisiae	2,3-Butanediol production	Shi et al. (2016)
S. cerevisiae	Ethanol production from orange peel	Yang et al. (2018)
Microalgae		
Nannochloropsis sp.	Petroleum-based fuel production	Wang et al. (2016b)
Nannochloropsis sp.	Bio-oil production	Kilian et al. (2011)
Fungi		
Myceliophthora thermophila	Biomass production for biofuel	Liu et al. (2017

Targeting specificity of Cas9 is tightly controlled by the SgRNA containing about 20 nucleotides and the presence of protospacer adjacent motif (PAM) adjacent to the target sequence in the gene, potential off-target cleavage could still occur (50% chance) on DNA sequence with even 3–5 base pair mismatches (Omodamilola and Ibrahim 2018).

14.4.6 Metabolic Flux Analysis

Metabolic flux analysis (MFA) is a recently developed analytical technique used to quantify the intracellular metabolic fluxes that are produced due to the catalytic and transcriptional interactions in a cell. MFA is based on the stoichiometry of metabolic reactions and mass balances that happens around intracellular metabolites under pseudo-steady state. In order to study the metabolic fluxes in a biological system, two methods have been employed namely ^{13}C-based flux analysis and constraints-based flux analysis.

14.4.6.1 Mechanism

^{13}C-Based Flux Analysis

The ^{13}C-based flux analysis is used in determining the intracellular fluxes in a metabolic network by labeling the carbon substrate with an isotope such as ^{13}C. Later, the enrichment patterns of the metabolites are analyzed using Nuclear

Magnetic Resonance imaging (NMR) or Gas Chromatography-Mass Spectrometry (GC-MS) (Sauer 2006). The isotope-labeled carbon substrates are fed to the proliferating cells till the ^{13}C-labelled isotope reaches the complete metabolic pathway. The interference pattern data of the isotope-labeled carbon and certain physiological data obtained during proliferation, such as flux exchange that is determined by the extracellular metabolite concentration and composition of the biomass data, are compiled using computational analysis. Further, the intracellular fluxes are estimated by fitting the simulated fluxes in stoichiometric models to the measured data. The difference between simulated and measured labeled pattern is reduced (Sauer 2006).

Constraints-Based Flux Analysis

Constraints-based flux analysis is an optimization-based simulation technique that works with a mathematical background. It is used to analyze cellular metabolism in a specified environmental or genetic condition and to predict the metabolic capacity of the organism when these conditions are altered (Park et al. 2009). To employ constraints-based flux analysis, a balanced model needs to be designed based on the genetic information, databases and literature surveys, and the genomic sequencing of most of the organisms that is already sequenced in silico via computer simulation. The in silico genome-scale metabolic model is reconstructed using the genome annotation to create a collection of metabolic reactions and the metabolite profile which gives rise to the linear mass balance equations for cellular metabolites describing the metabolism. This is the foundation of the metabolic network.

14.4.6.2 Advantages and Drawbacks

Metabolic flux analysis has been used to understand the variation in the intracellular fluxes under a particular condition. It also has been used to quantify the activities of unusual pathways within complex metabolic networks and to identify the pathways in less characterized species. Other advantages include the characterization of conditions-based regulatory circuits that control the metabolic pattern.

Though this approach is highly useful in metabolic engineering research, on application of this technique various hurdles have been faced. Accurate determination of the pathways holds back the reliability of this approach. Various experimental calculations and computational analysis are required in order to come to a single conclusion about a pathway. These problems limit the application of metabolic flux analysis for large-scale analysis in industries (Sauer 2006).

14.4.7 Protein Engineering

Protein engineering is a related branch to metabolic engineering in industrial biotechnology and synthetic biology (Stephanopoulos 2012). Basically, protein engineering undergoes the alteration of protein structure by site-specific or random mutations to gain desired functional changes namely reduction in product inhibition, increased substrate specificity, increased catalytic rates, desired cofactor use, and substrate competition (Pleiss 2011; Foo et al. 2012).

14.4.7.1 Mechanism

There are various mechanisms employed in protein engineering with different strategies such as rational design, computational approach, and directed evolution. Rational design is the direct observation of the analysis of protein structure for the presence of desired mutation. There are three steps described for the rational design approach such as choice of appropriate scaffold, identification of specific residue to change, and selection of desirable mutants by characterization.

The computational approach was grouped into three main categories such as bioinformatics, molecular modeling, and de novo design and engineering enzymes (Damborsky and Brezovsky 2014). Bioinformatics involved software like ZEBRA, SWISS-Model, etc., to analyze homology models and multiple sequence alignments. It depends on the sequence and structure prediction. Molecular modeling includes details of the structure of the target protein to examine the shape and size of the tunnel to adapt substrate and product entry or release. It enclosed the software like MOLE (Petřek et al. 2007), CAVER (Petřek et al. 2006), and POREWALKER (Pellegrini-Calace et al. 2009) to alter the substrate specificity and enantioselectivity. The de novo protein design is the advanced stage in protein engineering to predict the tertiary structure of proteins using primary structures as the precursor. Computational protein engineering will be helpful to prevent the limitations in experimental methods, by observing protein folds in in silico and designing novel proteins from them. With the emergence of computational tools, it becomes easy to predict enzyme thermostability before engineering the enzymes in wet lab experiments. By combining protein engineering with computational methods, we can design synthetic proteins as same as native enzymes (Barrozo et al. 2012).

Directed evolution is based on random mutations, by creating a library of mutagenized genes using error-prone PCR. Improved beneficial mutants can be screened by genetic selection and screening. Both in vitro recombination and DNA shuffling was employed to generate beneficial mutations to expand sequence diversity from homologous genes (Chen 2001). This made the advantage by showing 80% methylation in the target region and less than 1% methylation at off-target sites (Chaikind and Ostermeier 2014). It was well-known to demonstrate the efficiency and threshold of industrially valuable strains and proteins (d'Oelsnitz and Ellington 2018).

14.4.7.2 Advantages and Drawbacks

Yeast and bacterial cell systems expressing proteins or peptides on the cell exterior were reported to be used as biocatalysts, biosorbents, and biostimulants. Among these, the yeast cells were broadly used for surface display of enzymes from various microbial sources like *S. cerevisiae*, *B. stearothermophilus*, *T. reesei, S. bovis,* etc., for biofuel production (Wu et al. 2008).

Another important environmental application of protein engineering involves fungal enzymes particularly peroxidases isolated from fungi can transform xenobiotics and many pollutants. Peroxidase from *Coprinus cinereus* was subjected to variant mutations to make it stable and it was commercialized to rinse the excess dyes in liquor (Cherry et al. 1999). Thus, many protein engineering strategies were

Table 14.5 List of protein engineering studies carried out in different microorganisms for bioenergy applications

Organism	Protein	Strategy followed	Applications	Reference
P. syringae	N-terminal His-tagged EFE (*EFEh*)	Synthetic quorum sensing promotor system	Ethylene production	Guerrero et al. (2012)
P. furiosus	NADP-dependent [*NtFe*]-hydrogenase	Heterologous expression and maturation	Biofuel production	Sun et al. (2010)
P. torridus	Esterases (*EstA* and *EstB*)	Expression of recombinant protein *E. coli*	Fatty acid esters hydrolysis	Hess et al. (2008)
C. acetobutylicum	Acetyl-CoA acetyltransferase (*THL*), Beta-hydroxybutyryl-CoA dehydrogenase (*HBD*), 3-hydroxybutyryl-CoA dehydratase (*CRT*), Butyryl-CoA dehydrogenase (*BCD*), Butyraldehyde dehydrogenase (*BYDH*), and butanol dehydrogenase (*BDH*)	Expression of recombinant protein in *E. coli*	Butanol production	Inui et al. (2008)
M. jannaschii	Citramalate synthase (*CimA*)	Directed evolution	1-propanol and 1-butanol production	Atsumi and Liao (2008)
P. stipitis	Xylose reductase (XI)	Expression of recombinant protein in *S. cerevisiae*	Ethanol production	Jeppsson et al. (2006)

identified such as improvement of hydrogen peroxide stability, increasing the redox potential to broaden the substrate range, heterologous expression, and industrial production development (Ayala et al. 2008).

Role of protein engineering in biofuel production is improving day by day. Specifically, enzyme hydrolysis was a tragedy in the case of extracting energy from biomass. Recent discoveries of protein engineering like lignocellulose degrading enzymes and biofuel-producing enzymes facilitate biofuel production (Wen et al. 2009). Biocatalyst engineering in combination with rational design and directed evolution provides efficient electrical communication between biocatalyst and electrode (Güven et al. 2010). The protein engineering studies carried out in microbes for bioenergy production were listed in Table 14.5.

Limitations will vary according to the method employed during protein engineering. Rational design was structure-based; structure–function interaction of the target enzyme should be particular. There are many chances for protein aggregation; it leads to damage in a liposome. Computational approaches were low in substrate specificity and catalysis and had difficulties in modeling. Directed evolution may lead to the low feasibility to engineer domain and secondary structure and it will become highly random. Some limitations were inaccuracy in modeling, low catalytic activity, and high failure rates. Those emerging advanced computational methods like algorithms, software, and calculation methods may diminish the limitations (Rahman et al. 2015).

14.4.8 Metabolic Process Engineering

In a controlled fermentation, the culture conditions such as pH, temperature, and nutrients are controlled at the optimum range. Any disturbance in these conditions can lead to either increase or decrease in the flux balance or metabolite production. This change in metabolite production on manipulation of the process parameters is exploited and developed into a technique called metabolic process engineering (MPE). MPE is a non-genetic engineering approach where the metabolic networks and their production are controlled by regulating the bio-production process parameters. The major goal of MPE is to reach high productivity, scalable, robust, and through less expenditure on molecular and other tedious methods. The MPE mainly focuses on controlling the physiology and metabolic responses in the cell by a change in the process parameters of the cell (Table 14.6).

Similarly, process engineering methods are bioaugmentation and consolidated bioprocessing is the processing technique in which the microbial community was introduced to improvise efficiency (Valdez-Vazquez et al. 2019). Dynamic sensor regulator system (DSRS) was adapted transcription factor to visualize intermediate

Table 14.6 List of process engineering studies carried out in different microorganisms for bioenergy applications

Microorganism involved	Process engineering applied	Applications	Reference
C. Cellulovorans and *C. acetobutylicum*	Bioaugmentation	Hydrogen production	Valdez-Vazquez et al. (2019)
C. Cellulolyticum and *C. cellulovorans*	Consolidated bioprocessing	Biobutanol production	Yang et al. (2015)
E.coli	Dynamic sensor-regulator system (DSRS)	Biodiesel production	Zhang et al. (2012)
E.coli JCL16	In situ product removal	Isobutanol production	Baez et al. (2011)
C. protothecoides	Direct methanogenesis of cellular biomass	Biodiesel production	Liu and Zhao (2007)

and regulation of expression genes (Zhang et al. 2012). Other techniques are in situ product removal and direct methanogenesis are also employed.

An emerging strapping approach in MPE is metabolomics where the regulatory mechanisms of metabolic flux balance are identified. This reveals the comprehensive knowledge about different cellular components such as proteins, genes, metabolites, transcripts, and other intermediary factors such as gene regulation, metabolic pathway, and interprotein interaction. Thus, metabolomics combined with knowledge about metabolic flux analysis is the key to MPE. Various software tools such as Open Flux and Fiat-Flux are available in open source and also allow a user-friendly calculation of metabolic flux which can be merged successfully with the data acquired from the metabolomic analysis.

The parameter involved in MPE in microorganisms for biofuel production is bioreactor operation parameters and metabolic process parameters. The bioreactor operation parameters include agitation rate, temperature, pH, and dissolved oxygen which can be controlled by the bioreactor controllers and probes. Like fuzzy-PI controller was developed to control the temperature fluctuation in commercial ethanol production (Fonseca et al. 2013). Mathematical modeling was highly implemented to analyze the dynamic process of biobutanol fermentation consisting of fermenter, cell retention system, and vacuum vessel (Mariano et al. 2010).

Still, it is puzzling to regulate the metabolic process parameters like basal medium, substrate, feed rate, and feed formulation in fed-batch fermentation due to variant behavior and activities of microorganisms. Optimal feeding is highly important to gain cell growth to achieve fuel production and to prevent nutrient depletion and accumulation of byproducts. To optimize the feeding strategy, we have to collect and analyze metabolic parameters. Analytical tools like HPLC, GC-MS, and other tools were used to evaluate the metabolites and biomass changing over the course of time interval. Current development of in situ probes including biomass probe, dissolved oxygen probe, extracellular oxidoreduction potential probe, and gas monitors minimized the issues by online samples analysis (Beutel and Henkel 2011).

14.4.8.1 Advantages and Drawbacks

Metabolic process engineering includes the benefits to convert complex hexose and pentose sugars and enhanced fermentation yields, and increased biofuel by using decreased biomass (Lynd et al. 2008). A few examples of MPE in research are the utilization of reduced substrates such as glycerol and mannitol for enhanced NADH availability for increased butanol production (Sabra et al. 2014; Luo et al. 2015). Similarly, the increased partial pressure of hydrogen, application of carbon monoxide, reduction of the iron concentration, and usage of artificial electrons assist in inhibiting hydrogen production (Girbal et al. 1995). The artificial electrons deviate from the electrons for hydrogen production and therefore help in NADH accumulation. Substrate pretreatment also needed to entangle the inhibition factor with substrate to make it suits to yield biofuel to large extent. Likewise, lignocellulosic biomass was pretreated with saturated steam at 200 °C, explosion with ammonia,

and cooked with warm dilute acid to relieve the complex of lignin and cellulose (Stephanopoulos 2007).

Recent transcriptomic analysis of *Clostridium acetobutylicum* ATCC 824 was used to understand the butanol stress to formulate the suitable medium to gain cell growth and butanol production (Heluane et al. 2011). *C. bejierinckii* NCIMB was also subjected to transcriptional analysis to evaluate the butanol metabolism with the butyrate supplementation (Wang et al. 2012). Maintenance will become the drawback to optimize the environment and bioreactor. Analyses are cost-consuming techniques to identify the suitable strain for further fermentation studies.

14.5 Conclusion

Several microbes have the potential to be a major organism for large-scale use if given a proper substrate. The currently available methods are still a work in progress which would need intensive labor and several trial and error to give an efficient output. However, improvement of the available techniques and further increasing the feasibility of these molecular approaches will allow researchers all over the world to give a contribution towards bioenergy.

One constant difficulty in all these techniques is the low transformation efficiency. However, various researchers have taken onerous efforts to improvise these protocols and resulted in higher transformation efficiencies. Future directions in the molecular approaches can include library-based methods with high transformation efficiency and appropriate screening methods. Synthetic biology is another developing field which makes a reliable toolkit for further expanded technologies with easier approaches in terms of handling and mechanism. Developments in these areas in the future will facilitate the improvement towards a sustainable bioenergy-based economy.

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Exploitation of Marine Waste for Value-Added Products Synthesis

15

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Abbreviation

ACE	Angiotensin-converting enzyme
EDS	Electron dispersive spectroscopy
EDTA	Ethylenediaminetetraacetic acid
ESI-MS/MS	Electrospray ionization tandem-mass spectrometry
FTIR	Fourier transform infrared spectroscopy
LC-MS/MS	Liquid chromatography tandem-mass spectrometry
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight
NMR	Nuclear magnetic resonance
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
sp.	Species
TGA	Thermal gravimetric analysis
TLC	Thin layer chromatography
UF-DF	Ultrafiltration-diafiltration
XRD	X-Ray diffraction

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B. Samuel Jacob et al. (eds.), *Applied Biotechnology for Emerging Pollutants Remediation and Energy Conversion*, https://doi.org/10.1007/978-981-99-1179-0_15

15.1 Introduction

The ocean covers approximately 71% of Earth's surface (NOAA 2012). The fish industry has an international market share of more or less 140 million tons of fish production (including aquaculture) of which 110 million is for human consumption (Benhabiles et al. 2012). From 1961 to 2013, there was a significant increase (3.2%) at an average annual rate for fish food supply (FAO 2016). During the processing of raw materials, fishing industries generate massive waste materials (Lan et al. 2012). These biological wastes have been exceeded to almost 20 million tons which is comparable to 25% of total marine species caught (FAOSTAT 2001). Furthermore, these industries produce biological wastes such as head, skin, trimmings, fins, bones, viscera, shells, and roe which can be utilized for generating bioactive peptides, compounds, and various biopolymers. Additionally, marine visceral mass is composed of more or less 20% of biomass and is found to be a supplementary source of digestive enzymes, bioactive peptides, and compounds which are un-knowingly discarded as low-value by-products; thus, producing extra waste disposal and environmental complications (Bougatef 2013; Joshi and Nazeer 2020).

Besides this, another serious issue is the by-catch marine species. They are collectively brought from the deep ocean to the fish harbor where the edible species are segregated and the remaining treated as by-catch. Therefore, currently marine by-catch and waste create a serious environmental problem which needs proper management. However, marine by-products are now getting into serious consideration as a significant supplementary source in the nutraceutical field. Also, marine by-products are found in a wide range of other products including oils, collagen, gelatin, hydrolysates, bioactive peptides, chitin and chitosan, etc. This chapter highlights the marine waste-derived important by-products as possible nutraceuticals with their involved extraction processes.

The phylum Chondrichthyes and Osteichthyes (also referred as "bony fish") are vertebrates and commonly comprise of bone tissues as well as skeletons. Stingrays are among the common by-catch fish due to their estuarine habit. Moreover, these by-catch fish parts cannot be consumed completely although they have a high protein content as compared to other crustaceans due to the presence of venom or bony dorsal fins (da Rocha et al. 2018). They are diverse and abundantly present in nature; also, the Osteichthyes class is considered for consumption worldwide which causes the maximum amount of biological trash waste. Mollusks are a family of marine invertebrates; they act as a potent source of seafood which contains eminent nutritional and medicinal properties (Benkendorff 2010). The muscle part is widely consumed but the viscera and their shells are discarded as wastes. These mollusks shells were powdered and widely consumed for their ethnomedicinal properties by people across the globe during different eras (Ahmad et al. 2018). According to Kumari et al. (2017), the mollusks shell and nonedible portions such as gladius, liver, intestine, mouth apparatus, eyes, and other organs can contribute to valuable by-products which are used for chitin, protein extraction, enzyme, flavor, collagen, and gelatin.

Further, the phylogenic position of Echinodermata closely resembles that of chordates and hemichordates. Sea urchins contain hard spines all over the body which in some cases are venomous with potentially deadly effects. These spines, foot, and mouth part of sea urchin are inedible and hence removed before consumption. Moreover, they are found in most parts of the world which makes them readily available and economical for the isolation of molecules. On another hand, low-molecular weight peptides and bioactive compounds can be isolated from the inedible discarded part such as the gut and body wall of sea cucumber. Additionally, star fish have saponins on the body surface and they are occasionally used as food due to unpleasant odor. Also, starfish usually feed on shellfish therefore causing loss to farmers. Hence, they are caught and dumped near the waterfront. Therefore, they can be utilized for the isolation and production of various biological compounds from there hard body and gut parts. Coelenterates are one of the simplest invertebrates' phyla in the animal kingdom and are found mostly in the rocks at the ocean bed. This phylum includes corals, sea anemones, and jellyfish and can be used for the extraction and isolation of various bioactive peptides from their waste body parts. To date, there are several bioactive peptides which are isolated from by-catch fish muscle and marine waste. However, additional research is needed to identify these peptides' applicability as bioactive peptides/compounds for Human health care (Fig. 15.1).

15.2 Bioactive Peptides

Proteins are a dynamic component which play a vital role in performing various operations in our body such as protecting and regulating biological functions. Our body consists of enzymes which hydrolyzes the protein into lower molecular weight molecules known as peptides. These peptides after hydrolysis consist of 2–20 both nonessential and essential amino acids chains which are commonly named hydrolysates. These peptides vary based on their peptide sequence composition and physiological behavior. Moreover, the bioactive peptides remain free or encrypted within the sequence of a parent protein but show wide biological activities when hydrolyzed or processed using enzymes (Udenigwe and Aluko 2012). It has been testified that the small molecular weight bioactive peptides can easily penetrate and get circulated in the circulatory system as well as can be absorbed in the intestine which can certify an outstanding effect at the targeted site (Erdmann et al. 2008). Lately, studies confirmed that amino acids such as glycine, histidine, cysteine, glutamine, and tryptophan can show anti-inflammatory effects; histidine as well as glutamine suppresses inflammation by downregulating NF-κB activation (Tsune et al. 2003; Son et al. 2005; Kim et al. 2009, 2010; Liboni et al. 2005). Glycine and histidine also display antioxidant effects (Wheeler and Thurman 1999). According to Furukawa et al. (2012), substances containing polar amino acid like aspartic acid, cysteine, glutamine, lysine, proline, serine, and tryptophan can exhibit potent antioxidant activity against hydroxyl radicals. Especially aspartic acid, cysteine, and hydrophobic amino acids play a key role in numerous antioxidant peptides

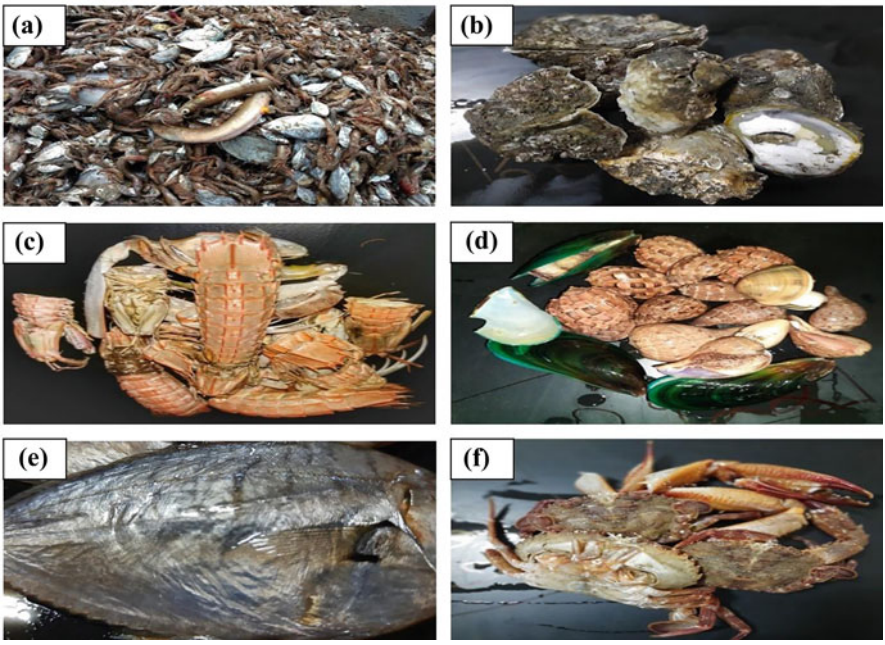

Fig. 15.1 Different sources of marine waste: (**a**) by-catch fish waste, (**b**) Oyster shells, (**c**) shrimp shells, (**d**) mollukc shells, (**e**) fish scales, and (**f**) crab exoskeleton. These waste sources are utilized to isolate several bioactive compounds, novel peptide therapeutics showing various antimicrobial, anticancer, anti-inflammatory activities, and polymers which can be used as drug delivery carriers

(Mendis et al. 2005a; Rajapakse et al. 2005). As bioactive peptides have tremendous functions, they can be useful in food and pharmaceutical drug agents. Though chemically synthesized drugs have successful curing history, these drugs also have drawbacks. For instance, antioxidants such as butylated hydroxyl anisole and butylated hydroxyl toluene long usage can cause liver damage and carcinogenesis (Ito et al. 1986); The extensive use of nonsteroidal anti-inflammatory drugs is associated with cardiovascular, renal, or gastrointestinal damage whereas synthetically designed angiotensin-converting enzyme (ACE) inhibitors can cause cough, taste disturbance, skin rashes, and deformations or death of the fetus (Wallace 2001; Torruco-Uco et al. 2009).

Hence, researchers are frequently trying to find alternative from natural sources which can diminish these side-effects. In recent years, the interest in marine bioprospecting has increased among researchers around the whole world. Previous studies have confirmed that the bioactive peptides derived from natural sources through enzymatic hydrolysis can generate notable alternative drugs and can exhibit biological as well as functional properties (Narayanasamy et al. 2020; Naqash and Nazeer 2013; Fujita and Yoshikawa 1999). Similarly, peptides as well as algal polysaccharides also have been found to reveal anticancer, antidiabetic, anticoagulant, antimicrobial, and anti-hypercholesterolemic activities (Lordan et al. 2011). Commercial anti-hypertensive peptide "Katsuo-bushi oligopeptide" with the amino

acid sequence of LKPNM was derived from dried bonito hydrolysate; it has been officially permitted by the ministry of health and welfare in Japan for specified health use (Fujita and Yoshikawa 1999).

The marine ecosystem varies from the land-based ecosystem and gives strong chemical diversity with better biochemical specificity. Comparatively lower octanol-water partition coefficient, extra routable bonds and stereo genic centers are one of the chemical properties of smaller molecular weight marine-derived products which makes them advantageous for drug discovery (Koehn and Carter 2005; Newman and Cragg 2012). Overall, researchers are showing more interest in protein hydrolysates and peptides production due to their cost-effective construction and their incredible physiological as well as physiochemical properties.

15.2.1 Bioactive Peptides Generation

There are several techniques to generate effective marine as well as its by-product hydrolysates, i.e., enzymatic hydrolysis, autolysis, and thermal hydrolysis; from which enzymatic hydrolysis is the most practiced methodology (Chalamaiah et al. 2012). The general steps for the production of bioactive peptides are shown in Fig. 15.2.

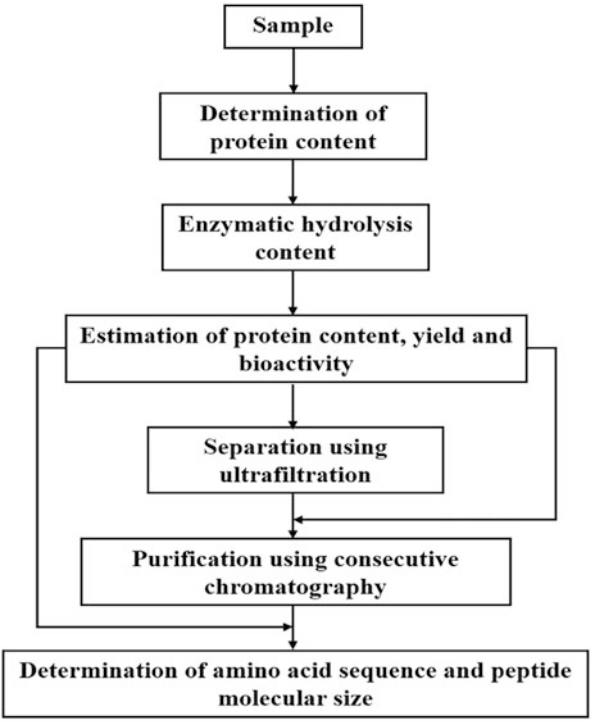

Fig. 15.2 Flowchart of bioactive peptides' isolation from marine scrape. In detail, enzymatic hydrolysis can be achieved by numerous enzymes (commonly digestive enzymes). Then it is tested for their bioactivity with specific target assays and purified based on their size, charge, or highly specific interaction between antigen-antibody, enzyme-substrate, receptor-ligand, or protein-nucleic acid. Finally, the peptide sequence along with the molecular size can be determined using Electrospray ionization tandem-mass spectrometry (ESI-MS/MS) or liquid chromatography tandem-mass spectrometry (LC-MS/MS)

15.2.2 List of Bioactive Peptides from Marine Species

Source	Peptide sequence with molecular weight	Bioactivity	References
Chondrichthyes			
Squalus acanthias heart	–	Natriuretic	Schofield et al. (1991)
Potamotrygon gr. orbignyi venom	HGGYKPTDK	Vasoconstrictor	Conceição et al. (2006)
Potamotrygon falkneri and *Dasyatis guttata* stinger tissue	–	Immunogenic and anti-inflammatory	Barbaro et al. (2007)
Potamotrygon gr. orbignyi venom	ESIVRPPPVEAKVEETPE	Microcirculatory environment	Conceição et al. (2009)
Chiloscyllium plagiosum liver	NH2-Met-Leu-Val-Gly-Pro-Ile-Gly-Ala-Ala-Lys-Val-Val-Tyr-Glu-Gln	Antidiabetic	Huang and Wu (2010)
Potamotrygon cf. *henlei* stingray mucus	–	Antimicrobial	Conceição et al. (2012)
Prionace glauca cartilage	–	Antioxidant	Weng et al. (2014)
Dasyatis sephen venom	–	Antiproliferative	Rajeshkumar et al. (2015)
Raja porosa cartilages	IVAGPQ, GPAGDY, and FIMGPY	Antioxidant	Pan et al. (2016)
Dasyatisa kajei cartilages	VPR, IEPH, LEEEE, and IEEEQ	Antioxidant	Pan et al. (2019)
Osteichthyes			
Theragra chalcogramma frame	FGASTRGA	Antihypertensive	Je et al. (2004)
Limanda aspera frame	MIFPGAGGPEL (1.3 kDa)	Antihypertensive	Jung et al. (2006)
Tuna backbone	VKAGFAWTANQQLS (1519 Da)	Antioxidant	Je et al. (2007)
Tuna liver	–	Antioxidant and antihypertensive	Je et al. (2009)
Gadus morhua backbone	–	Antioxidant	Šližytė et al. (2009)
Tuna frame	GDLGKTTTVSNWSPPKYKDTP (2482 Da)	Antihypertensive	Lee et al. (2010)
Parastromateus niger visceral mass	AMTGLQA (701.9 Da)	Antioxidant	Nazeer and Kumar (2011)
Caranx ignobilis skin	–	Antioxidant	Nazeer and AnilaKulandai (2012)
Sphyraena barracuda and *Lepturacanthus savala* backbone	–	Antioxidant	Nazeer et al. (2011)
Skate skin	PGPLGLTGP (975.38 Da) and QLGFLGPR (874.45 Da)	Antihypertensive	Lee et al. (2011)
Salmo salar skin	AP and VR (<1000 Da)	Antihypertensive	Gu et al. (2011)

(continued)

Source	Peptide sequence with molecular weight	Bioactivity	References
Exocoetus volitans backbone	–	Antioxidant, antiproliferative, and antimicrobial	Naqash and Nazeer (2011)
Threadfin bream surimi wastes (frame, bone, and skin) as well as refiner discharge	–	Antioxidant	Wiriyaphan et al. (2012)
Magalaspis cordyla visceral mass	ACFL (518.5 Da)	Antioxidant	Sampath Kumar and Nazeer (2013)
Channa striatus and *Labeo rohita* roe	–	Antioxidant	Galla et al. (2012)
Nemipterus japonicus backbone	–	Antioxidant and antiproliferative	Naqash and Nazeer (2012)
Salmon pectoral fin	1000–2000 Da	Antioxidant and anti-inflammatory	Ahn et al. (2012)
Pacific cod skin	LLMLDNDLPP (1301 Da)	Antioxidant and antihypertensive	Himaya et al. (2012)
Ctenopharyngodon idella skin	PYSFK (640.74 Da), GFGPEL (618.89 Da), and VGGRP (484.56 Da)	Antioxidant	Cai et al. (2015)
Navodon septentrionalis skin	GSGGL (389.41), GPGGFI, (546.63) and FIGP, (432.52 Da)	Antioxidant	Chi et al. (2015)
Monkfish liver	–	Antioxidant and anti-fatigue	Xu et al. (2017)
Oncorhynchus mykiss skin	–	Antioxidant and Anticancer	Yaghoubzadeh et al. (2019)
Mollusks			
Elysia rufescens (Kahalalide F)	–	Anti-tumor activity	Rademaker-Lakhai et al. (2005), Martín-Algarra et al. (2009)
Haliotis discus hannai intestine digest	–	Antioxidant and anti-inflammatory	Qian et al. (2012)
Harpa ventricosa visceral mass	AKGTWK (690.2 Da)	Anti-inflammatory	Joshi et al. (2016)
Meretrix meretrix visceral mass	HKGQCC (675.5 Da)	Anti-inflammatory	Joshi et al. (2021)
Echinoderms and arthropods			
Strongylocentrotus nudus gonad	AAVPSGASTGIYEALELR and NPLLEAFGNAK (<2 kDa)	Antioxidant	Zhao et al. (2018)
Harpiosquilla raphidea muscle	MSN (350 Da) and MTH (388 Da)	Antioxidant and ACE-1 inhibiting	Noorani and Nazeer (2020)
Charybdis natator leg muscle	LGLGAAVL (713.4 Da)	Anti-inflammatory	Narayanasamy et al. (2020)

(continued)

Source	Peptide sequence with molecular weight	Bioactivity	References
Coelenterates			
Rhipolema esculentum	–	Antioxidant	Yu et al. (2005)
Chrysaora quinquecirrha venom	–	Antitumor	Balamurugan et al. (2010)
Nemopilema nomurai	YI	Antihypertensive	Lim et al. (2013)
Palythoa caribaeorum venom	–	Hemolytic, anticancer, and Antigiardial	Lazcano-Pérez et al. (2018)

15.3 Chitin and Chitosan

Chitin is an amino-polysaccharide found most abundantly in nature after cellulose. It is synthesized by the main pivotal enzyme chitin synthase and uses acetaglucosamine in the chitin synthesis pathway present in many organisms. Although chitin cannot be found in most cellulose-producing organism, it is sometimes considered as a cellulose derivative due to similar structure (Elieh-Ali-Komi and Hamblin 2016). Moreover, chitin can be found abundantly in nature in algae, protozoa, mollusks, arthropoda, and tunicata. Whereas, mollusks and arthropoda and most widely used for the extraction of low-molecular weight polymers (Shamshina et al. 2019). It is usually present in the exoskeleton of most of the crustaceans and mollusks which protects these animals from predators as the hard chitin layer does not allow the large animals to prey on these small ones. Moreover, their shells also consist of abundant amount of calcium and other compounds due to which metabolism of this chitin using proteolytic enzymes in humans is still not confirmed. Marine animals such as crabs, shrimps, and lobsters are one of the most important seafood delicacies while the shells are discarded and thrown into the trash or dumped into the deep ocean due to their recalcitrant nature causing land and water pollution. Hence, the shells can be used for the extraction of various biopolymers and compounds showing different biological activities. Chitin extracted from the shell waste is shown to have low toxicity, high activity, high biodegradability, and biocompatibility (Mohammed et al. 2017).

On the other hand, chitosan is one of the major polymers that can be extracted from this waste apart from chitin is chitosan which is the deacetylated product of chitin. Chitosan is a copolymer of N-acetyl D-glucosamine and D-glucosamine units which on the basis of the degree of deacetylation has a number of bioactivities such as antimalarial, anticancer, and antimicrobial for the formation of biofilms (Rubini et al. 2018). Moreover, it has uses in various fields of science such as agriculture, food processing, and pharmaceuticals (Elieh-Ali-Komi and Hamblin 2016). Chitin is water-soluble while chitosan being insoluble in water needs to be dissolved in any other solvent such as acetic acid for its further usage (Shamshina et al. 2019).

Molecular weight distribution and intrinsic viscosity play a major role in the functionality of chitosan. The chitosan extracted from marine shell waste showed a high degree of deacetylation which makes it a most suitable biopolymer for the production of mesosphere, nanosphere, and scaffolds. The nanoparticle is a spherical nanocarrier system which can be loaded with the drug for sustained release and tissue-specific delivery. Due to their low molecular weight, they are able to pass through the cells easily and deliver the drug in a controlled concentration (Mohammed et al. 2017; Agarwal et al. 2018).

15.3.1 Extraction of Chitin from Shell Waste

Chitin can be extracted using different methods described by Younes and Rinaudo (Younes and Rinaudo 2015) involving steps such as demineralization where minerals like calcium and magnesium are removed using strong alkali treatment, deproteinization where proteins and fatty acids are removed using acid treatment as well as decoloration is the removal of pigments such as astaxanthins using acetic acid and anionic detergents. Apart from this chitin can also be extracted using fermentation technique by lactic acid or non-lactic acid fermentation. Prior to this the shells are enzymatically deproteinized using bacterial and alkaline proteases (Ghorbel-Bellaaj et al. 2012). Many marine crustacean shells are used for the extraction of chitin and exploited as biopolymer films, cryogels scaffolds, etc., which can be used in various applications such as tissue engineering, textile, wound dressing, and as biosensors (Elieh-Ali-Komi and Hamblin 2016).

15.3.2 Extraction of Chitosan from Shell-Derived Chitin

Chitosan is obtained by deacetylation treatment of chitin using 40–50% strong alkali such as NaOH. Deacetylation is the removal of acetyl groups from the chitin structure and replacing it with amino groups. Moreover, the degree of acetylation is time- and temperature-dependent where to obtain complete deacetylation the alkali treatment is repeated (Kurita 2006). Although chemical treatment is the most widely used method for this process, chitin deacetylase enzyme which catalyzes the hydrolysis of N-acetamino bonds in chitin is also used for the deacetylation of chitin to form chitosan (Zhao et al. 2010). Various characterization methods are available to test the purity and functionality of extracted chitosan from the shells such as Fourier Transform Infrared Spectroscopy (FTIR) to analyze the availability of functional groups, X-Ray Diffraction (XRD) to study the crystalline or amorphous nature of the polymer, Scanning Electron Microscopy (SEM) equipped with Electron dispersive spectroscopy (EDS) to see the morphological characteristics and elemental analysis, respectively (Hassan et al. 2018) (Fig. 15.3).

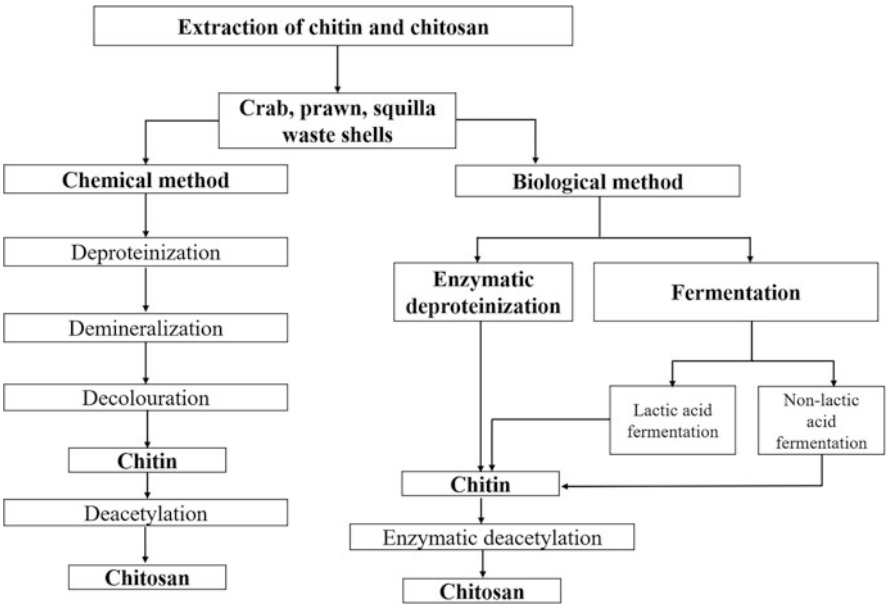

Fig. 15.3 Methodology chart for the extraction of chitin and chitosan using different processes. Marine shell waste is processed using a chemical or biological method where chemical steps involved the use of NaOH (deproteinization), HCl (demineralization), NaOCl (decoloration), and NaOH (deacetylation) in different concentrations and time of incubation. Biological procedures involve deproteinization/deacetylation using protease enzymes as well as fermentation processes to extract pure chitosan

15.3.3 List of Marine Waste Sources for Chitin and Chitosan

Species/Source	Polymer	Method of extraction	Applications	Reference
Prawn shell waste	Chitosan	Chemical deacetylation—45–72 h, 60% NaOH	–	Nessa et al. (2010)
Fenneropenaeus indicus	Chitin and chitosan	Chemical deacetylation—2 h, 50% NaOH	–	Paul et al. (2014)
Scylla serrata shell waste	Chitosan	Chemical deacetylation—30 min, 50% NaOH	–	Gaikwad and Koli (2015)
Scylla olivicea shells	Chitosan	Chemical deacetylation—2 h, 40% NaOH	Antioxidant activity	Sarbon et al. (2015)
Carcinus mediterraneus shells	Chitosan	Enzymatic deproteinization using crude protease (from *Bacillus mojavensis* A21) and chemical deacetylation—4 h, 12.5 M NaOH	Antimicrobial and antioxidant activity	Hajji et al. (2015)
Penaeus kerathurus exoskeleton				
Sepia officinalis bones				

(continued)

Species/Source	Polymer	Method of extraction	Applications	Reference
Callinectes sapidus exoskeleton	Chitin and chitosan	Chemical deacetylation—4 h, 50% NaOH	Chitosan cryogel scaffolds	Bölgen et al. (2016)
Portunus pelagicus shells	Chitin	Chemical process	Chitin-based polymer films	Fernando et al. (2016)
Crangon crangon shell	Chitosan	Chemical deacetylation—6 h, 50% NaOH	–	Kumari et al. (2016)
Labeo rohita scales				
Lutjanus sp. Scale	Chitosan	Chemical deacetylation—4–6 h, 60–80% NaOH	–	Takarina et al. (2017)
Scylla serrata shells	Chitosan	Chemical deacetylation—20 h, 50% NaOH	–	Parthiban et al. (2017)
Squilloidesleptosquill shells				
Fenneropenaeus indicus shells				
Tachypleus gigas exoskeleton	Chitosan	Chemical process	Antibacterial activity	Kassim et al. (2018)
Metacarcinus magister shell	Chitosan	Chemical deacetylation—42% NaOH	Drug delivery	Samrot et al. (2018)
Plaemon serratus exoskeleton	Chitosan	Chemical deacetylation—10 min, 50% NaOH	For lentic water bodies	Díaz et al. (2018)
Fish scales, prawns and crab shells, oyster and mussel waste	Chitin	Chemical deproteinization—18 h, 10% NaOH, and demineralization—16 h, 10% HCl	–	Alabaraoye et al. (2018)
Callinectes sapidusi shell waste	Chitosan	Chemical deacetylation—4 h, 50% NaOH	Antioxidant and antimicrobial	Metin et al. (2019)
Crab, prawn, oyster shell waste	Chitin	Liquid fermentation using *B. parabrevis* TKU046 for 4 days	Wound dressing, tissue engineering	Doan et al. (2019)
Conus inscriptus shells	Chitin	Chemical demineralization—30 min, 1 M HCl, and deproteinization—120 min, 3 M NaOH	–	Mohan et al. (2019)
Carinosquilla multicarinata shell waste	Chitosan	Chemical deacetylation—12 h, 50% NaOH	Chitosan nanoparticle-based drug delivery	Balde et al. (2020)

15.4 Collagen

Collagen is a fibrous protein which provides structure and mechanical strength to the body tissues. The extracellular matrix contains deposits of collagen, which benefits the organization, flexibility, and plasticity of a tissue. It has numerous applications in the pharmaceutical, cosmetics, and nutraceutical industries. The prime source of collagen are bovine and porcine skin and bones which contains type-I collagen but, due to ethical issues and the onset of various diseases such as foot and mouth disease and encephalopathy they are not used. Aquatic fish are a better substitute for collagen rather than pigs and cows. Fish are widely consumed for their protein content and they are rich in omega-3 fatty acids which are present in the edible fillet (muscle) part and the other parts such as skin, cartilage, and fins are discarded. The discarded aquatic waste cartilage and skin constitute almost 30% of the total fish weight and this can be utilized for collagen extraction. They are also cheaper and easily procured sources for collagen rather than porcine and bovine sources (Silvipriya et al. 2016).

The structure of collagen comprises of a triple helix domain, in which the α-chains undergo trimerization into different isoforms and supramolecular structures giving rise to 28 members in the collagen superfamily each with unique protein interactions. The α-chains in collagen contain amino acids in a range of 662–3152 chains. Each collagen type differs in the X and Y amino acid linked as Gly-X-Y. Amino acids proline and hydroxyproline are distinctly found in the sequence along with glycine. Among the 28 members of the superfamily, α4 (VI) cannot be synthesized in the human body, whereas type-I collagen comprises 90% of the organic bone mass in the animal kingdom. The superfamily can be further classified into four types based on their supramolecular interactions: (1) Fibrils, (2) Beaded fibrils, (3) Anchoring fibrils, and (4) Networks. Collagen type-II and III are present abundantly in the cartilages, and collagen I and III are found in the skin (Ricard-Blum 2011). However, collagen type-I is primarily found in fish and has wide applications in the nutraceutical and pharmaceutical fields. Collagen binds to various glycoproteins and other non-collagenase proteins to provide stability to the tissues and cells. They generally exist in two native forms: (a) a soluble form, released by the cells as a result of shredding and (b) a transmembrane form. Based on the type of collagen required they are extracted using acid extraction or enzymatic extraction.

Each part requires a different method of extraction based on its structure and composition. Traditionally, extraction of collagen was done by using 0.5M acetic acid, EDTA is used to decalcify the sample and later dialysis was done using 0.1 acetic acid to remove the salts (acid extraction), which provided a low yield of collagen (Nagai and Suzuki 2000a). However, pepsin (enzymatic method) has a unique quality of cleaving the collagen at the telopeptide region present in the tropocollagen without affecting the triple helix structure. This establishes pepsin as the common enzyme used for the extraction of collagen from porcine, bovine, and fish waste (Ali et al. 2018). Pepsin is used in an enzyme/substrate ratio of 1:20 (w/w) to the samples before adding 0.5M acetic acid. The samples are all minced into

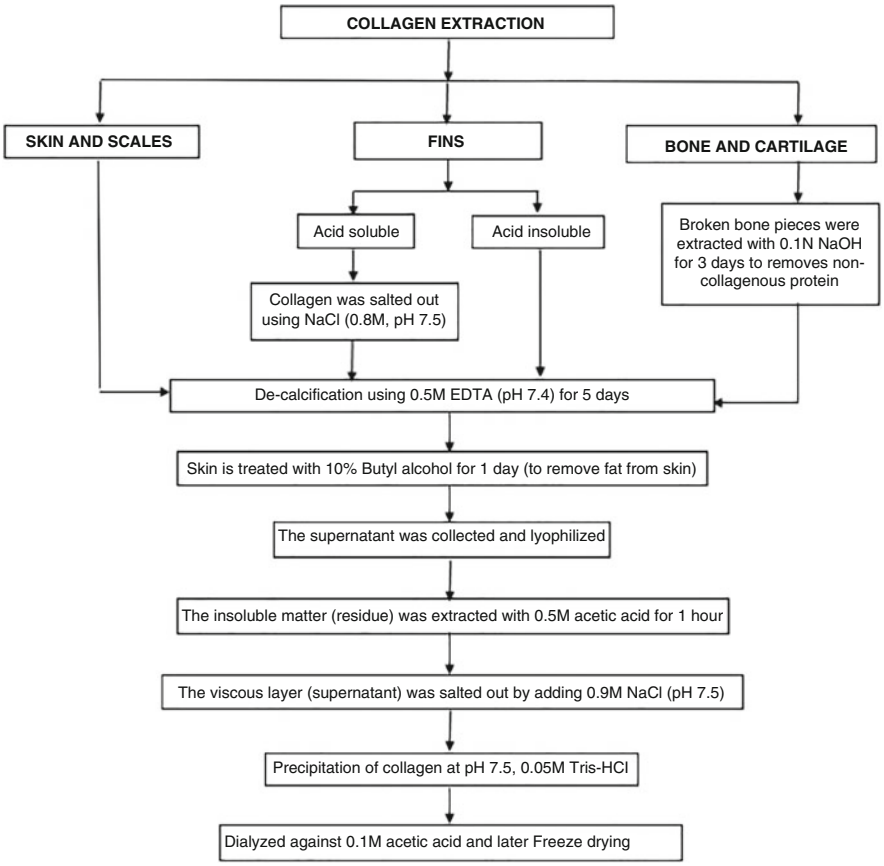

Fig. 15.4 General extraction procedure of collagen from skin, scale, fins, bone, and cartilage. Initially, the process requires the separation of crude collagen from non-collagenous protein either by salting out with NaCl or by NaOH and later decalcification in Ethylenediaminetetraacetic acid (EDTA) solution. The simplest way for collagen purification is dialysis

smaller bits and extracted at 4 °C. The yield (%) of collagen is identified and amino acid profiling is done to check the concentration of Gly-X-Y in the sample. SDS-PAGE is executed to check the conformational change in the triple helix domains. The extraction procedure of collagen is shown in Fig. 15.4.

15.4.1 Skin and Scales Collagen

The calcium hydroxyapatite crystals on the scales and skin have collagen encapsulated within them. The denaturation temperature of collagen is observed for designing medical implants and sustained drug release where the collagen should

not dissolve in the blood stream during high temperature causing unsuccessful implants and improper release of drugs (Leikina et al. 2002). The fish scales have lower denaturation temperature in comparison with commercial porcine collagen which is 313 K (Nomura et al. 1996). The collagen having less denaturation temperature are utilized for food preparations.

15.4.2 Bone, Cartilage, and Fin Collagen

Bone is a growing tissue which provides structure and support to the internal organs. Bone contains calcium phosphate, a mineral which on accumulation increases bone density. The collagen and calcium phosphate combination provide strength, and flexibility, and enhances stress tolerance. Cartilages and bones are considered offal since they are nonedible. Bone belongs to type-I collagen, whereas cartilages are of the type-II collagen family (Schmidt et al. 2016). The bone collagen when cross-linked along with glutaraldehyde expressed efficient wound-healing properties in Wistar rats (Kumar et al. 2012). Also, these types of collagens are found to have applications in the cosmetic, biomedical, and pharmaceutical industries.

15.4.3 List of Collagens from Different Sources of Various Species

Name of the species	Applications	References
Lateolabrax japonicus, Trachurus japonicus, Plecoglossus altivelis bone	Food, cosmetic, and biomedical materials	Nagai and Suzuki (2000b)
Takifugu rubripes skin	Tissue engineering	Nagai et al. (2002)
Pagrus major and *Oreochromis niloticas* scales	Biomaterials	Ikoma et al. (2003)
Pogonia cromis, Archosargus probatocephalus bone	Biomedicals and pharmaceuticals	Ogawa et al. (2004)
Sardinops melanostictus, Lateolabrax japonicus scales	Tissue engineering	Nagai et al. (2004)
Priacanthus tayenus bone	Food, cosmetics, pharmaceutical, and biomedical materials	Kittiphattanabawon et al. (2005)
Siganus fuscescens, Kyphosus bigibbus, Myliobatis tobijei, Dasyatisa kajei, Dasyatis laevigata skin	Substitute for mammalian collagens	Bae et al. (2008)
Sebastes mentella skin, scales, and bone	Food, cosmetics, pharmaceutical, and biomedical materials	Wang et al. (2008)
Cyprinus carpio skin and bone	Food, cosmetics, pharmaceutical, and biomedical materials	Duan et al. (2009)
Chiloscyllium punctatum, Carcharhinus limbatus cartilages	Food, cosmetics, pharmaceutical, and biomedical materials	Kittiphattanabawon et al. (2010)

(continued)

Name of the species	Applications	References
Diodon holocanthus skin	Biomaterials	Huang et al. (2011)
Parupeneus heptacanthus scales	Tissue engineering	Matmaroh et al. (2011)
Pangasianodon hypophthalmus skin	Wound dressing and drug delivery	Singh et al. (2011)
Magalaspis cordyla, Otolithes ruber bone	Wound healing	Kumar et al. (2011)
Hypophthalmichthys nobilis bone and fin	Food, cosmetics, pharmaceutical, and biomedical materials	Liu et al. (2012)
Sphyrna lewini skin	Substitute for mammalian collagens	Chi et al. (2014a)
Magalaspis cordyla, Otolithes ruber skin	Food, cosmetics, pharmaceutical, and biomedical materials	Kumar and Nazeer (2013)
Pseudosciaena crocea scales	Antioxidant	Wang et al. (2013)
Scomberomorous niphonius scales	Food, cosmetics, pharmaceutical, and biomedical materials	Chi et al. (2014b)
Catla catla, Cirrhinus mrigala skin	Food, cosmetics, pharmaceutical, and biomedical materials	Mahboob (2015)
Cyprinus carpio scales	Plant growth promotion	Bhagwat and Dandge (2016)
Probarbus jullieni skin	Food, cosmetics, pharmaceutical, and biomedical materials	Ali et al. (2018)

15.5 Gelatin

Gelatin is basically derived from collagen by partial hydrolysis consisting of protein and peptides using destruction of cross-linkages between polypeptide chains of collagen along with some level of breakage of polypeptide bonds. Gelatin can be applied in the food and pharmaceutical industries and can be isolated from fibrous protein present in skin, cartilage, and bone, respectively. It has been reported that factors such as extraction source, age of the animal, physiochemical properties as well as collagen type are some of the fundamental factors which tremendously affect gelatin's properties; also, gelatin conversion depends on pretreatment and the warm-water extraction process (Johnston-Banks 1990). It has been reported that factors like amino acid composition and molecular weight are responsible for the quality of gel strength and thermostability (Gómez-Guillén et al. 2002). Gelatin's amino acid sequence is most of the time observed similar to its parent collagen because during the collagen-gelatin conversion, there will not be any rearrangements of the amino acids. Gelatin can be used for the production of bioactive peptides/products, emulsifiers, foaming agents, etc.

15.5.1 Extraction of Gelatin

There are two types of pretreatment procedures by which we can generate gelatin at isoelectric point: type-A gelatin obtained at pH 8–9 and type-B gelatin at pH 4–5. In brief, type-A gelatin requires acid pretreatment in which gelatin from the raw skin and hide of animals can be generated with in a day. Marine species with warm-water habitats can be pretreated at 27 ± 2 °C, whereas for cold-water habitat species gelatin can be extracted at lower than 10°C (Hou and Regenstein 2004). On the other hand, type-B gelatin requires alkali treatment to produce gelatin from frames and bones. The general methodology of extracting gelatin is shown in Fig. 15.5.

15.5.2 List of Gelatin Applications from Marine Species

- Gelatin is in high demand for water-gel desserts.
- Gelatin is used as a stabilizer and a texturing agent in dairy products.
- In pharmaceutical industries, gelatin is used in capsules, and tablet coating.

Gelatin peptides are also used for various bioactivities.

15.5.3 List of Bioactive Peptides from Marine Species-Based Gelatin

Source	Peptide sequence with molecular weight	Bioactivity	References
Chondrichthyes			
Carcharhinus limbatus skin	–	Antioxidant	Kittiphattanabawon et al. (2012)
Pangasius sutchi skin and bone	–	Antihypertensive	Mahmoodani et al. (2014)
Prionace glauca skin	EGP, GPR, GY, GF	Antioxidant	Weng et al. (2014)
Okamejei kenojei skin	MVGSAPGVL (829 Da), and LGPLGHQ (720 Da)	Antihypertensive	Ngo et al. (2014)
Osteichthyes			
Theragra chalcogramma skin	Gly-Pro-Hyp	Antioxidant	Kim et al. (2001)
Theragra chalcogramma skin	GPL and GPM	Antihypertensive	Byun and Kim (2001)
Johnius belengerii skin	HGPLGPL (797 Da)	Antioxidant	Mendis et al. (2005a)
Sole skin	–	Antioxidant	Giménez et al. (2009)
Thunnus spp. skin	–	Antioxidant	Alemán et al. (2011a)

(continued)

Source	Peptide sequence with molecular weight	Bioactivity	References
Hypoglossus spp. Skin	–	Antioxidant	Alemán et al. (2011a)
Oreochromis niloticus skin	EGL (317.33 Da) and YGDEY (645.21 Da)	Antioxidant	Zhang et al. (2012)
Cyprinus Carpio skin	AY (253.2 Da)	Antioxidant	Tkaczewska et al. (2019)
Mollusks			
Dosidicus gigas skin	FDSGPAGVL (880.18 Da) and NGPLQAGQPGQR (1241.59 Da).	Antioxidant	Mendis et al. (2005b)
Dosidicus gigas skin	GPLGLLGFLGPLGLS	Antioxidant and antihypertensive	Alemán et al. (2011b)
Coelenterates			
Rhopilema esculentum	–	Antioxidant	Zhuang et al. (2010)

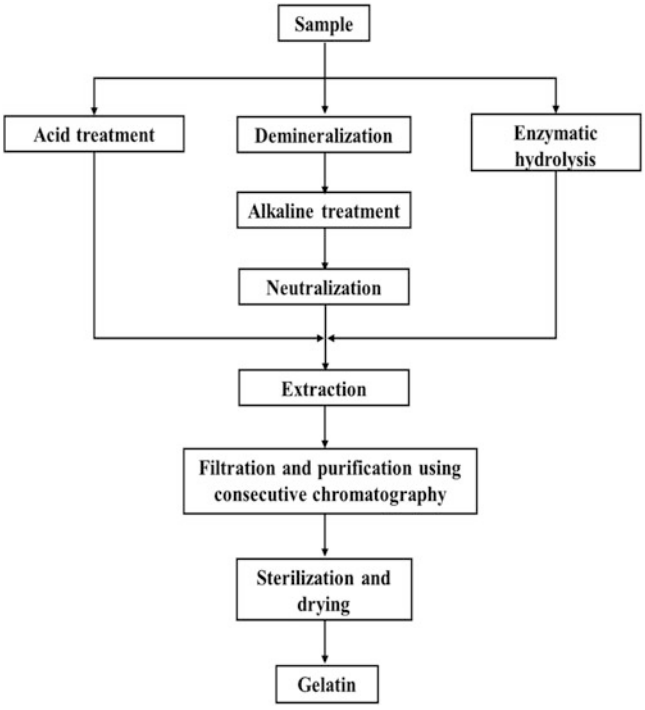

Fig. 15.5 Extraction of gelatin from marine waste samples. The gelatin can be extracted by three ways, i.e., acid treatment, demineralization, and enzymatic hydrolysis. The filtration can be done using a molecular weight cut-off ultrafiltration unit and purification can be performed by various chromatographic techniques. Also, the fish gelatin can be sterilized with different methods of sterilization which also include microfiltration or elevating at high temperature

15.6 Biopolymers

Biopolymers are a class of naturally occurring compounds which can be derived from polysaccharides, proteins, and lipids produced by living microorganisms, plants, and animals. These biodegradable polymers are biocompatible with the environment and serve a wide range of applications in the field of agriculture, petrochemical industries, medical and pharmaceutical industries, etc. (Kaplan 1998). Among the several sources of biopolymers, marine wastes have played a significant role in the generation of these biopolymers. The exploitation of these marine wastes has led to proper control of fisheries and aquaculture, low cost, and environmentally friendly removal of marine wastes, and profitability in the field production of several value-added products. Some of the biopolymers extracted from certain species of marine organism wastes such as skeleton, skins, viscera, and heads have been listed below along with their production process.

15.6.1 Polymeric 3-Alkylpyridinium Salts

3-Alkylpyridinium salts are derived from the marine sponge species *Reneirasarai,* which have expressed its activity as an antifouling agent by inhibiting the growth of algae and fungi in the marine microenvironment due to its water-soluble nature as a marine toxin. These naturally occurring polymers have expressed potent anticholinergic properties useful in the treatment of Alzheimer's disease along with cytotoxic and hemolytic activities against various tumor cell lines and inhibition of epidermal growth factor (Sepčić et al. 1997).

The process of extraction of this biopolymer is a simple process where the supernatant of the ethanolic extract of a homogenized marine sponge after centrifugation is passed through an Amicon Y3 membrane (Eleršek et al. 2008). The retentate was then passed through a Sephadex G-50 column and eluted. The eluted fractions were freeze-dried to obtain pure powdered 3-alkylpyridinium salts. This compound is then tested on fungal and algal cells for their growth inhibition. The mass of the compound has been detected by MALDI-TOF mass spectrometry method and structural analysis has been done using nuclear magnetic resonance (NMR) technology.

15.6.2 Glycosaminoglycans

Chondroitin sulfate and Dermatan sulfate are units of glycosaminoglycans which are heteropolysaccharides occurring in the extracellular matrix of animal cells and following purification can be used in the production of cosmetics and food substances. These sulfate polymers have been isolated from potent producers of glycosaminoglycans like *Salmo salar* species of salmon, *Channa argus* (snakehead), *Katsuwonus pelanis* (skipjack tuna), etc. (Lin et al. 2017). Hyaluronic acid is isolated from marine bivalve mollusk species *Amussium pleuronectus* is also a unit of

glycosaminoglycan from which the sulfur group has been removed (Saravanan and Shanmugam 2010). It is a linear polymer which consists of repeated units of N-acetyl-D-glucosamine and D-glucuronic acid linked by glycosidic linkages (Kanchana et al. 2013). Several species of sponges, sea cucumbers, squids as well as fish like shark and ray are potent producers of glycosaminoglycans which further lead to the production of chondroitin sulfate and hyaluronic acid. These compounds have played a role in the effectivity of antiviral drugs as well as in the production of malaria vaccines (Vázquez et al. 2013).

The alkaline hydrolysis of cartilage, for the recovery of chondroitin sulfate, a high concentration of NaOH, urea, and guanidine HCl has been used. Non-ionic detergents like Tween, Triton, or Brij can be used for the removal of proteins from the proteoglycan core. Similarly, in the extraction process of dermatan sulfate and hyaluronic acid, enzymes like Trypsin, Papain, or Alcalase can be used for the enzymatic hydrolysis of the skin and the vitreous humor from the eyeballs. Purification of these compounds is done by size exclusion chromatography, ion-exchange chromatography, and ultrafiltration-diafiltration (UF-DF) techniques (Fig. 15.6).

Chondroitin sulfate is mainly used in the treatment of inflammation and osteoarthritis and fish-derived chondroitin sulfate has been seen to consistently suppress osteoclast activities which is a major contributor to osteoarthritis (Cantley et al. 2013). The processing of these polymers from fish waste is economical and convenient and reduces environmental pollution; therefore, chondroitin sulfate is essentially derived from fish.

15.6.3 Omega-3-Fatty Acid

Omega-3-polyunsaturated fatty acids are usually derived from marine oil, plant oil, algal oil, flaxseed oil, etc. These fatty acids cannot be synthesized in our body and have to be taken from external dietary sources and have played a significant role in modulating immune response and in the treatment of cardiovascular diseases, ischemic stroke, psoriasis, inflammatory bowel disease, etc. (Mori and Beilin 2004). Three of the most important omega-3 fatty acids are alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid and the most common and prominent sources of these fatty acids are fish like mackerel, salmon, cod liver oil, sardines, etc. Apart from fish, certain algal sources and egg albumin, chia seeds, and sprouts also contain a decent amount of eicosapentaenoic and docosahexaenoic acid.

The fatty acid isolation was done by removing the skin of the fish and dissolving them in chloroform and phosphate buffer. Following centrifugation and dissolving the supernatant in proteinase K, the purity of the sample was analyzed by TLC method. The color and retention factor of each spot on the TLC paper separated the various omega-3 fatty acids from each other. After the isolation of the fatty acids, the biopolymer was prepared by dissolving the acid aliquots in water and adding 1.0 M

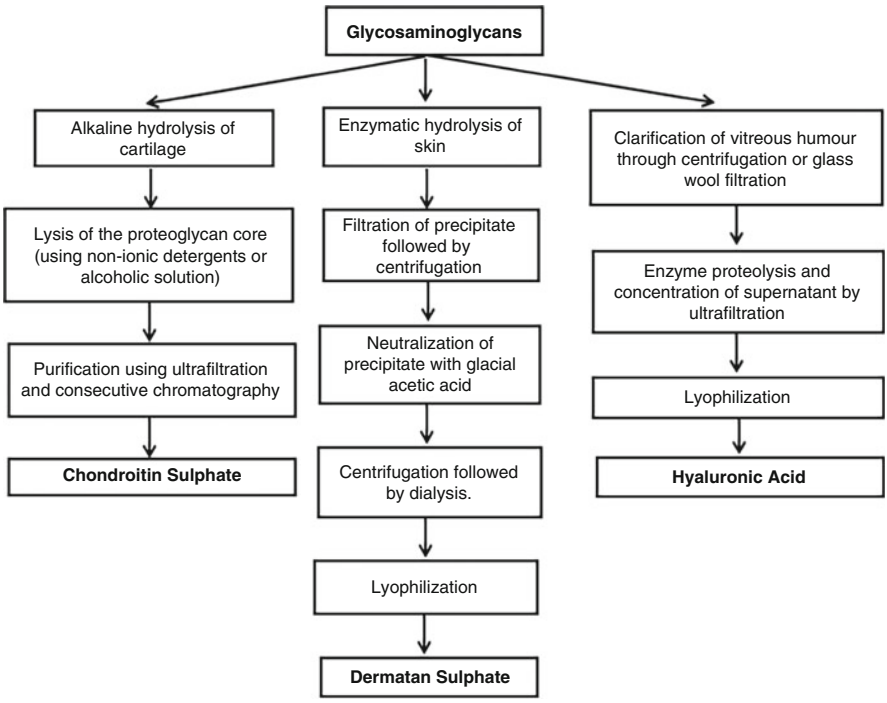

Fig. 15.6 Extraction procedure of glycosaminoglycan from marine waste skin and cartilage. These are extracted through alkaline or enzymatic hydrolysis/proteolysis with further purification procedures. Chromatographic separation of these compounds is based on affinity towards the mobile phase while ultrafiltration separates according to the molecular weight of the compounds

NaOH. The pH was adjusted to pH 11 by adding the required amount of 1.0 M HCl and vortexed. The biopolymers were formed upon instant vortexing (Fig. 15.7).

15.6.4 Polyhydroxy Butyrate

Polyhydroxy butyrate is a class of biodegradable and biocompatible thermoplastics which have played a potential role in tissue engineering, aquaculture, and medical field and have also expressed antifouling properties (Kavitha et al. 2018). These bioplastics have been obtained from different sources of marine fish as well microorganisms and the optimal conditions for the production of these bioplastics using microbes include providing an excess carbon source and limiting the availability of a single nutrient source like nitrogen or phosphorus (Mahitha and Madhuri 2015). Fish solid waste has also been used for the production of these biopolymers to reduce environmental pollution. These wastes including scales and visceral organs like intestines can be converted to polyhydroxy butyrate with the help of the bacteria *Bacillus subtilis* (Mohapatra et al. 2017). Following the process of submerged

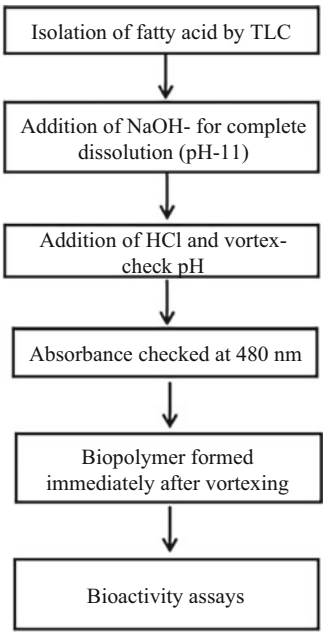

Fig. 15.7 Omega 3-fatty acid isolation from fish oil. The main analytical method used to analyze the component present in the mixture is thin layer chromatography (TLC) equipped with densitometry detection which separates the mixture based on the affinity of substance towards the mobile phase. It is a quantification technique with a rapid process and a high degree of precision.

fermentation, the polyhydroxy butyrate granules are extracted using sodium hypochlorite and these extracted polyhydroxy butyrates after structural analysis by FTIR, NMR, and thermal analysis by TGA are tested for various bioactive properties on RAW 264.7 cell lines.

Fish solid waste-derived polyhydroxy butyrate tends to show much better cell surface adhesion properties than standard polyhydroxy butyrate or polylactic acid derived from other sources. Therefore, fish waste forms an important source for the extraction of such polymers (Fig. 15.8).

Apart from these biopolymers, several other species, most prominently the soft coral species from the phylum Coelentrata have been exploited to isolate a large number of value-added products like cerberanoids, a naturally occurring diterpene isolated from *Lobophytum crassum* which plays a role in human immunodeficiency virus-inhibitory activity (Aboutabl et al. 2017), sesquiterpenoid metabolites isolated from *Lemnalia cervicornis* which has played a significant role in anticancer and antiviral activities (Bowden et al. 1986).

15.7 Conclusion

The diversity and abundance of marine sources enable the exploitation of value-added products on a commercial level. Along with the edible nature of these marine species, a large part is disposed as waste which can be of great significance in the industrial, pharmaceutical, agricultural, and cosmetic industries. These wastes

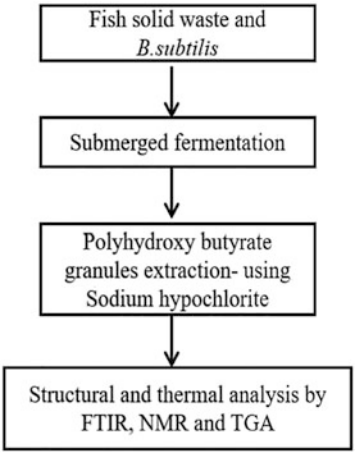

Fig. 15.8 Schematic representation of polyhydroxy butyrate extraction from fish solid waste using fermentation. Structural characterization involves FTIR spectroscopy which is an analytical technique used in order to identify organic and inorganic materials, and to observe chemical properties by identifying the chemical bonds in molecules. Further, NMR spectroscopy is used to study the purity of a given sample and to study the structure of a given molecule. On the other hand, TGA is used to determine the thermal stability of a given compound and the amount of volatile components present in the compound by measuring the occurrence of weight change when the compound is heated constantly

generated compounds are preferred over chemically synthesized products therefore several processes of recovery of these products have been carried out and we have discussed some of them such as the use of bioactive peptides in the treatment of diseases rather than using synthetic drugs, chitosan nanoparticles used in targeted drug therapy, chitin in the field of cosmetology for skin treatment and hair supplements, gelatin as food thickeners and stabilizers, collagen in tissue engineering for bone repairment and the use of biopolymers as dietary supplements and widely in the treatment of osteoarthritis. Further advancements in marine waste processing will lead to cost-effective and greater product quality reducing the land and water pollution created by dumping this discard in the ocean and harbor.

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